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CHAPTER ONE

Microbiota Regulation of the Mammalian Gut–Brain Axis

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Abstract

The realization that the microbiota—gut—brain axis plays a critical role in health and disease has emerged over the past decade. The brain—gut axis is a bidirectional communication system between the central nervous system (CNS) and the gastrointestinal tract. Regulation of the microbiota—brain—gut axis is essential for maintaining homeostasis, including that of the CNS. The routes of this communication are not fully elucidated but include neural, humoral, immune, and metabolic pathways. A number of approaches have been used to interrogate this axis including the use of germ-free animals, probiotic agents, antibiotics, or animals exposed to pathogenic bacterial infections. Together, it is clear that the gut microbiota can be a key regulator of mood, cognition, pain, and obesity. Understanding microbiota—brain interactions is an exciting area of research which may contribute new insights into individual variations in cognition, personality, mood, sleep, and eating behavior, and how they contribute to a range of neuropsychiatric diseases ranging from affective disorders to autism and schizophrenia. Finally, the concept of psychobiotics, bacterial-based interventions with mental health benefit, is also emerging.

1. INTRODUCTION

A bidirectional communication between the brain and gut exists that is referred to as the gut—brain axis (Rhee, Pothoulakis, & Mayer, 2009; Wood, Alpers, & Andrews, 1999). Mammals live in a coevolutionary association with huge quantities of commensal microorganisms resident on the exposed and internal surfaces of their bodies. The entirety of microorganisms in a particular habitat is termed microbiota, with the collective genomes of all the microorganisms in a microbiota referred to as the microbiome (Cryan & Dinan, 2012). Accumulating evidence from both clinical and basic medical research is driving our increased awareness of the significance of the human microbiota in maintaining a healthy central nervous system (CNS) (Mayer, Knight, Mazmanian, Cryan, & Tillisch, 2014).

It is estimated that 10^{14} microorganisms reside in the adult gastrointestinal (GI) tract which amounts to 10 times the number of human cells in the body, the majority of which are comprised of bacteria from 500 to

1000 different species that vary in stability, diversity, and number throughout development and across different human populations (Arumugam et al., 2011; Eckburg et al., 2005; Frank & Pace, 2008). Interestingly, the catalogue of microbial genes living in the human gut contains 3.3 million microbial genes which amount to 150-fold more than the human gene complement (Qin et al., 2010; Zhu, Wang, & Li, 2010). Though considerably smaller in size, these approximately 100 trillion cells add up to a mass of almost 1–2 kg in an adult individual—approximately the weight of a full-grown human brain (Forsythe & Kunze, 2013; Parent, 1996; Stilling, Dinan, & Cryan, 2014).

The human microbiota composition is host specific, and relatively stable (Zoetendal, Akkermans, & De Vos, 1998). The microbiome is composed of two major bacterial phyla, Bacteroidetes and Firmicutes, with others such as Proteobacteria, Actinobacteria, Fusobacteria, Archaea, and Verrucomicrobia phyla also present but in relatively small quantities (Grenham, Clarke, Cryan, & Dinan, 2011). The microbiota matures in the first three years of life (Gonzalez et al., 2011; Wopereis, Oozeer, Knipping, Belzer, & Knol, 2014) and has a physiological fundamental role in intestinal motility and in the development of the metabolic and immune systems (mucosal and systemic) (Cebra, 1999), thus protecting the host against pathogens, participating in the digestion of meals and drugs, and influencing fat absorption and distribution (Musso, Gambino, & Cassader, 2010; Patterson et al., 2014; Serino et al., 2012). In early development factors that shape the bacterial landscape include mode of delivery, feeding regime, environment, gestational age, host genetics, exposure to infections (both maternal and infant), and antibiotic usage (Cryan & Dinan, 2015). Moreover, stress, especially that in early life and prenatally, can have marked effects on microbiota composition. Shaping of the microbiota occurs in parallel with neurodevelopment and they have similar critical developmental windows (Borre, O'Keeffe, et al., 2014). Hosts also benefit from several other properties of the intestinal microbiota: vitamin K synthesis, trophic effects on intestinal epithelial cells, energy salvaging from unabsorbed food by short-chain fatty acids (SCFAs) production, growth inhibition of pathogens, maintenance of the intestinal barrier integrity and mucosal immune homeostasis and participation in the xenobiotic metabolism system (Backhed, Ley, Sonnenburg, Peterson, & Gordon, 2005; Patterson et al., 2014). The microbiota critically supports host metabolism and yields a source of metabolites, many of which would otherwise not be available to host cells.

It has been suggested that the human gut microbiota may fall into different compositional categories or enterotypes, characterized by

differences in the abundance of signature taxa, (Arumugam et al., 2011) but this concept has been challenged more recently (Knights et al., 2014). What is clear is the enrichment of specific bacterial communities in our GI tract appears to be directly linked to their function in degrading the type of food we are consuming (Lakshminarayanan, Stanton, O'Toole, & Ross, 2014; Power, O'Toole, Stanton, Ross, & Fitzgerald, 2014).

There is a rapidly increasing amount of evidence implicating host–microbe interactions at virtually all levels of complexity, ranging from direct cell-to-cell communication to extensive systemic signaling, and involving various organs and organ systems, including the CNS (Stilling, Bordenstein, Dinan, & Cryan, 2014). Thus, the sole presence of microorganisms as well as the specific composition of this microbiota has multiple, critical consequences for host physiological and metabolic processes ranging from postnatal development and immunomodulation to, perhaps most surprisingly, behavior and cognition (Sommer & Backhed, 2013; Stilling, Dinan, et al., 2014). In this chapter, we highlight our current understanding on the underlying mechanisms of microbiota–gut–brain interactions and associated behavioral alterations.

2. PATHWAYS OF MICROBIOTA–GUT–BRAIN COMMUNICATION

The bidirectional signaling between the GI tract and the brain is vital for maintaining homeostasis and is regulated at the neural (both central and enteric nervous systems (ENSs)), hormonal, and immunological levels. Interest in the potential involvement of gut microbiota in brain function emerged, in part, due to the well-described pathways of communication between the brain and the GI tract (brain–gut axis) which has been heavily studied in the area of food intake, satiety, and the regulation of the digestive tract (Cryan & O'Mahony, 2011; Davey et al., 2011; Sam, Troke, Tan, & Bewick, 2012; Schellekens, Finger, Dinan, & Cryan, 2012). The brain–gut axis plays an important role in maintaining homeostasis and its dysfunction has been linked to various psychiatric and nonpsychiatric disorders (Cryan & O'Mahony, 2011; Grenham et al., 2011; O'Mahony, Hyland, Dinan, & Cryan, 2011). Moreover, modulation of the brain–gut axis is also linked to the stress response and altered behavior with the microbiome being an important factor in the brain–gut axis communication network (Bercik, Denou, et al., 2011; Clarke et al., 2013; Dinan & Cryan, 2012). Recently, the microbiota–gut–brain axis is becoming recognized in

biomedical research, creating multidisciplinary approach in the fields of neuroscience, psychiatry, gastroenterology, immunology, and microbiology (Collins, Surette, & Bercik, 2012; Cryan & Dinan, 2012; Rhee et al., 2009).

This complex network of communication between the gut microbiota and the brain comprises the CNS, and both the sympathetic and parasympathetic branches of the autonomic nervous system (ANS) and the enteric nervous system in addition to the neuroendocrine and neuroimmune systems (Grenham et al., 2011; Moloney, Desbonnet, Clarke, Dinan, & Cryan, 2014). However, the exact mechanism of the microbiota role is not determined as not much is known of the microbial composition and the vast quantity, diversity, and the functional capabilities all these gut microorganisms (Moloney et al., 2014).

2.1 Neural Pathways

The neuronal control of the brain–gut axis transits between the CNS and the ENS via the ANS and peripheral nervous system (Jones, Wessinger, & Crowell, 2006; Rhee et al., 2009). Afferent signals of sensation, nociception, proprioception, or satiety, transmit from the GI tract to the brain, notably via vagal (vagus nerve), spinal (dorsal ganglia root), and somatosensory afferents (Grundy et al., 2006; O'Mahony et al., 2011). These signals reach relay structures such as the brain stem and periaqueductal gray, that further synapse into regulative, emotional, associative, motor, or sensory brain areas, such as the hypothalamus, thalamic nuclei, the limbic system (hippocampus, amygdala), somatosensory cortex (Grundy et al., 2006). Efferent responses or control messages are sent back to the ENS via spinal (ventral motor root) or vagal efferent. The ENS constitutes a secondary sensory, interneuronal, and motoneuronal network working on its own, allowing to be referred to as the "brain of the gut" or the "second brain" (Galligan, 2002). The ENS is important for good coordination of gut functions and maintaining the general homeostatic state of the organism (control of colon motility, the GI blood flow, and interaction with intraluminal and epithelial gut cells signaling) in order to maintain the optimal performance even in situations of threat (Galligan, 2002; Holzer, 2007). This continuous communication with the brain is facilitated by neurotransmitters, such as acetylcholine, noradrenaline, adrenaline, gamma-amino butyric acid (GABA), neuropeptides, such as substance P, neuropeptide Y, and opioids (Grundy et al., 2006; Mertz, 2002). The ENS is divided into two neuronal networks or plexus. The myenteric plexus, embedded into two layers of circular and longitudinal muscles, controls colon motility, whereas the submucous plexus,

lying on the submucosa layer of the digestive tube, directly controls GI blood flow and interacts with intraluminal and epithelial gut cells signaling (Goyal & Hirano, 1996; Wood et al., 1999). Electrophysiological studies in the area of microbiota reveal its role in ENS functioning. Previous work demonstrated that the probiotic, *Lactobacillus reuteri* targets an ion channel in enteric sensory nerves which may be mediating its effects on gut motility and pain perception (Kunze et al., 2009). Interestingly, it has been shown that electrophysiological properties of myenteric neurons are altered in germ-free mice specifically; decreased excitability in myenteric sensory neurons was found in the absence of intestinal microbiota. Upon colonization of germ-free mice with normal gut microbiota, excitability of after-hyperpolarization sensory neurons in germ-free mice was increased (McVey Neufeld, Mao, Bienenstock, Foster, & Kunze, 2013). A more recent study has shown that the gut contains intrinsic primary afferent neurons that relay or gate signals originating from the lumen to the vagal sensory ganglia via an intramural nicotinic sensory relay synapse (Perez-Burgos, Mao, Bienenstock, & Kunze, 2014).

The vagus nerve is the major nerve of the parasympathetic division of the ANS and has been shown to be an important pathway for bidirectional communication between the gut microbes and the brain (Bercik, Park, et al., 2011; Perez-Burgos et al., 2013). For example, introduction of *Lactobacillus rhamnosus* (JB-1) in the jejunal lumen of mice resulted in vagal afferents being activated and that this activation was recorded as an increase in the spontaneous frequency (Perez-Burgos et al., 2013). Moreover, chronic treatment with *L. rhamnosus* induced region-dependent alterations in GABA receptor expression in the brain and reduced stress-induced corticosterone and anxiety- and depression-like symptoms via vagus nerve signaling in mice (Bravo et al., 2011). In the same line, the anxiolytic effect of *Bifidobacterium longum* was observed only in nonvagotomized mice in an animal model of colitis (Bercik, Park, et al., 2011). Therefore, the brain—gut axis not only constitutes a purely mechanistic axis, but is a dynamic system in permanent interaction and adjustment with both internal and external milieu and factors, such as physical, physiological, and psychological events (Enck, Martens, & Klosterhalfen, 2007).

2.2 Serotonin and Tryptophan Metabolism

Serotonin [5-hydroxytryptamine] is a biogenic amine that functions as a neurotransmitter in the body, both in the CNS and the gut. Approximately, 95% of serotonin in the body is contained within the gut, specifically, in the

enterochromaffin cells of the mucosa and in the nerve terminals of the ENS neurons. Peripheral serotonin is involved in the regulation of GI secretion, gut motility, and pain perception (Costedio, Hyman, & Mawe, 2007; McLean, Borman, & Lee, 2007) and it plays an important role in maintaining mood and cognition (Cryan & Leonard, 2000). Alterations in serotonin transmission may underlie the pathological symptoms of both GI and some psychiatric disorders, and may explain their high comorbidity (O'Mahony, Clarke, Borre, Dinan, & Cryan, 2015). Actually, selective serotonin reuptake inhibitors and tricyclic antidepressants modulating serotonergic neurotransmission, have been shown to be effective in the treatment of both affective and GI disorders such as irritable bowel syndrome (IBS) (Chua, Keating, Hamilton, Keeling, & Dinan, 1992; Tack et al., 2006; Weilburg, 2004).

Serotonin synthesis in the brain depends on the availability of its precursor, tryptophan which is an essential amino acid and must be supplied in sufficient quantities in the diet (Le Floc'h, Otten, & Merlot, 2011). The evidence of a relationship between the microbiota and tryptophan metabolism has emerged from germ-free mice studies, whereby the absence of the microbiota in early life resulted in increased plasma tryptophan concentrations, reduced kynurenine:tryptophan ratio, and induced increases in hippocampal serotonin levels in adulthood. Moreover, these effects were restored following the introduction of bacteria in germ-free mice post-weaning (Clarke et al., 2013). Furthermore, inhibition of the enzyme that initiates the first and rate-limiting step of tryptophan breakdown along the kynurenine pathway indoleamine-(2,3)-deoxygenase, in rats resulted decreased concentrations of serotonin in brain and associated change in anxiety behavior in the elevated plus maze, demonstrating that peripheral tryptophan can influence brain activity and, more importantly, behavior (Naslund, Studer, Nilsson, Westberg, & Eriksson, 2013). The enzymes indoleamine-(2,3)-deoxygenase and tryptophan 2,3-dioxygenase are regulated by inflammatory mediators like proinflammatory cytokine IFN-c and corticosteroids, respectively (Ruddick et al., 2006; Taylor & Feng, 1991). Excessive immune-mediated tryptophan degradation may induce depressive symptoms when the availability of tryptophan is insufficient for normal serotonin synthesis. Moreover, depressive illness has been associated with reduced plasma tryptophan concentrations and enhanced enzymatic activity, as reflected by an increase in the kynurenine:tryptophan ratio (Myint et al., 2013). Interestingly, there is evidence to suggest that the probiotic *Bifidobacterium infantis* affects tryptophan metabolism (Desbonnet, Garrett, Clarke, Bienenstock, & Dinan, 2008). Moreover, ingestion of

Lactobacillus helveticus, for the treatment of hyperammonemia, in rats significantly reduced the level of inflammatory markers, decreased serotonin metabolism and restored cognitive function and improved anxiety-like behavior (Luo et al., 2014).

The specific mechanisms underlying this putative modulatory interaction remain unknown and much work has yet to be done to elucidate the processes involved in this potentially important pathway of communication between the gut microbiota and the CNS (See Figure 1).

2.3 Immune System

The maturation of the immune and neurological systems, as well as the microbial colonization, initiated within the fetal period, are dynamic in their character and are expanding in time through the first months and even years of human's life (Li, Wang, & Donovan, 2014). Naturally, the immune system plays an important intermediary role in the dynamic equilibrium that exists between the brain and the gut (Bengmark, 2013). The gut itself is an important immune organ forming a crucial defensive barrier between externally derived pathogens and the internal biological environment. The gut-associated lymphoid tissues, together, form the largest immune organ of the human body (Qiao, Thornton, & Shevach, 2007). In animals, infectious microorganisms have been well documented to affect behavioral measures through activation of the central immune response. For example, the pathogenic bacteria, *Campylobacter jejuni*, when administered to mice at subclinical doses, resulted in anxiety-like behavior (Lyte, Varcoe, & Bailey, 1998). In addition, peripheral administration of proinflammatory cytokines in rodents induces depressive-like behaviors, disturbances of sleep, reduced appetite and fatigue, symptoms collectively referred to as sickness behaviors (Bilbo & Schwarz, 2012). More recently, specific effects of the intestinal microbiota on the local and distal immune systems have been uncovered with important consequences for health and disease. Indeed, alterations in intestinal microbial composition have been associated with various disease states (Lei, Nair, & Alegre, 2014). The immunoregulatory effects of probiotic microorganisms may occur through the generation of T regulatory cell populations and the synthesis and secretion of the anti-inflammatory cytokine, IL-10 (Amar et al., 2011). In healthy individuals, the microbiota may constantly calibrate and arm the immune system to be ready to fight potential infections. For example, the microbiota has been shown to confer protection against *Escherichia coli*-induced sepsis, where antibiotic-induced dysbiosis resulted in reduced production of IL-17, granulocyte-colony

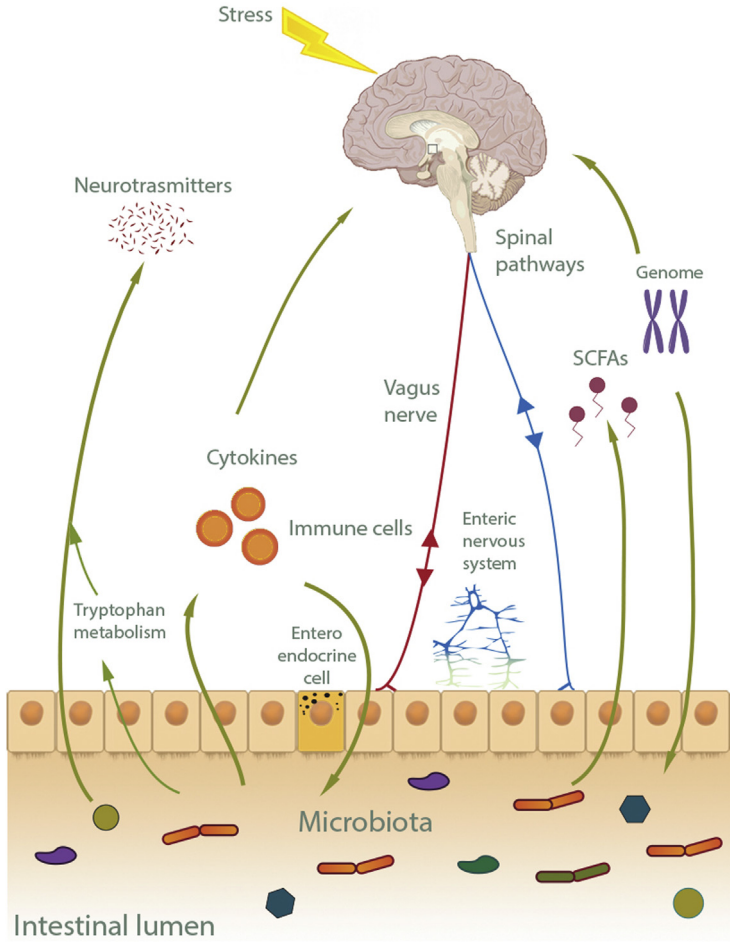

Figure 1 ***Pathways involved in bidirectional communication between the gut microbiota and the brain.*** There are many potential direct and indirect pathways through which the gut microbiota can modulate the gut—brain axis. They include endocrine (cortisol), immune (cytokines), and neural (vagus and enteric nervous system) pathways. The gut microbiota and probiotic agents can alter the levels of circulating cytokines, and this can have a marked effect on brain function. Both the vagus nerve and modulation of systemic tryptophan levels are strongly implicated in relaying the influence of the gut microbiota to the brain. Stress at the level of the CNS can also impact on gut function and lead to perturbations of the microbiota. In addition, short-chain fatty acids (SCFAs) are neuroactive bacterial metabolites of dietary fibers that can also modulate brain and behavior. Harnessing such pathways may provide a novel approach to treat various brain disorders. Neurotransmitters: serotonin, dopamine, norepinephrine, GABA. *Adapted from Cryan and Dinan (2015).*

stimulating factor (Deshmukh et al., 2014). Additionally, in a model of influenza-induced lung infection, antibiotic-treated mice exhibited a reduction in influenza-specific $CD4^+$ and $CD8^+$ T cells, resulting in increased pulmonary viral titers (Ichinohe et al., 2011). This was associated with decreased migration of dendritic cells after viral infection, an IL-1β-inflammasome-dependent event, suggesting that the microbiota may participate in priming the distal immune system (Ichinohe et al., 2011; Lei et al., 2014). Moreover, oral consumption of *B. infantis* 35624 in humans is associated with enhanced IL-10 expression in human peripheral blood (Konieczna, Akdis, Quigley, Shanahan, & O'Mahony, 2012; Konieczna, Groeger, et al., 2012). Indeed, mononuclear phagocytes in germ-free mice were less capable of producing type I and type II interferons (IFN), which resulted in defects in antiviral immunity, implicating microbiota signals in making the distal immune system competent (Abt et al., 2012; Ganal et al., 2012).

In immunocompromised patients, intestinal dysbiosis may occur as a result of antibiotic therapy and perhaps reduced immune function, such that opportunistic pathogenic bacteria may rise and possibly translocate and provoke systemic infections (Taur & Pamer, 2013). Correction of microbial imbalances may help prevent outgrowth of pathogens and infectious complications (Lei et al., 2014). Therefore, it is likely that the intestinal microbial balance closely regulates inflammatory responses in the host, and disturbances to this microbial balance, particularly in early life (O'Mahony et al., 2009), may result in a chronic inflammatory state that can lead to maladaptive changes in mood and behavior (See Figure 1).

2.4 Gut Hormonal Response

The gut can also communicate with the brain via hormonal signaling pathways that involve the release of gut peptides from enteroendocrine cells, which can act directly on the brain (Forsythe & Kunze, 2013). Gut peptides, such as ghrelin, gastrin, orexin, galanin, pancreatic polypeptide, cholecystokinin, and leptin, modulate feeding behavior, energy homeostasis, circadian rhythm, sexual behavior, arousal, and anxiety (Cameron & Doucet, 2007; Kirchgessner, 2002; Wren & Bloom, 2007). For example, galanin is suggested to modulate the hypothalamic—pituitary—adrenal axis (HPA) response to stress and may act as a link between stress, anxiety, and memory given the established adverse effects of galanin on cognitive function (Rustay et al., 2005; Wrenn et al., 2006). Similarly, ghrelin may be involved in the modulation of the HPA response to stress or changes in metabolic status (Finger, Dinan, & Cryan, 2011; Giordano et al., 2006). Indeed, ghrelin

which is released from the upper GI tract under conditions of hunger, reduces both anxiety-like and depression-related behavior (Lutter et al., 2008; Schellekens et al., 2012). The subsequent identification of orexins and their receptors in the ENS (including the myenteric and the submucosal plexuses) as well as in mucosa and smooth muscles has suggested that these neuropeptides may also exhibit local action (Baccari, 2010). In summary, ghrelin may contribute to the stress-induced rise in glucocorticoids, activating the negative feedback loop in an attempt to prevent HPA axis overstimulation (Schellekens, Dinan, & Cryan, 2013). Leptin receptors can be found in limbic structures, and chronic leptin treatment reverses stress-induced behavioral deficits (Lu, Kim, Frazer, & Zhang, 2006), suggesting a potential role for this hormone in emotional processes (Finger, Dinan, & Cryan, 2010). The idea that changes in enteric microbiota composition can alter gut hormone release is supported by probiotic studies (Di Giancamillo et al., 2008; Lesniewska et al., 2006). Furthermore, germ-free studies suggest that the gut microbiota mediates and regulates the release of gut peptides (Schele et al., 2013), yet little is known about the underlying mechanism of the hormonal aspect of the microbiota–gut–brain communication. NPY is another target thought to be involved in microbiome–brain interactions as it is sensitive to microbiota manipulations and functions both as a neural and endocrine messenger (Holzer, Reichmann, & Farzi, 2012). NPY is present at numerous locations throughout the microbiota–gut–brain axis and have a broad array of functions such as regulation of mood, stress resilience, and GI motility (Holzer et al., 2012).

Not only do bacteria in the gut produce hormone-like substances and regulate hormonal output, they can also potentially respond to the hormonal secretions of the host (Lyte, 2013). Elevations in noradrenaline concentrations after acute stress can, for example, stimulate the growth of nonpathogenic commensal *E. coli* as well as other gram-negative bacteria (Freestone et al., 2002). NPY has been found to exhibit a direct antimicrobial effect against various gut bacteria including *E. coli*, *Enterococcus faecalis*, and *Lactobacillus acidophilus* (El Karim, Linden, Orr, & Lundy, 2008). The role of the gut hormonal response in the microbiota–gut–brain cross talk is clearly an area of research that demands more attention and may offer novel therapeutic targets for the brain–gut axis disorders.

2.5 Bacterial Metabolites: Short-Chain Fatty Acids

Under the anaerobic conditions of the large intestine, undigested carbohydrates are fermented mainly to SCFAs (such as butyrate and acetate) and gases

(hydrogen, carbon dioxide, methane, and hydrogen sulphide). SCFAs have multiple effects on the host, as the major anions in the colon and as energy sources for the host, with butyrate being consumed mainly by the colonic epithelium and acetate becoming available systemically (Pomare, Branch, & Cummings, 1985). It has also been recognized that SCFAs signal to the gut receptors such as free fatty acid receptor 2 (FFAR2, formerly known as GPR43) and free fatty acid receptor 3 (FFAR3, formerly known as GPR41). These receptors are involved in controlling anorectic hormones—including peptide YY and glucagon-like peptide 1—that have roles in appetite control, thus providing a potential link between microbial SCFA formation and food intake (Sleeth, Thompson, Ford, Zac-Varghese, & Frost, 2010). Moreover SCFAs may modulate enteroendocrine serotonin secretion (Evans, Morris, & Marchesi, 2013). Other reported influences include anticancer effects (especially for butyrate), anti-inflammatory properties (Hamer et al., 2008), and changes in gut motility and energy expenditure (Gao et al., 2009; Lewis & Heaton, 1997). Circulating SCFAs, such as butyrate and propionate, travel to sites far removed from their site of production and can be carried by monocarboxylate transporters, which are abundantly expressed at the blood–brain barrier (Maurer, Canis, Kuschinsky, & Duelli, 2004). This provides a plausible mechanism through which they can cross the blood–brain barrier and enter the CNS. Once available in the CNS, they can be taken up via these same monocarboxylate transporters on glia and neurons (Pellerin, 2005; Pierre & Pellerin, 2005), and they are thought to comprise a major energy source in cellular metabolism, particularly during early brain development (Rafiki, Boulland, Halestrap, Ottersen, & Bergersen, 2003). Interestingly, intraventricular administration of propionic acid in rats induces a variety of behavioral alterations relevant to autism, although it is unclear whether this occurs via similar mechanisms as seen in the periphery (Macfabe, 2012). It is worth noting that FFAR3, a receptor activated by propionic acid, is highly expressed in rat brain tissue (Bonini, Anderson, & Steiner, 1997). SCFA interaction with FFAR2 can profoundly affect inflammatory responses (Maslowski et al., 2009). Sodium butyrate, injected systemically induced a shortlasting transient acetylation of histones in frontal cortex and hippocampus in conjunction with dynamic changes in expression of the brain-derived neurotropic factor (BDNF), thereby resulting in an antidepressant-like behavioral response in mice (Schroeder, Lin, Crusio, & Akbarian, 2007). Therefore, changes in the relative production rates of the major SCFAs by the colonic microbiota are likely to have important physiological consequences (Clarke, Stilling, et al., 2014) (See Figure 1).

There is some evidence that microbes via SCFAs do have a significant impact on epigenetic regulation in the host's gut epithelium and immune system. The effects were reported to be largely mediated by butyrate and related to altered activity of histone deacetylases (Chang, Hao, Offermanns, & Medzhitov, 2014; Smith et al., 2013). Propionate and other SCFAs, as well as lactate and pyruvate, have activity histone deacetylase inhibitory functions as well, but to a much lesser degree compared to butyrate (Latham et al., 2012; Waldecker, Kautenburger, Daumann, Busch, & Schrenk, 2008). Inhibition of histone deacetylases leads to increased histone acetylation and thereby promotes stimulus-driven transcription in active neurons. This has been shown to facilitate long-term memory consolidation and neuroprotection/-regeneration in animal models of learning and memory and neurodegenerative diseases (Govindarajan, Agis-Balboa, Walter, Sananbenesi, & Fischer, 2011; Graff & Tsai, 2013; Peleg et al., 2010). Though the effect of SCFAs that reach the CNS may be rather subtle, cumulative chronic delivery of SCFAs by the gut microbiota may result in longlasting, stable effects on gene expression (Stilling, Dinan, et al., 2014). Indeed, intracerebroventricular administration of relatively high doses of the SCFA propionic acid results in some autistic-like behaviors in rats (MacFabe, Cain, Boon, Ossenkopp, & Cain, 2011; Thomas et al., 2012). Moreover, it has been proposed that the microbiota may even be viewed as an epigenetic entity itself as it exhibits similar features in its interaction with the host as compared to classical epigenetic mechanisms such as histone modifications and DNA methylation (Stilling, Dinan, et al., 2014). Thus, the fields of (neuro)epigenetics and microbiology have the potential to converge at many levels and more interdisciplinary studies are necessary to unravel the full range of this interaction.

2.6 Host Genetics

The gut microbiome is environmentally acquired from birth (Costello, Stagaman, Dethlefsen, Bohannan, & Relman, 2012), therefore, it may function as an environmental factor that interacts with host genetics to shape phenotype, as well as a genetically determined attribute that is shaped by, and interacts with, the host (Bevins & Salzman, 2011; Spor, Koren, & Ley, 2011). Importantly, the human microbiota has been extensively investigated in recent years using the advances in next-generation sequencing and related omics technologies (Clarke, O'Toole, Cryan, & Dinan, 2014; Cotter, Stanton, Ross, & Hill, 2012). These have provided essential information not only on the microbial composition in health and disease, but

also on its impact on host metabolism and physiology (Cotter et al., 2012; Fouhy, Guinane, et al., 2012; Kim, Jeon, & Chun, 2013). Although gut microbiota can differ markedly in diversity across adults (Goodrich et al., 2014; Huttenhower et al., 2012; Qin et al., 2010), family members are often observed to have more similar microbiota profiles than unrelated individuals (Lee, Sung, Lee, & Ko, 2011; Tims et al., 2013; Yatsunenko et al., 2012). Familial similarities are usually attributed to shared environmental influences, such as dietary preference, a powerful shaper of microbiota composition (David et al., 2014; Wu et al., 2011; Zhao et al., 2014). Support for a host genetic effect on the microbiota comes mostly from studies taking a targeted approach. For instance, the concordance rate for carriage of the methanogen *Methanobrevibacter smithii* is higher for monozygotic than dizygotic twin pairs (Hansen et al., 2011), and studies comparing microbiota fingerprints between human subjects differing at specific genetic loci have shown gene–microbiota interactions (Frank et al., 2011; Khachatryan et al., 2008; Rausch et al., 2011; Rehman et al., 2011; Wacklin et al., 2011). A more general approach to this question has linked genetic loci with abundances of gut bacteria in mice (Benson et al., 2010; McKnite et al., 2012). In humans, the heritability of the gut microbiome was assessed in a well-powered twin study, by comparisons made between monozygotic and dizygotic twin pairs. This allowed identification of the most heritable taxon, the family Christensenellaceae, that also formed a cooccurrence network with other heritable bacteria and with methanogenic Archaea (Goodrich et al., 2014). A notable component of this network was the archaeal family Methanobacteriaceae. Furthermore, Christensenellaceae and its partners were enriched in individuals with low body mass index (Goodrich et al., 2014). The host alleles underlying the heritability of gut microbes, once identified, should allow to understand the nature of the association with these health-associated bacteria and eventually to exploit them to promote health.

3. MICROBIOTA REGULATION

Although a stable core microbiome is shared among individuals, certain gut microbial populations fluctuate over time, depending on several factors, such as mode of delivery, feeding regime, maternal diet/weight, probiotic and prebiotic use, and antibiotic exposure pre-, peri-, and postnatally (Fouhy, Ross, Fitzgerald, Stanton, & Cotter, 2012).

3.1 Germ-Free Studies

Germ-free animals are important for examining the relationship of the gut microbiota and brain function (Grover & Kashyap, 2014; Kirk, 2012). The use of germ-free animals enables the direct assessment of the role of the microbiota on all aspects of physiology (Cryan & Dinan, 2012). Germ-free animals are maintained in a sterile environment in gnotobiotic units, eliminating the chance of the postnatal colonization of their GI tract, thus, being a microbiota-free control group for the conventionally colonized gut of their counterparts. In adult germ-free mice, exposure to a mild restraint stress induced an exaggerated release of adrenocorticotropic hormone and corticosterone compared with control mice with a normal composition of microbiota. The stress response in germ-free mice could be partially reversed by colonization with fecal matter from control animals and was fully reversed by *B. infantis* (Sudo et al., 2004). Despite, exaggerated neuroendocrine responses to stress as demonstrated by increased basal levels of plasma corticosterone (Neufeld, Kang, Bienenstock, & Foster, 2011; Sudo et al., 2004), several independent laboratories have demonstrated consistent decreases in anxiety-like behavior in germ-free mice when exposed to novel and aversive environments (elevated plus maze, light/dark box, open field) (Clarke et al., 2013; Diaz Heijtz et al., 2011; Neufeld et al., 2011). Decreased anxiety in germ-free mice is normalized following postweaning bacterial colonization (Clarke et al., 2013). Moreover, germ-free mice also show social deficits characterized by reduced sociability with a novel stimulus mouse, in the three compartment arena compared with their conventional counterparts. Furthermore, germ-free mice exhibited repetitive grooming behavior and social cognitive deficits (Desbonnet, Clarke, Shanahan, Dinan, & Cryan, 2014), suggesting that the gut microbiota plays a role in socially driven behaviors which may be of relevance to certain psychiatric and/or neurodevelopmental disorders (see Section 4.5). Additionally, germ-free mice demonstrate impairments in nonspatial and working memory tasks (novel object recognition and spontaneous alternation assessed in the T-maze) in contrast to conventionally reared, control mice with an intact intestinal microbiota (Gareau et al., 2011). A recent study in rats has demonstrated that germ-free rats have enhanced anxiety-like behavior and neuroendocrine response to acute stress (Crumeyrolle-Arias et al., 2014).

The behavioral findings in germ-free mice correlate with molecular changes, as it has been found that germ-free mice have reduced levels of N-methyl-D-aspartate (NMDA) receptors, specifically the NR1 and

NR2A subunits, in the hippocampus (Sudo et al., 2004), or NR2B subunits in the amygdala (Neufeld et al., 2011). These molecular targets have been shown to play a key role in neuropsychiatric disorders (Lakhan, Caro, & Hadzimichalis, 2013). Interestingly, a decrease in hippocampal *BDNF* mRNA expression was observed in male germ-free animals, a qualitative increase in *BDNF* mRNA expression was present in female germ-free mice, suggesting that *BDNF* expression differences are related to sex (Clarke et al., 2013). More recently an increase in adult hippocampal neurogenesis has been shown in germ-free mice (Ogbonnaya et al., 2015). Adult neurogenesis has been shown to be an important factor in cognitive and emotional responses (O'Leary & Cryan, 2014) and understanding how the microbiota regulates it will be an important direction for future research. Using the germ-free animal authors have shown that the integrity of the blood–brain barrier is also dependent on the microbiota and especially microbial produced SCFAs (Braniste et al., 2014). In summary, germ-free studies demonstrate utility in teasing apart the mechanisms underlying the microbiota–gut–brain axis communication relevant to brain function. Further behavioral studies in germ-free animals, including the use of other species, such as rats (Crumeyrolle-Arias et al., 2014), will greatly expand our knowledge of the role of microbiota in stress-related disorders.

3.2 Prebiotics

Prebiotics are nondigestible food ingredients that selectively stimulate the growth of probiotic bacteria such as *Lactobacilli* and *Bifidobacteria* in the gut (Saulnier et al., 2013). Increasing the proportion of these bacteria with prebiotics such as galactooligosaccharides or fructooligosaccharides has many beneficial effects on the gut, the immune system, and on brain function, specifically, increased BDNF expression and NMDA receptor signaling, providing initial support for further investigations of the utility of prebiotics in mental health and potential treatment of psychiatric disorders (Drakoularakou, Tzortzis, Rastall, & Gibson, 2010; Savignac et al., 2013; van Vlies et al., 2012; Vulevic, Drakoularakou, Yaqoob, Tzortzis, & Gibson, 2008). Recently, a human study has shown that subjects supplemented with galactooligosaccharides displayed a suppression of the neuroendocrine stress response and an increase in the processing of positive versus negative attentional vigilance, showing an early anxiolytic-like profile (Schmidt et al., 2014). Inulin-type fructans and lactulose modulate gut transit, decrease putrefactive activity within the gut lumen, prevent GI infections, and mitigate

inflammatory responses (Casellas et al., 2007; Lewis, Burmeister, & Brazier, 2005; de Preter et al., 2008).

3.3 Probiotics

Probiotic bacteria are living organisms that can inhabit the gut and contribute to the health of the host (Bravo et al., 2011). In the last decade in particular, there has been an abundance of studies investigating the impact of probiotics on behaviors (Bravo et al., 2011; Desbonnet et al., 2010; Diop, Guillou, & Durand, 2008; McKernan, Fitzgerald, Dinan, & Cryan, 2010; Messaoudi, Violle, et al., 2011; Savignac, Kiely, Dinan, & Cryan, 2014). Work in animal models has assessed a range of probiotics, however, not all bacterial populations show efficacy in modulating behavior. The two most promising types of probiotics are from the bifidobacteria and *Lactobacillus* and are the main genera thus far investigated for beneficial effects on health. However, it is also pertinent to note that, not all probiotics even within bacterial genera, will produce positive effects. Moreover, the status of the host itself is critical for the efficacy of probiotics in that some probiotics will only exhibit beneficial effects in disease states such as IBS and may show no positive effects in healthy individuals. This area of research is an exciting and rapidly growing field with numerous probiotics now widely available on the market.

There is an ever-increasing number of studies in animal models assessing the impact of probiotics on behavior. Specifically, in a model of early life stress, the maternal separation model, chronic treatment with *B. infantis* in adulthood-attenuated immune system abnormalities and depressive-like behaviors in the forced swim test to a similar extent as the antidepressant citalopram (Desbonnet et al., 2010). Moreover, *L. helveticus* ROO52 has also been shown to reduce anxiety-like behavior and alleviate memory dysfunction in the Barnes maze in both naïve and western diet fed mice (Ohland et al., 2013). Work from our lab and collaborators has shown that *L. rhamnosus* reduced anxiety- and depression-related behaviors in the elevated plus maze and forced swim test respectively (Bravo et al., 2011). Similarly, another study has demonstrated reduced anxiety and improved performance on a complex maze task following treatment with live *Mycobacterium vaccae* prior to and during the test (Matthews & Jenks, 2013). *Bifidobacterium longum* has been shown to normalize anxiety-like behavior in the dextran sodium sulfate-induced colitis model (Bercik, Park, et al., 2011). Furthermore *B. longum*, but not *L. rhamnosus* normalizes anxiety-like behavior in *Trichuris muris* infection (Bercik et al., 2010). Memory dysfunction occurs as a result

of *Citrobacter rodentium* infection in C57BL/6 mice exposed to acute stress. This was prevented by daily treatment of infected mice with probiotics (*L. rhamnosus* (R0011) + *L. helveticus* (R0052)) (Gareau et al., 2011). Probiotic treatment has also proved efficacious in alleviating visceral pain responses (Johnson, Greenwood-Van Meerveld, & McRorie, 2011; McKernan et al., 2010; Rousseaux et al., 2007; Verdu et al., 2006).

There are some studies showing effects of probiotics on brain function in healthy humans. For example, women who had taken a fermented milk product containing four probiotics (*Bifidobacterium animalis* subsp. *lactis*, *Streptococcus thermophiles*, *Lactobacillus bulgaricus*, and *Lactococcus lactis* subsp. *lactis*) showed reductions in brain responses to an emotional task, particularly in sensory and interoceptive regions that were measured with functional magnetic resonance imaging (Tillisch et al., 2013). Moreover, in another study, global psychological distress and anxiety symptoms, as measured by the Hospital Anxiety and Depression Scale, were improved in the group taking a *Lactobacillus* and Bifidobacterium-containing probiotic compared with those taking a matched control product (Messaoudi, Lalonde, et al., 2011). Importantly, probiotic supplementation of the mother during and after pregnancy has been shown to alter the infant's microbiota (Lahtinen et al., 2009; Mueller, Bakacs, Combellick, Grigoryan, & Dominguez-Bello, 2014). There is a need for future trials focusing on the best combinations of probiotic strains, the timing of administration, and whether these probiotics are more efficacious in conjunction with prebiotics (Mueller et al., 2014). Also the mechanisms of action of probiotics are understudied and further investigating why certain bacterial strains have positive effects on brain health will be an important area into the future.

3.4 Antibiotics

Modern society has seen a massive increase in the prescription and use of antibiotics, however, emerging research has found that chronic antibiotic use can be both detrimental and beneficial to the host depending on the context. Rifaximin, a nonabsorbable antibiotic, has been shown to infer positive effects in IBS patients (Saadi & McCallum, 2013). However, chronic exposure to other antibiotics can have serious effects on the host while also contributing to the growing concern of antibiotic resistance and the emergence of "superbugs" (Davies & Davies, 2010). The role of antibiotics and their impact of behavior has been demonstrated in animal models. It was found that antibiotic-treated mice showed more exploratory and less apprehensive behavior which was reversible after a 2 week washout period

(Bercik, Denou, et al., 2011). However, the strain of mouse used in these studies was BALB/c, an innately anxious and stress-sensitive strain, which may impact on outcome measures. When germ-free mice were treated with antibiotic, no alteration in anxiety and exploratory behavior was observed, further emphasizing the role of the host microbiota. Increased visceral hypersensitivity has also been shown in antibiotic-treated animals, however, this effect was reversed by administration of *Lactobacillus paracasei* (Verdu et al., 2006). Indeed, data from our own group show that the early postnatal period was important for the development of antibiotic-induced visceral hypersensitivity (O'Mahony et al., 2014). Moreover, this effect was sex specific with the phenotype only being observed in male animals. Interestingly, all other behaviors assessed were unchanged by antibiotic exposure (O'Mahony et al., 2014). On the other hand, animals treated with antibiotics in adulthood display an attenuation of visceral pain-related responses elicited by intraperitoneal acetic acid or intracolonic capsaicin (Aguilera, Cerda-Cuellar, & Martinez, 2014).

3.5 Bacterial Infection

Investigating the impact of infections caused by enteric pathogens on brain and behavior has been an important strategy to interrogate the function of the microbiota–gut–brain axis. Indeed, the link between infection and psychiatric illness has long been known, mainly through the observation that syphilis and Lyme disease are often accompanied by neurological deficits (van Eijk et al., 1987; Steere, Pachner, & Malawista, 1983). Recently, it has been shown that mice infected with *T. muris* (close to human parasite *Trichuris trichiura*) increase anxiety-like behavior, decrease hippocampal levels of *BDNF* mRNA, and increase plasma kynurenine:tryptophan ratio (which is indicative of alterations in tryptophan metabolism (Section 2.2)), and increase plasma levels of the proinflammatory cytokines tumor necrosis factor-α and IFN-γ (Bercik et al., 2010). Vagotomized animals infected with *T. muris* did not prevent anxiety-like behavior induced by infection, indicating that the vagus nerve does not mediate the behavioral effects of the infection. Treatment with anti-inflammatory agents normalized behavior, reduced circulating cytokine levels, and increased tryptophan metabolism, but did not alter *T. muris*-induced changes in hippocampal *BDNF* mRNA expression (Bercik et al., 2010). Administration of the probiotic *B. longum* also normalized behavior. In addition, it restored hippocampal *BDNF* mRNA levels, but did not affect plasma cytokine or kynurenine levels (Bercik et al., 2010). Clearly, the mechanism of action

of these pharmacological and probiotic interventions differ, nevertheless, all three reversed infection-induced behavioral changes, indicating that the gut microbiota may signal to the brain through multiple routes. *Citrobacter rodentium* is another common infectious agent to investigate gut—brain axis function. Although infection with this bacterium does not affect baseline behavior in mice nor have long-term consequences (Gareau et al., 2011), it does, however, induce an increase in anxiety-like behavior short term (Lyte, Li, Opitz, Gaykema, & Goehler, 2006). In addition, a pretreatment regime with a combination of probiotics initiated 7 days before infection reduced the increase in serum corticosterone levels and prevented cognitive dysfunction (similar cognitive deficits were observed in germ-free mice). Alterations in hippocampal BDNF and central c-Fos expression (a marker for neural activity) induced by *C. rodentium* infection were also reversed by probiotic treatment (Gareau et al., 2011). All of this data suggest that the effects of infection and stress can converge and synergize to alter CNS function, behavior, and cognitive function (Cryan & Dinan, 2012). Indeed, there is a growing appreciation of the effect of gut—brain signaling on cognitive function in both animals and patients with functional GI disorders such as IBS (Kennedy et al., 2012). Similarly, there is a growing body of research aimed at increasing our understanding, at a molecular, cellular and in vivo level, of the relationship between dysregulated stress responses and immune system alterations (either individually or in combination) in the etiology of IBS (O'Malley, Quigley, Dinan, & Cryan, 2011). Other bacteria apart from infectious *C. rodentium* that also use the vagus nerve for gut-to-brain signaling is *C. jejuni*, a foodborne pathogen. It has been shown in mice to increase c-Fos levels in visceral sensory nuclei in the brain stem and stress response centers such as the paraventricular nucleus of the hypothalamus (Gaykema, Goehler, & Lyte, 2004; Lyte et al., 2006). In addition, the animals showed increased anxiety-like behavior in the holeboard test, and the level of anxiety was correlated with neuronal activation as assessed by the number of c-Fos-expressing cells in the amygdala, a key region in fear response processing (Goehler, Park, Opitz, Lyte, & Gaykema, 2008). Moreover, it has been demonstrated by vagotomy studies that *Salmonella enterica* subsp. *enterica serovar Typhimurium* also uses the vagus nerve for the transmission of signals from the GI tract to the CNS (Wang et al., 2002). In related studies, epidemiological evidence suggests that an association exists between prenatal maternal infection and the increased risk of neurodevelopmental brain disorders in rat and mouse pups (Meyer, Feldon, & Fatemi, 2009). Maternal

infection of pregnant rats with *E. coli* is associated with altered cognitive development in the offspring, and is associated with increased hippocampal neuronal apoptosis (Jiang et al., 2013). Although such studies with pathogens do not directly address the ability of the microbiota per se to signal to the brain, they offer key insights in elucidating the pathways through which microorganisms can signal to the brain and affect behavior (Cryan & Dinan, 2012).

3.6 Genetically Modified Bacteria

One potential strategy to beneficially alter the gut microbiota is to incorporate bacteria that have been genetically modified to express therapeutic factors. This strategy could also be used to sustainably deliver other therapeutic molecules beneficial in the treatment of many microbiota–gut–brain disorders or indeed in conditions where therapeutics cannot be readily delivered by other routes. This innovatory approach was recently demonstrated by incorporating genetically modified bacteria, that biosynthesize N-acyl-phosphatidylethanolamines (the immediate precursors of N-acylethanolamides, a family of the potent anorexigenic lipids), into the gut of mice which resulted in lasting attenuation of high-fat diet-induced obesity (Chen et al., 2014). Moreover, administration of N-acyl-phosphatidylethanolamines expressing bacteria to TallyHo mice, a polygenic mouse model of obesity, inhibited weight gain (Chen et al., 2014). Another application of genetically modified bacteria is to act as sensors and reporters of local gut environment (Kotula et al., 2014). This approach was demonstrated in an elegant study, where *E. coli* was engineered with a synthetic memory system based on the phage lambda cI/Cro genetic switch, that allowed the ability to sense and record antibiotic exposure during passage through the mouse gut (Kotula et al., 2014). This work lays the foundation for the use of synthetic genetic circuits in living diagnostics. One clear advantage of using genetically modified gut microbiota rather than wild-type probiotics is the ability to choose both an appropriate carrier bacteria that can colonize the gut of the diseased individual and an appropriate therapeutic compound, whereas most probiotic bacteria are poor colonizers and the actual bioactive metabolites that confer their benefit are poorly characterized (Chen et al., 2014). Without appropriate characterization of the bioactive metabolites, quality control during production and continuous culture of these probiotics may be very difficult. Another final possible advantage of genetically modified gut bacteria is that they could be engineered to be responsive to temporal cues such as food intake by use of appropriate

promoters. Such food-dependent biosynthesis would mimic physiological regulation of many metabolic responses, potentially improving efficacy (Kotula et al., 2014).

3.7 Fecal Microbiota Transplantation

Fecal transplantation can be seen as the most extreme intervention of gut microbiota. However, its use has proved lifesaving in the context of patients with *Clostridium difficile* infection (van Nood et al., 2013). The aim of fecal transplantation is to replace or replenish the intestinal microbiota of a sick individual by transplanting the microbiota from a healthy donor. One of the most intriguing and direct ways of studying the impact of microbiota on behavior is via fecal transplantation to germ-free mice (Dinan & Cryan, 2013). For example, it has been showed that colonization of germ-free BALB/c mice (anxious strain) with microbiota from NIH Swiss mice (normal anxiety levels), increased exploratory behavior and hippocampal levels of BDNF, whereas colonization of germ-free NIH Swiss mice with BALB/c microbiota reduced exploratory behavior (Bercik, Denou, et al., 2011). More recently, it has been shown that fecal transplantation from obese mice to mice whose microbiota is abrogated with antibiotics had significant and selective disruptions in exploratory, cognitive, and stereotypical behavior compared with mice with control diet microbiota in the absence of significant differences in body weight (Bruce-Keller et al., 2014).

From the literature it is clear that the majority of human studies to date have been performed in the context of *C. difficile* infection (Aroniadis & Brandt, 2013; Udayappan, Hartstra, Dallinga-Thie, & Nieuwdorp, 2014). A pilot study assessed the effects of fecal microbiota transplantation from lean healthy donors to individuals with metabolic syndrome (Vrieze et al., 2012). Regarding other potential treatable conditions, evidence for fecal microbiota transplantation at present is lacking. There are isolated case reports of fecal microbiota transplantation in relation to multiple sclerosis (MS) (Smits, Bouter, de Vos, Borody, & Nieuwdorp, 2013).

Many unanswered questions remain, including fecal microbiota transplantation methodology—for example, optimal route of administration, what makes a "good donor," safety issues, and long-term effects of fecal microbiota transplantation (Borody, Paramsothy, & Agrawal, 2013). The development of novel technologies such as artificial microbiota stool and novel delivery devices will help advance this field (Petrof et al., 2013; Youngster et al., 2014).

4. DISORDERS OF THE MICROBIOTA–GUT–BRAIN AXIS

4.1 Metabolic Disorders: Obesity, Metabolic Syndrome, Diabetes

During the past ~160 million years, mammals have coevolved with a vast and diverse microbial community that colonizes our cutaneous and mucosal surfaces. Most of these microorganisms reside within our GI tract, and their constituency is determined by host phylogeny and diet (Ley et al., 2008). Obesity is now classically characterized by a cluster of several metabolic disorders and by a low grade inflammation (Rastmanesh, 2011). Abnormal or pathological changes in gut microbiota promote gut permeability, increase metabolic endotoxemia, and increase the risk to develop metabolic disorders such as obesity (Cani & Delzenne, 2009). Individuals with type 2 diabetes mellitus and/or obesity often display a chronic low grade inflammation in the gut combined with an altered composition of the gut microbiota (Delzenne, Neyrinck, Backhed, & Cani, 2011). The gut microbiota influences whole-body metabolism by affecting energy balance and metabolic inflammation associated with obesity and related disorders (Tremaroli & Backhed, 2012). Moreover, the intestinal microbiota is altered in humans and animal models of obesity (de Vos & de Vos, 2012). The mechanisms by which gut microbiota contribute to the pathophysiology of obesity have been investigated in many mouse studies and, thanks to their results, we can now draw a clearer picture of the impact of gut microbiota on maintaining energy balance of the host (Nguyen, Vieira-Silva, Liston, & Raes, 2015). The intestinal (cecum-derived) microbiota of ob/ob mice has a 50% reduction in levels of Bacteroidetes and an increased proportion of Firmicutes compared with wild-type mice (Ley et al., 2005). The composition of the fecal microbiota of obese human subjects is similarly affected but changes with weight loss (Ley, Turnbaugh, Klein, & Gordon, 2006). Studies in germ-free mice provide insights into the effects of the intestinal microbiota on host metabolism. Germ-free mice fed high-fat, high-sugar diets did not have the same metabolic disturbances as their conventional littermates (Backhed et al., 2004). Transfer of intestinal microbiota from obese mice resulted in significantly greater adiposity in recipients than transfer of microbiota from lean donors (Backhed, Manchester, Semenkovich, & Gordon, 2007).

One way in which intestinal microbes might affect host metabolism is by extracting calories from otherwise indigestible carbohydrates which are fermented by intestinal microbes to produce SCFAs (Gao et al., 2009; Tilg,

2010). SCFAs may act as an energy substrate as they are absorbed by the intestinal epithelium and metabolized in the liver (Turnbaugh et al., 2006). Mouse models of obesity and human obese subjects have increased intestinal (cecal) levels of SCFAs and decreased energy content in their feces (Schwiertz et al., 2010).

Studies have associated changes in proportions of Bacteroidetes and Firmicutes with obesity and metabolic syndrome. Metagenome-wide association studies in Europe and in China reported metagenomic differences between a cohort of patients with type 2 diabetes mellitus and a group of healthy subjects (Karlsson et al., 2013; Qin et al., 2012). Clusters of genomic sequences were used as signatures for specific groups of bacteria, and each study found independently that the microbiota of subjects with type 2 diabetes mellitus had a lower proportion of butyrate-producing *Clostridiales* (*Roseburia* and *Faecalibacterium prausnitzii*), and greater proportions of *Clostridiales* that do not produce butyrate, as well as pathogens such as *Clostridium clostridioforme*. These studies raise interest in the association between type 2 diabetes mellitus and reduced production of butyrate because diets supplemented with butyrate were previously shown to prevent and reverse insulin resistance in mice that became obese on high-calorie diets and increase energy expenditure (Gao et al., 2009). Combined results from human and animal studies of obesity suggest that reduced butyrate production by the microbiota contributes to the development of insulin resistance (Nieuwdorp, Gilijamse, Pai, & Kaplan, 2014). Individuals with type 2 diabetes mellitus and/or obesity often display a chronic low grade inflammation in the gut combined with an altered composition of the gut microbiota (Delzenne et al., 2011). Moreover, in a rat model of type 1 diabetes, it was found that intestinal microbiota composition and microbial diversity were altered over time (Patterson et al., 2015). Furthermore, conventionalization of adult germ-free C57BL/6 mice with a normal microbiota harvested from conventionally raised animals has shown to increase the body fat content and insulin resistance within 14 days despite reduced food intake (Backhed et al., 2004). Taken together, modulation of the gut microbiota may be promising nutritional and pharmacological target in the management of obesity and obesity-related disorders (Torres-Fuentes, Schellekens, Dinan, & Cryan, 2014) (See Figure 2).

4.2 Functional GI Disorders

4.2.1 Irritable Bowel Syndrome

IBS is a common functional GI disorder with an estimated prevalence of 10–20% in the general population (Longstreth et al., 2006). Symptoms

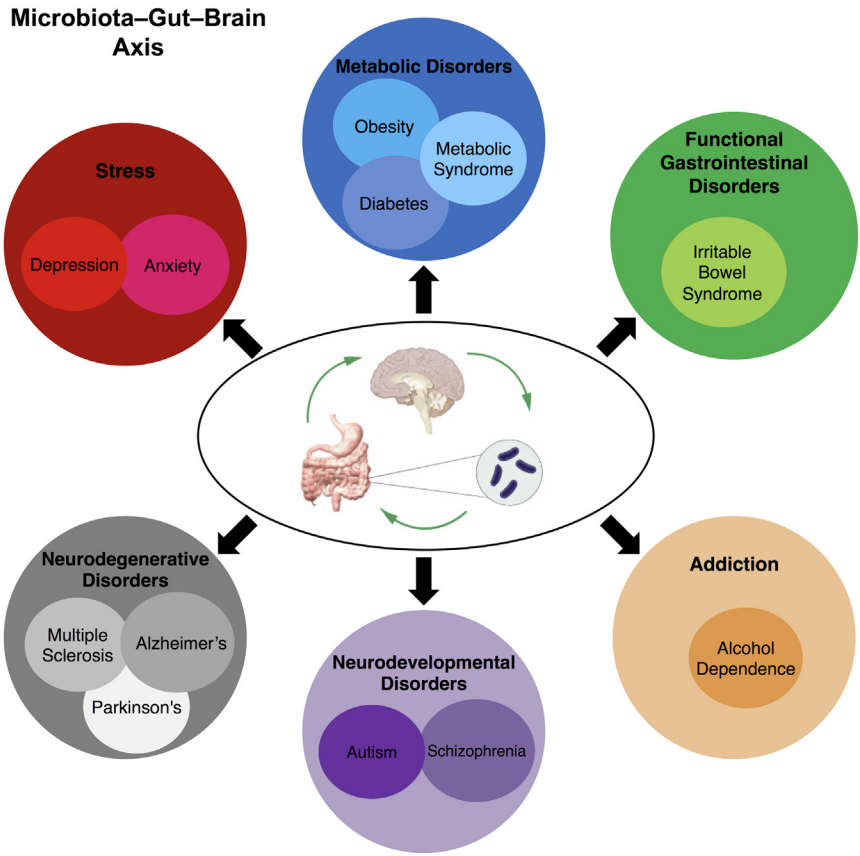

Figure 2 ***Disorders of the microbiota–gut–brain axis.*** The microbiota–gut–brain axis plays an important role in maintaining homeostasis and its dysfunction has been linked to various psychiatric and nonpsychiatric disorders.

include abdominal pain, altered bowel habit, and bloating. Moreover, IBS patients in a recent study displayed a subtle but significant deficit on a hippocampal-mediated test of visuospatial episodic memory, which was related to morning cortisol levels and independent of psychiatric comorbidity (Kennedy et al., 2014, 2012). Although the pathophysiology of IBS is not fully understood, it is best regarded as a disorder caused by dysregulation of the complex interactions along the microbiota–gut–brain axis. The role of the gut microbiota in IBS has been reviewed by many (Collins, 2014; Dupont, 2014; Grenham et al., 2011; Major & Spiller, 2014; Mayer, Savidge, & Shulman, 2014; Moloney et al., 2014; Ohman & Simren, 2013) and the utility of microbiota-based therapies for this

disorder is now an area of intense research (Clarke, Cryan, Dinan, & Quigley, 2012).

The most striking evidence for the role of the gut microbiota in the onset of IBS symptoms is in the case of postinfectious IBS, whereby symptoms emerge after a bout of gastroenteritis or the occurrence of enteric infection (Spiller & Garsed, 2009). Indeed, fecal microbiota composition of postinfectious IBS patients was significantly different from both general IBS patients and healthy controls (Sundin et al., 2014). It has also been demonstrated that subgroups of IBS patients may have an altered microbiota composition relative to healthy individuals, mainly based on the analysis of fecal microbiota (Salonen, de Vos, & Palva, 2010; Tana et al., 2010). In a recent study, fecal communities were monitored over 6–8 weeks from diarrhea-predominant IBS patients and compared with that of their healthy spouse. It was found that a higher temporal instability in the fraction of active microbiota, related to the IBS condition and fluctuations in symptoms, than that seen in healthy spouses (Durban et al., 2013). This highlights that the composition of the gut microbiota in IBS patients is unstable temporally and may explain the findings described below. Both increases (Rajilic-Stojanovic, Smidt, & de Vos, 2007) and decreases (Codling, O'Mahony, Shanahan, Quigley, & Marchesi, 2010) in the diversity of the microbiota have been reported in IBS patients (Jeffery et al., 2012). Independent of the change in direction, this abnormal variation likely reflects a loss of homeostasis within the gut, thus altering the environment of the bacterial community leading to altered composition.

Although the specific mechanisms by which changes in the gut microbiota lead to IBS symptoms remain unclear, it is hypothesized that higher numbers of microbes such as *Lactobacilli* and *Veillonella* spp. in IBS patients result in a high level of organic acids such as acetic and propionic acid, which in turn may contribute to abdominal pain, bloating, anxiety and poor quality of life (Tana et al., 2010).

In light of these findings examining gut microbiota composition and diversity in IBS, it is not surprising that many are now investigating the potential of microbial-based therapeutics (both nonabsorbable antibiotics and probiotics) in IBS (Clarke et al., 2012; Moayyedi et al., 2010; Parkes, Sanderson, & Whelan, 2010; Saulnier et al., 2013). These approaches have recently been reviewed by (Shanahan & Quigley, 2014).

The most promising findings have been in the application of probiotics in IBS (Clarke et al., 2012; Dai, Zheng, Jiang, Ma, & Jiang, 2013; Ford et al., 2014; Theodorou, Ait Belgnaoui, Agostini, & Eutamene, 2014). Multispecies probiotics are effective in IBS patients and induce positive alterations in

the composition of intestinal microbiota (Yoon et al., 2014). However, inconsistencies in trial design including dosage, probiotic strain selection, and sample size need to be addressed in future clinical studies so that a true evaluation of probiotics for the treatment of IBS can be made.

Moreover, the proposal that fecal transplantation may be an effective treatment strategy in IBS has recently been appreciated but warrants further investigation (Borody, Brandt, & Paramsothy, 2014; Pinn, Aroniadis, & Brandt, 2014; Smits et al., 2013).

4.3 Stress, Anxiety, and Depression

Despite the well-established association between stress and psychiatric disorders, the struggle to understand the complex processes by which stress mediates pathological changes that increase vulnerability to disease is on-going (Hornig, 2013). Severe, chronic, and uncontrollable stressors can trigger maladaptive changes in brain structure and function that can have long-term consequences on one's physical and mental wellbeing (Burokas et al., 2014; Lupien, McEwen, Gunnar, & Heim, 2009; Moloney et al., 2014; Nutt & Malizia, 2004). Moreover, it is clear that stress at different periods in life can have different physiological consequeces (Cryan & Dinan, 2013; Hyland et al., 2015; Lupien et al., 2009; O'Connor & Cryan, 2014; Prenderville, Kennedy, Dinan, & Cryan, 2015).

Microbiota–gut–brain axis dysregulation in stress-related CNS disorders recently has received increasing recognition in the studies of GI disorders (Bested, Logan, & Selhub, 2013; Bravo et al., 2012; Cryan & Dinan, 2012; Cryan & O'Mahony, 2011; Foster & McVey Neufeld, 2013; Sherman, Zaghouani, & Niklas, 2014). The stress response is generated by the complex integration of a series of interconnected brain regions, most notably the amygdala, the hippocampus, and the paraventricular nucleus of the hypothalamus, which also receive modulatory inputs from higher cortical regions such as the prefrontal cortex (Moloney et al., 2014, 2012). The major output of the central stress circuitry consists of the neuroendocrine HPA axis, and the ANS. The association between microbiota and stress response is further supported by experiments with germ-free mice and rodents treated with probiotics and/or antibiotics. It has been demonstrated an enhanced HPA axis activity in germ-free mice following an acute psychological stress, providing first convincing evidence of the essential role played by microbiota in programming of the stress response (Sudo et al., 2004). Another study has shown that treatment with a probiotic strain, *Lactobacillus farciminis* attenuates intestinal

permeability and the HPA axis response to an acute stress in rats (Ait-Belgnaoui et al., 2012).

Depression and general anxiety are disorders with well-established etiological links to the traumatic life events, particularly when experienced in early life and during periods of chronic stress in both humans and animals (Burokas et al., 2014; Caspi et al., 2003; Kendler, Thornton, & Gardner, 2000). Animal studies suggest that microbiota may play a major role in lifetime stress, anxiety, and mood regulation (Fond et al., 2014). It has been demonstrated that the experimental administration of the endotoxin lipopolysaccharide in healthy humans can be associated with increased rates of anxiety and depression, in turn associated with increased salivary cortisol, plasma norepinephrine, and proinflammatory cytokines (Grigoleit et al., 2011). Low-gastric acid secretion has been reported in patients with severe depressive disorders and has been associated with reversible small intestinal bacterial overgrowth, increased intestinal barrier permeability, malabsorption syndrome, diarrhea, abdominal pain, and constipation (Addolorato et al., 2008). In a clinical study focused on further exploration of the link between microbiota composition and depression, researchers observed a general underrepresentation of the Bacteroidetes phylum in depressed patients and an association of the Lachnospiraceae family with the depression group, and interestingly, even with a decrease in Bacteroidetes, specific operational taxonomic units identified as members of the Bacteroidetes phylum correlated with depression (Naseribafrouei et al., 2014).

Indeed, probiotic treatment in animal studies during the postnatal stress period in maternally separated rat offspring has been shown to normalize basal corticosterone levels (Gareau, Jury, MacQueen, Sherman, & Perdue, 2007). When administered to mice, *L. rhamnosus* reduced stress-induced corticosterone, which was paralleled by region-dependent alterations in GABA receptor gene expression levels in the brain (Bravo et al., 2011). Moreover, the neurochemical effects were not found in vagotomized mice, identifying the vagus nerve as a major modulatory communication pathway between the bacteria exposed to the gut and the brain (Bravo et al., 2011). *Bifidobacterium infantis* altered peripheral cytokine levels and concentrations of the serotonin precursor, tryptophan, which may allude to the development of possible protective mechanisms prior to stress exposure (Desbonnet et al., 2008). The therapeutic potential of probiotics in psychiatric conditions has been the topic of intense discussion and additional investigations are required to fully elucidate the role of probiotics in brain function (Clarke et al., 2012; Davis et al., 2008; Mayer, Knight, et al., 2014).

Despite the lack of clinical data to support the idea to utilize probiotics in the treatment of mood disorders, there are sufficient preclinical data to support this view. One of the principal mechanisms proposed to underlie stress-induced alterations is the "leaky gut" phenomenon, which has been described by in major depression (Maes, Kubera, & Leunis, 2008). Thus, increased intestinal permeability and the consequent translocation of gram-negative bacteria across the mucosal lining to sites where direct interaction with immune cells and the ENS can occur (Gareau, Silva, & Perdue, 2008). This may lead to activation of an immune response characterized by increased production of inflammatory mediators. Indeed, it has been shown that patients with major depression had higher serum concentrations of IgM and IgA against lipopolysaccharide of enterobacteria than healthy controls (Maes et al., 2008). Potential psychobiotics are delivery vehicles for neuroactive compounds, and have a capacity to reduce inflammatory response and reduce HPA activity, a much broader profile than of existing antidepressant treatment options (Dinan, Stanton, & Cryan, 2013). As not all probiotics are equal in their effects and may not have psychobiotic potential, a careful examination of their efficacy is warranted. There is no doubt that many patients, particularly those with milder symptom profiles, would value the rise of nonconventional antidepressants in the form of psychobiotics (Borre, Moloney, Clarke, Dinan, & Cryan, 2014).

Taken together, the enteric microbiota has a significant impact on the behavioral, neurochemical, and immunological measures relevant to the brain–gut axis disorders with psychobiotics as a promising emerging treatment (Dinan et al., 2013) (See Figure 2).

4.4 Neurodegenerative Disorders

4.4.1 Alzheimer's

Alzheimer's disease (AD) is the leading cause of dementia in the elderly. The most common early symptom is difficulty in remembering recent events (short-term memory loss) (Querfurth & LaFerla, 2010). As the disease advances, symptoms can include: problems with language, disorientation (including easily getting lost), mood swings, loss of motivation, not managing self-care, and behavioral issues (Burns & Iliffe, 2009) There is strong epidemiological and clinical evidence that abnormality in inflammatory signals in the brain contributes to the slow degeneration of neurons, deposition of amyloid protein, and early dysfunction in the brains of AD patients (Huang & Mucke, 2012). Meanwhile, accumulating evidence has led to the realization that the mediators of neurodegeneration behind cognitive decline and memory loss could also derive from the periphery (Perry & Holmes, 2014).

Interestingly, induction of AD in mice could increase inflammatory responses both in the brain and blood, suggesting that inflammatory events in the peripheral system are closely associated with AD pathogenesis (Aso, Sanchez-Pla, Vegas-Lozano, Maldonado, & Ferrer, 2015; Jiang et al., 2009). Dysregulation of serotonergic and kynurenine routes of tryptophan metabolism influences the CNS pathological conditions of dementia, Huntington's disease, and AD (Ruddick et al., 2006). Recent studies propose association of the gut microbiome with development of obesity and metabolic syndromes, such as type 2 diabetes mellitus where the vascular effects of obesity play a role in the development of AD (Alam, Alam, Kamal, Abuzenadah, & Haque, 2014; Naseer et al., 2014; Noble et al., 2014). It is generally believed that gut microbiota control obesity that is the cause of type 2 diabetes mellitus, which consequently may cause AD (Naseer et al., 2014). Studies are providing the solid evidence that expression and phosphorylation of tau protein (AD is also characterized by intracellular tangles containing hyperphosphorylated tau protein) are regulated by insulin and insulin growth factor signaling cascades and the critical aspects of AD can be explained on the basis of impaired insulin signaling (de la Monte & Wands, 2008).

4.4.2 Multiple Sclerosis

MS is a devastating autoimmune disease that is characterized by the progressive deterioration of neurological function. This damage disrupts the ability of parts of the nervous system to communicate, resulting in a wide range of signs and symptoms, including physical, mental, and sometimes psychiatric problems (Compston & Coles, 2008). It has been suggested that the gut microbiota may have a role in MS (Berer et al., 2011). Indeed, it has been shown that the induction of experimental autoimmune encephalomyelitis (EAE), by myelin oligodendrocyte glycoprotein (MOG) peptide, an animal model of MS, was greatly attenuated in germ-free mice (Lee, Menezes, Umesaki, & Mazmanian, 2011). This relative resistance is probably due to the reduced immune responses to MOG in the germ-free animals (Lee, Menezes, et al., 2011), further exemplifying the extent of the effects of the gut microbiota on CNS function via the immune system.

Similar effects were shown in another study (Berer et al., 2011), in which mice that were genetically predisposed to spontaneously develop EAE were housed under germ-free or specific-pathogen-free conditions and, as a result, remained fully protected from EAE throughout their life. This protection dissipated upon colonization with conventional microbiota in adulthood. These data illustrate a key role for the gut microbiota in

immunomodulatory mechanisms underlying MS, and further studies should also investigate whether other aspects of MS pathophysiology, especially at the spinal cord level, are affected by the gut microbiota.

4.4.3 Parkinson's Disease

The motor symptoms of Parkinson's disease (PD) result from the death of dopamine-generating cells in the substantia nigra, a region of the midbrain (Dickson et al., 2009). Extranigral pathology is related to a broad spectrum of nonmotor symptoms that have been increasingly recognized as an important feature of PD (Muller, Assmus, Herlofson, Larsen, & Tysnes, 2013). GI dysfunction, in particular constipation, affects almost 80% of PD patients and may precede the onset of motor symptoms by years (Cersosimo & Benarroch, 2012). In a recent study, the intestinal microbiota has been shown to be altered in PD and related to motor phenotype (Scheperjans et al., 2014). Another study suggests that variation in genes encoding peptidoglycan recognition proteins which modulates the immune response to advantageous and harmful gut bacteria and plays a major role in the development and maintenance of a healthy commensal microbiota (Royet, Gupta, & Dziarski, 2011) affects the risk of PD (Goldman et al., 2014). The gut is a site of early involvement in PD and peptidoglycan recognition proteins influence the host immune response to gut bacteria and the makeup of the gut microbiota, they could play a role in PD cause and pathogenesis (Goldman et al., 2014). Further characterization of these mechanisms may lead to novel early approaches to delay or prevent onset of PD.

4.5 Neurodevelopmental Disorders

The microbiota modulates a range of neurotrophins and proteins, such as BDNF, synaptophysin, and PSD-95 all previously shown to be involved in brain development and plasticity (Diaz Heijtz et al., 2011; Douglas-Escobar, Elliott, & Neu, 2013). A decrease in BDNF and NR2a receptor expression was found in the cortex and hippocampus of germ-free animals compared with controls (Sudo et al., 2004). BDNF is a neurotrophin growth factor associated with neurogenesis and brain plasticity, and NMDA receptors are a group of ionotropic glutamate receptors involved in the control of synaptic plasticity in memory function (See Figure 2).

4.5.1 Autism

Autism is a neurodevelopmental disorder characterized by impaired social interaction, verbal and nonverbal communication, and restricted and

repetitive behavior (Caronna, Milunsky, & Tager-Flusberg, 2008). Autism affects information processing in the brain by altering how nerve cells and their synapses connect and organize; how this occurs is not well understood (Levy, Mandell, & Schultz, 2009). The appellation "autism spectrum disorder" (ASD) refers to a group of heterogeneous neurodevelopmental disorders with multiple causes and courses, a significant range in severity of symptoms, and several associated comorbid disorders, including anxiety and GI symptoms (Mayer, Padua, & Tillisch, 2014). Microbiota dysbiosis has mostly been studied in ASD, a collective term used for a diverse group of neurodevelopmental conditions, characterized by impaired social interactions and communication, restricted and repetitive behavior, and frequently accompanied by digestive disorders (Fond et al., 2014; Kushak, Lauwers, Winter, & Buie, 2011; de Magistris et al., 2010; Rogers, Viding, Blair, Frith, & Happe, 2006; Torrente et al., 2002; Williams, Hornig, Parekh, & Lipkin, 2012). While genetics play a major role in the etiology of ASD, recent years have seen an emerging interest in the potential role of environmental factors in this disorder (Grabrucker, 2012). Among the associated environmental risk factors, GI abnormalities and altered microbiota composition have been identified in a number of small-scale studies on children with ASD (Finegold et al., 2010; Williams et al., 2011) that directly correlate with symptom severity (Adams, Audhya, et al., 2011; Adams, Johansen, Powell, Quig, & Rubin, 2011). Several teams have studied the intestinal microbiota of the autistic population and found a different composition of various microbial species in comparison to healthy controls. Compared to healthy children, children with autism have been found to have increased *Clostridium*, Bacteroidetes, and *Desulfovibrio*, and decreased Firmicutes and *Bifidobacterium* species (Y. Song, Liu, & Finegold, 2004). However, there is much controversy in the field and varying results have emerged. Whereas increases in Bacteroidetes and decreases in Firmicutes have been reported in autistic children presenting with GI symptomology (Finegold et al., 2010), examination of the fecal flora in a similar cohort revealed no differences in microbiota composition relative to neurotypical sibling measures (Gondalia et al., 2012).

Intestinal permeability disorders have also been described in autism (Fond et al., 2014; Torrente et al., 2002). For example, it has shown the increased levels of lipopolysaccharide in the blood of individuals with ASD, a finding that corresponded to increased peripheral IL-6 levels, a neuromodulating cytokine (Emanuele et al., 2010). Some studies found increased intestinal permeability in autistic subjects and in their first-degree

relatives, suggesting that these changes may be involved in the pathogenesis of the disease rather than in the consequences of autistic behaviors (Finegold et al., 2010; Yap et al., 2010). However, the existence of a GI pathology specific to ASD subjects remains a controversial topic. Interpretation of results from these studies is complicated by the knowledge that individuals suffering from ASD have high rates of antibiotic usage and consume diets which often differ from those of healthy populations and may account for reported microbial changes (Cryan & Dinan, 2012). For example, a parent survey indicating that children who are not breast-fed are at higher risk of developing ASD, suggests that diet-related factors with the capacity to alter gut microbiota composition at a very early age are more likely to play a direct causative role in ASD (Schultz et al., 2006). An additional factor such as mode of delivery, which is known to alter gut microbiota composition (Curran et al., 2014) may also impact on the development of ASD.

When tested, antibiotic treatment of ASD children did not only lead to GI improvements, but also to improvements in cognitive skills (Sandler et al., 2000). Additionally, altered fecal concentrations of SCFAs, which are neuroactive microbial fermentation products, have also been reported in ASD (Wang et al., 2012).

To date, this area of research had not been extensively explored in the preclinical field. Nevertheless, a recent study conducted in germ-free mice demonstrated robust and reproducible social deficits characterized by social avoidance and deficits in social cognition in addition to increases in repetitive grooming behaviors in these microbiota-depleted mice when compared to mice with conventional bacterial colonization (Desbonnet et al., 2014). Interestingly, reconstitution of microbiota from weaning onward normalized social interest in germ-free mice but had no effect on social cognition in the three-chambered social test, indicating that the adolescent period is particularly important in the programming of specific aspects of normal social behavior (Desbonnet et al., 2014; Moloney et al., 2014).

In summary, these studies provide promising evidence indicating a more direct role for the microbiota–gut–brain axis in the pathogenesis of ASD than previously considered. This is an area of research that has received greater attention in the field of autism in recent years and will without doubt generate more interest and fruitful results in the coming years that may impact on treatment strategies in ASD (Fond et al., 2014; Louis, 2012; Mulle, Sharp, & Cubells, 2013; de Theije et al., 2011).

4.5.2 Schizophrenia

Schizophrenia has been recognized as a devastating neuropsychiatric disorder for over a century, but the mechanism of the disease remains elusive. Common symptoms include false beliefs, unclear or confused thinking, auditory hallucinations, reduced social engagement and emotional expression, and inactivity (Picchioni & Murray, 2007). Schizophrenia is often described in terms of positive and negative (or deficit) symptoms. Positive symptoms are delusions, disordered thoughts and speech, and tactile, auditory, visual, olfactory and gustatory hallucinations, typically regarded as manifestations of psychosis and generally respond well to medication (Jensen et al., 2008; Picchioni & Murray, 2007). Negative symptoms are deficits of normal emotional responses or of other thought processes, and respond less well to medication (Brunet-Gouet & Decety, 2006). Recent genome-wide association studies have shown that thousands of small single nucleotide polymorphisms carry weak-effect associations but cumulatively could explain approximately 30% of the underlying genetic risk (Singh, Kumar, Agarwal, Phadke, & Jaiswal, 2014). The strongest findings in genome-wide association studies thus far have been from immune-related genes (Schizophrenia Working Group of the Psychiatric Genomics, 2014; Stefansson et al., 2009). The findings from clinical studies demonstrate an upregulated immune and inflammatory status in patients with schizophrenia (Song et al., 2013) and a correlation between the level of inflammatory markers and severity of clinical symptoms (Hope et al., 2013). It has been suggested that the uncontrolled neuroinflammation by proinflammatory cytokines is involved in the pathogenesis of schizophrenia (Dennison, McKernan, Cryan, & Dinan, 2012; Nemani, Hosseini Ghomi, McCormick, & Fan, 2014). Chronic macrophage activation and secretion of interleukin-2 and interleukin-2 receptors has been proposed as the basic biological mechanism of schizophrenia in earlier papers (Smith, 1991, 1992). For example, the protozoa *Toxoplasma gondii* is known to cause major perturbation to the gut microbiota and is a recognized environmental risk factor for schizophrenia (Bhadra, Cobb, Weiss, & Khan, 2013; Molloy et al., 2013). More recently a chlorovirus (family Phycodnaviridae) has been identified in humans that affects cognitive function relevant to schizophrenia in animal models (Yolken et al., 2014).

NMDA receptor hypofunction is believed to be central to the pathophysiology of schizophrenia, as NMDA receptor antagonists produce schizophrenia-like symptoms while agents that enhance NMDA receptor function reduce negative symptoms and improve cognition (Coyle, 2012).

Variation in BDNF expression is believed to play a role in the molecular mechanism underlying cognitive dysfunction in schizophrenia (Nieto, Kukuljan, & Silva, 2013). Given that normal development of the microbiota is necessary to stimulate brain plasticity through the appropriate expression of BDNF and NMDA receptors, it is possible that altered microbiota may contribute to the NMDA receptor dysfunction seen in schizophrenia (Dinan, Borre, & Cryan, 2014; Nemani, Hosseini Ghomi, McCormick, & Fan, 2015). In animal model of schizophrenia (chronic NMDA antagonist treatment), it has been shown that the gut microbiota profile correlated to memory performance, suggesting an influence of the microbiota on cognition in the model, which was supported by restoration of cognition through oral ampicillin administration (Pyndt Jorgensen et al., 2014).

Evidence showing possible microbiota alteration in schizophrenia includes structural damage to the GI tract, a heightened immune response to infectious pathogens and food antigens, and known differences in the microbiome in other neuropsychiatric disorders (Nemani et al., 2014). Further investigation into the microbiota and how the gut–brain axis may mediate the link between neuropsychiatric disease and the immune system is needed.

It is also worth noting that one of the most important side effects of treatments for schizophrenia is weight gain and metabolic syndrome. We have recently shown that the microbiota plays a critical role in olanzapine-induced weight gain in rats (Davey, Cotter, et al., 2013; Davey, O'Mahony, et al., 2012) which has been confirmed in germ-free mice study (Morgan et al., 2014).

4.6 Addiction

4.6.1 Alcohol Dependence

Alcohol dependence is a substance-related disorder in which an individual is physically or psychologically dependent upon drinking alcohol. Studies suggest a role for inflammation in the development of several psychiatric diseases (Felger & Lotrich, 2013; Grenham et al., 2011), including alcohol dependence (Kelley & Dantzer, 2011), a disorder that affects 5–7% of the population in developed countries (Anderson & Baumberg, 2007). Preclinical studies have shown that chronic ethanol administration induces microbial dysbiosis in rats (Mutlu et al., 2009) and mice, with a decrease in the level of *Ruminococcaceae* (Bull-Otterson et al., 2013), or a decrease in the level of Firmicutes and an increase in Bacteroidetes (Yan et al., 2011). In humans, a decrease in *Bifidobacterium* and *Lactobacillus* was observed in the stool cultures

of alcohol-dependent subjects compared with those of healthy controls (Kirpich et al., 2008). Inflammation in alcohol dependence has been attributed to a local proinflammatory effect of ethanol, either in the brain or in the liver (Kelley & Dantzer, 2011). However, heavy chronic alcohol consumption induces gut mucosal damage, increases intestinal permeability (Keshavarzian et al., 2009; Leclercq et al., 2012), induces changes in the composition of the gut microbiota (Kirpich et al., 2008; Mutlu et al., 2009), and induces bacterial overgrowth in the small intestine (Yan et al., 2011). The view is that systemic inflammation plays a role in alcohol dependence and be induced by increased intestinal permeability and permeation of lipopolysaccharide is supported by more recent data in humans (Leclercq et al., 2012). Chronic alcohol consumption activated the mitogen-activated protein kinase/activator protein 1 pathway, together with the inflammasome complex. This activity resulted in increased messenger RNA and plasma levels of IL-8, IL-1β, and IL-18 (Leclercq, De Saeger, Delzenne, de Timary, & Starkel, 2014). Activated proinflammatory pathways, in particular, IL-8 and IL-1β, were positively correlated with alcohol consumption and alcohol-craving scores (Leclercq, De Saeger, et al., 2014). Moreover, short-term alcohol withdrawal was associated with the recovery of lipopolysaccharide-dependent receptors (Leclercq, De Saeger, et al., 2014). Despite a relationship between the gut microbiota, depression and anxiety, it also frequently develops in actively drinking alcohol-dependent subjects and plays an important role in the negative reinforcement of drinking tendency (Koob & Le Moal, 2005). These factors are strongly related to the urge to drink, an important predictor of relapse after detoxification (de Timary et al., 2013). Thus, the gut microbiota seems to be a previously unidentified target in the management of alcohol dependence (Leclercq, Matamoros, et al., 2014).

5. IMPLICATIONS AND FUTURE PERSPECTIVES

5.1 Therapeutic Manipulation of the Microbiota—A New Hope?

There is considerable commercial interest in the gut microbiota as indexed by the expanding markets for probiotics, some of which have shown significant benefits in the setting of clinical trials of GI disorders (Aziz, Dore, Emmanuel, Guarner, & Quigley, 2013; Farmer, Randall, & Aziz, 2014). Recently, psychobiotics (live organisms that, when ingested in adequate amounts, produces a health benefit in patients suffering from

psychiatric illness) have received a lot of attention as a promising emerging treatment (Dinan et al., 2013).

We have showed that chronic administration of *L. rhamnosus* in mice induces region-dependent alterations in GABA receptor expression in the CNS, which has been implicated in the pathogenesis of anxiety and depression (Bravo et al., 2011). Similarly, it has been shown that probiotics may prevent the development of changes in brain activity in mice in response to chronic stress (Ait-Belgnaoui et al., 2014). In humans, the consumption of fermented milk product with probiotic, affected activity of brain regions that control central processing of emotion and sensation using functional brain imaging (Tillisch et al., 2013). These data suggest that certain organisms may prove to be useful therapeutic adjuncts in stress-related disorders, although well-designed controlled human trials are needed to further evaluate this interesting concept (Cryan & Dinan, 2012; Saulnier et al., 2013). When accounting for the potential of proinflammatory cytokines to affect aspects of central functioning such as depression and cognitive function, and the fact that the gut microbiota are key regulators of immune function and inflammatory responses (Shanahan, 2012), it is likely that a change in composition of the intestinal microbiota during aging plays a major role in the manifestation of immunosenescence and consequently inflammageing (Prenderville et al., 2015). As such, delineation of the nature of this link could identify novel therapies to treat cognitive decline and psychiatric disorders during old age (Prenderville et al., 2015).

Overall, it is becoming increasingly apparent that behavior, neurophysiology, and neurochemistry can be affected in many ways through modulation of the gut microbiota. Whether this translates to microbial-based CNS therapeutics remains a tempting possibility and one that is worthy of much further investigation (Borre, Moloney, et al., 2014; Cryan & Dinan, 2012). While rodent models suggest that the microbiota plays a fundamental role in the genesis of the HPA axis, the serotoninergic system, the immunoinflammatory system, and that the microbiota can affect the CNS through multiple pathways, few studies have been carried out on humans. Considering the gut's multifaceted capacity to communicate with the CNS, it is plausible that the gut and its components are playing a crucial role in resultant mood and behaviors. Some therapeutic opportunities targeting potential microbiota dysbiosis have already been explored such as probiotic administration, fecal transplantation, or diet modifications, with inconsistent results. Exciting evidence from animal studies has provided the rationale to warrant further exploration in humans, both in health and disease. Future research

should focus on delineating the relative contributions of immune, neural, and endocrine pathways through which the gut microbiota communicates with the brain. A better understanding of these pathways will improve our knowledge about the role of gut microbiota play in a range of neurological disorders, including neuropsychiatric diseases such as depression, anxiety, autism, as well as AD. Future work should focus on gut microbiota manipulations for treating metabolic diseases and neurological diseases. Further work is also needed to better understand the mechanism by which different bacterial groups can differentially affect CNS functioning.

List of Abbreviations

AD	Alzheimer's disease
ANS	Autonomic nervous system
ASD	Autism spectrum disorder
BDNF	Brain-derived neurotrophic factor
CNS	Central nervous system
EAE	Autoimmune encephalomyelitis
ENS	Enteric nervous system
FFAR	Free fatty acid receptor
GABA	Gamma-aminobutyric acid
GI	Gastrointestinal
HPA	Hypothalamic–pituitary–adrenal axis
IBD	Inflammatory bowel disease
IBS	Irritable bowel syndrome
IFN	Interferon
IL	Interleukin
MOG	Myelin oligodendrocyte glycoprotein
MS	Multiple sclerosis
NMDA	N-methyl-D-aspartate
NPY	Neuropeptide Y
PD	Parkinson's disease
SCFA	Short-chain fatty acid

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REFERENCES

Abt, M. C., Osborne, L. C., Monticelli, L. A., Doering, T. A., Alenghat, T., Sonnenberg, G. F., et al. (2012). Commensal bacteria calibrate the activation threshold of innate antiviral immunity. *Immunity, 37*(1), 158–170. http://dx.doi.org/10.1016/j.immuni.2012.04.011.

Adams, J. B., Audhya, T., McDonough-Means, S., Rubin, R. A., Quig, D., Geis, E., et al. (2011). Nutritional and metabolic status of children with autism vs. neurotypical children, and the association with autism severity. *Nutrition & Metabolism (London), 8*(1), 34. http://dx.doi.org/10.1186/1743-7075-8-34.

Adams, J. B., Johansen, L. J., Powell, L. D., Quig, D., & Rubin, R. A. (2011). Gastrointestinal flora and gastrointestinal status in children with autism–comparisons to typical children and correlation with autism severity. *BMC Gastroenterology, 11*, 22. http://dx.doi.org/10.1186/1471-230X-11-22.

Addolorato, G., Mirijello, A., D'Angelo, C., Leggio, L., Ferrulli, A., Abenavoli, L., et al. (2008). State and trait anxiety and depression in patients affected by gastrointestinal diseases: psychometric evaluation of 1641 patients referred to an internal medicine outpatient setting. *International Journal of Clinical Practice, 62*(7), 1063–1069. http://dx.doi.org/10.1111/j.1742-1241.2008.01763.x.

Aguilera, M., Cerda-Cuellar, M., & Martinez, V. (2014). Antibiotic-induced dysbiosis alters host-bacterial interactions and leads to colonic sensory and motor changes in mice. *Gut Microbes*. http://dx.doi.org/10.4161/19490976.2014.990790.

Ait-Belgnaoui, A., Colom, A., Braniste, V., Ramalho, L., Marrot, A., Cartier, C., et al. (2014). Probiotic gut effect prevents the chronic psychological stress-induced brain activity abnormality in mice. *Neurogastroenterology & Motility, 26*(4), 510–520. http://dx.doi.org/10.1111/nmo.12295.

Ait-Belgnaoui, A., Durand, H., Cartier, C., Chaumaz, G., Eutamene, H., Ferrier, L., et al. (2012). Prevention of gut leakiness by a probiotic treatment leads to attenuated HPA response to an acute psychological stress in rats. *Psychoneuroendocrinology, 37*(11), 1885–1895. http://dx.doi.org/10.1016/j.psyneuen.2012.03.024.

Alam, M. Z., Alam, Q., Kamal, M. A., Abuzenadah, A. M., & Haque, A. (2014). A possible link of gut microbiota alteration in type 2 diabetes and Alzheimer's disease pathogenicity: an update. *CNS & Neurological Disorders Drug Targets, 13*(3), 383–390.

Amar, J., Chabo, C., Waget, A., Klopp, P., Vachoux, C., Bermudez-Humaran, L. G., et al. (2011). Intestinal mucosal adherence and translocation of commensal bacteria at the early onset of type 2 diabetes: molecular mechanisms and probiotic treatment. *EMBO Molecular Medicine, 3*(9), 559–572. http://dx.doi.org/10.1002/emmm.201100159.

Anderson, P., & Baumberg, B. (2007). Alcohol policy: who should sit at the table? *Addiction, 102*(2), 335–336. http://dx.doi.org/10.1111/j.1360-0443.2006.01713.x.

Aroniadis, O. C., & Brandt, L. J. (2013). Fecal microbiota transplantation: past, present and future. *Current Opinion in Gastroenterology, 29*(1), 79–84. http://dx.doi.org/10.1097/MOG.0b013e32835a4b3e.

Arumugam, M., Raes, J., Pelletier, E., Le Paslier, D., Yamada, T., Mende, D. R., et al. (2011). Enterotypes of the human gut microbiome. *Nature, 473*(7346), 174–180. http://dx.doi.org/10.1038/nature09944.

Aso, E., Sanchez-Pla, A., Vegas-Lozano, E., Maldonado, R., & Ferrer, I. (2015). Cannabis-based medicine reduces multiple pathological processes in AbetaPP/PS1 mice. *Journal of Alzheimer's Disease, 43*(3), 977–991. http://dx.doi.org/10.3233/JAD-141014.

Aziz, Q., Dore, J., Emmanuel, A., Guarner, F., & Quigley, E. M. (2013). Gut microbiota and gastrointestinal health: current concepts and future directions. *Neurogastroenterology & Motility, 25*(1), 4–15. http://dx.doi.org/10.1111/nmo.12046.

Baccari, B. C. (2010). Orexins and gastrointestinal functions. *Current Protein & Peptide Science, 11*(2), 148–155.

Backhed, F., Ding, H., Wang, T., Hooper, L. V., Koh, G. Y., Nagy, A., et al. (2004). The gut microbiota as an environmental factor that regulates fat storage. *Proceedings of the National Academy of Sciences of the United States of America, 101*(44), 15718–15723. http://dx.doi.org/10.1073/pnas.0407076101.

Backhed, F., Ley, R. E., Sonnenburg, J. L., Peterson, D. A., & Gordon, J. I. (2005). Host-bacterial mutualism in the human intestine. *Science, 307*(5717), 1915–1920. http://dx.doi.org/10.1126/science.1104816.

Backhed, F., Manchester, J. K., Semenkovich, C. F., & Gordon, J. I. (2007). Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. *Proceedings of the National Academy of Sciences of the United States of America, 104*(3), 979–984. http://dx.doi.org/10.1073/pnas.0605374104.

Bengmark, S. (2013). Gut microbiota, immune development and function. *Pharmacological Research, 69*(1), 87–113. http://dx.doi.org/10.1016/j.phrs.2012.09.002.

Benson, A. K., Kelly, S. A., Legge, R., Ma, F., Low, S. J., Kim, J., et al. (2010). Individuality in gut microbiota composition is a complex polygenic trait shaped by multiple environmental and host genetic factors. *Proceedings of the National Academy of Sciences of the United States of America, 107*(44), 18933–18938. http://dx.doi.org/10.1073/pnas.1007028107.

Bercik, P., Denou, E., Collins, J., Jackson, W., Lu, J., Jury, J., et al. (2011). The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. *Gastroenterology, 141*(2), 599–609. http://dx.doi.org/10.1053/j.gastro.2011.04.052, 609 e591–593.

Bercik, P., Park, A. J., Sinclair, D., Khoshdel, A., Lu, J., Huang, X., et al. (2011). The anxiolytic effect of *Bifidobacterium longum* NCC3001 involves vagal pathways for gut-brain communication. *Neurogastroenterology & Motility, 23*(12), 1132–1139. http://dx.doi.org/10.1111/j.1365-2982.2011.01796.x.

Bercik, P., Verdu, E. F., Foster, J. A., Macri, J., Potter, M., Huang, X., et al. (2010). Chronic gastrointestinal inflammation induces anxiety-like behavior and alters central nervous system biochemistry in mice. *Gastroenterology, 139*(6). http://dx.doi.org/10.1053/j.gastro.2010.06.063, 2102–2112 e2101.

Berer, K., Mues, M., Koutrolos, M., Rasbi, Z. A., Boziki, M., Johner, C., et al. (2011). Commensal microbiota and myelin autoantigen cooperate to trigger autoimmune demyelination. *Nature, 479*(7374), 538–541. http://dx.doi.org/10.1038/nature10554.

Bested, A. C., Logan, A. C., & Selhub, E. M. (2013). Intestinal microbiota, probiotics and mental health: from Metchnikoff to modern advances: Part I – autointoxication revisited. *Gut Pathogens, 5*(1), 5. http://dx.doi.org/10.1186/1757-4749-5-5.

Bevins, C. L., & Salzman, N. H. (2011). The potter's wheel: the host's role in sculpting its microbiota. *Cellular and Molecular Life Sciences, 68*(22), 3675–3685. http://dx.doi.org/10.1007/s00018-011-0830-3.

Bhadra, R., Cobb, D. A., Weiss, L. M., & Khan, I. A. (2013). Psychiatric disorders in toxoplasma seropositive patients–the CD8 connection. *Schizophrenia Bulletin, 39*(3), 485–489. http://dx.doi.org/10.1093/schbul/sbt006.

Bilbo, S. D., & Schwarz, J. M. (2012). The immune system and developmental programming of brain and behavior. *Frontiers in Neuroendocrinology, 33*(3), 267–286. http://dx.doi.org/10.1016/j.yfrne.2012.08.006.

Bonini, J. A., Anderson, S. M., & Steiner, D. F. (1997). Molecular cloning and tissue expression of a novel orphan G protein-coupled receptor from rat lung. *Biochemical and Biophysical Research Communications, 234*(1), 190–193. http://dx.doi.org/10.1006/bbrc.1997.6591.

Borody, T. J., Brandt, L. J., & Paramsothy, S. (2014). Therapeutic faecal microbiota transplantation: current status and future developments. *Current Opinion in Gastroenterology, 30*(1), 97–105. http://dx.doi.org/10.1097/mog.0000000000000027.

Borody, T. J., Paramsothy, S., & Agrawal, G. (2013). Fecal microbiota transplantation: indications, methods, evidence, and future directions. *Current Gastroenterology Reports, 15*(8), 337. http://dx.doi.org/10.1007/s11894-013-0337-1.

Borre, Y. E., Moloney, R. D., Clarke, G., Dinan, T. G., & Cryan, J. F. (2014). The impact of microbiota on brain and behavior: mechanisms & therapeutic potential. *Advances in*

Experimental Medicine and Biology, 817, 373–403. http://dx.doi.org/10.1007/978-1-4939-0897-4_17.

Borre, Y. E., O'Keeffe, G. W., Clarke, G., Stanton, C., Dinan, T. G., & Cryan, J. F. (2014). Microbiota and neurodevelopmental windows: implications for brain disorders. *Trends in Molecular Medicine, 20*(9), 509–518. http://dx.doi.org/10.1016/j.molmed.2014.05.002.

Braniste, V., Al-Asmakh, M., Kowal, C., Anuar, F., Abbaspour, A., Toth, M., et al. (2014). The gut microbiota influences blood–brain barrier permeability in mice. *Science Translational Medicine, 6*(263), 263ra158. http://dx.doi.org/10.1126/scitranslmed.3009759.

Bravo, J. A., Forsythe, P., Chew, M. V., Escaravage, E., Savignac, H. M., Dinan, T. G., et al. (2011). Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proceedings of the National Academy of Sciences of the United States of America, 108*(38), 16050–16055. http://dx.doi.org/10.1073/pnas.1102999108.

Bravo, J. A., Julio-Pieper, M., Forsythe, P., Kunze, W., Dinan, T. G., Bienenstock, J., et al. (2012). Communication between gastrointestinal bacteria and the nervous system. *Current Opinion in Pharmacology, 12*(6), 667–672. http://dx.doi.org/10.1016/j.coph.2012.09.010.

Bruce-Keller, A. J., Salbaum, J. M., Luo, M., Blanchard, E.t, Taylor, C. M., Welsh, D. A., et al. (2014). Obese-type gut microbiota induce neurobehavioral changes in the absence of obesity. *Biological Psychiatry*. http://dx.doi.org/10.1016/j.biopsych.2014.07.012.

Brunet-Gouet, E., & Decety, J. (2006). Social brain dysfunctions in schizophrenia: a review of neuroimaging studies. *Psychiatry Research, 148*(2–3), 75–92. http://dx.doi.org/10.1016/j.pscychresns.2006.05.001.

Bull-Otterson, L., Feng, W., Kirpich, I., Wang, Y., Qin, X., Liu, Y., et al. (2013). Metagenomic analyses of alcohol induced pathogenic alterations in the intestinal microbiome and the effect of *Lactobacillus rhamnosus* GG treatment. *PLoS One, 8*(1), e53028. http://dx.doi.org/10.1371/journal.pone.0053028.

Burns, A., & Iliffe, S. (2009). Alzheimer's disease. *BMJ, 338*, b158. http://dx.doi.org/10.1136/bmj.b158.

Burokas, A., Martin-Garcia, E., Gutierrez-Cuesta, J., Rojas, S., Herance, J. R., Gispert, J. D., et al. (2014). Relationships between serotonergic and cannabinoid system in depressive-like behavior: a PET study with [11C]-DASB. *Journal of Neurochemistry, 130*(1), 126–135. http://dx.doi.org/10.1111/jnc.12716.

Cameron, J., & Doucet, E. (2007). Getting to the bottom of feeding behaviour: who's on top? *Applied Physiology, Nutrition, and Metabolism, 32*(2), 177–189. http://dx.doi.org/10.1139/h06-072.

Cani, P. D., & Delzenne, N. M. (2009). The role of the gut microbiota in energy metabolism and metabolic disease. *Current Pharmaceutical Design, 15*(13), 1546–1558.

Caronna, E. B., Milunsky, J. M., & Tager-Flusberg, H. (2008). Autism spectrum disorders: clinical and research frontiers. *Archives of Disease in Childhood, 93*(6), 518–523. http://dx.doi.org/10.1136/adc.2006.115337.

Casellas, F., Borruel, N., Torrejon, A., Varela, E., Antolin, M., Guarner, F., et al. (2007). Oral oligofructose-enriched inulin supplementation in acute ulcerative colitis is well tolerated and associated with lowered faecal calprotectin. *Alimentary Pharmacology & Therapeutics, 25*(9), 1061–1067. http://dx.doi.org/10.1111/j.1365-2036.2007.03288.x.

Caspi, A., Sugden, K., Moffitt, T. E., Taylor, A., Craig, I. W., Harrington, H., et al. (2003). Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science, 301*(5631), 386–389. http://dx.doi.org/10.1126/science.1083968.

Cebra, J. J. (1999). Influences of microbiota on intestinal immune system development. *American Journal of Clinical Nutrition, 69*(5), 1046S–1051S.

Cersosimo, M. G., & Benarroch, E. E. (2012). Pathological correlates of gastrointestinal dysfunction in Parkinson's disease. *Neurobiology of Disease, 46*(3), 559–564. http://dx.doi.org/10.1016/j.nbd.2011.10.014.

Chang, P. V., Hao, L., Offermanns, S., & Medzhitov, R. (2014). The microbial metabolite butyrate regulates intestinal macrophage function via histone deacetylase inhibition. *Proceedings of the National Academy of Sciences of the United States of America, 111*(6), 2247–2252. http://dx.doi.org/10.1073/pnas.1322269111.

Chen, Z., Guo, L., Zhang, Y., Walzem, R. L., Pendergast, J. S., Printz, R. L., et al. (2014). Incorporation of therapeutically modified bacteria into gut microbiota inhibits obesity. *Journal of Clinical Investigation, 124*(8), 3391–3406. http://dx.doi.org/10.1172/JCI72517.

Chua, A., Keating, J., Hamilton, D., Keeling, P. W., & Dinan, T. G. (1992). Central serotonin receptors and delayed gastric emptying in non-ulcer dyspepsia. *BMJ, 305*(6848), 280–282.

Clarke, G., Cryan, J. F., Dinan, T. G., & Quigley, E. M. (2012). Review article: probiotics for the treatment of irritable bowel syndrome–focus on lactic acid bacteria. *Alimentary Pharmacology & Therapeutics, 35*(4), 403–413. http://dx.doi.org/10.1111/j.1365-2036.2011.04965.x.

Clarke, G., Grenham, S., Scully, P., Fitzgerald, P., Moloney, R. D., Shanahan, F., et al. (2013). The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner. *Molecular Psychiatry, 18*(6), 666–673. http://dx.doi.org/10.1038/mp.2012.77.

Clarke, G., O'Toole, P., Cryan, J. F., & Dinan, T. G. (2014). Characterising the gut microbiome: role in brain–gut function. In G. Coppola (Ed.), *The omics: Applications in neuroscience*. Oxford University Press.

Clarke, G., Stilling, R. M., Kennedy, P. J., Stanton, C., Cryan, J. F., & Dinan, T. G. (2014). Minireview: gut microbiota: the neglected endocrine organ. *Molecular Endocrinology, 28*(8), 1221–1238. http://dx.doi.org/10.1210/me.2014-1108.

Codling, C., O'Mahony, L., Shanahan, F., Quigley, E. M., & Marchesi, J. R. (2010). A molecular analysis of fecal and mucosal bacterial communities in irritable bowel syndrome. *Digestive Diseases and Sciences, 55*(2), 392–397. http://dx.doi.org/10.1007/s10620-009-0934-x.

Collins, S. M. (2014). A role for the gut microbiota in IBS. *Nature Reviews Gastroenterology & Hepatology, 11*(8), 497–505. http://dx.doi.org/10.1038/nrgastro.2014.40.

Collins, S. M., Surette, M., & Bercik, P. (2012). The interplay between the intestinal microbiota and the brain. *Nature Reviews Microbiology, 10*(11), 735–742. http://dx.doi.org/10.1038/nrmicro2876.

Compston, A., & Coles, A. (2008). Multiple sclerosis. *Lancet, 372*(9648), 1502–1517. http://dx.doi.org/10.1016/S0140-6736(08)61620-7.

Costedio, M. M., Hyman, N., & Mawe, G. M. (2007). Serotonin and its role in colonic function and in gastrointestinal disorders. *Diseases of the Colon & Rectum, 50*(3), 376–388. http://dx.doi.org/10.1007/s10350-006-0763-3.

Costello, E. K., Stagaman, K., Dethlefsen, L., Bohannan, B. J., & Relman, D. A. (2012). The application of ecological theory toward an understanding of the human microbiome. *Science, 336*(6086), 1255–1262. http://dx.doi.org/10.1126/science.1224203.

Cotter, P. D., Stanton, C., Ross, R. P., & Hill, C. (2012). The impact of antibiotics on the gut microbiota as revealed by high throughput DNA sequencing. *Discovery Medicine, 13*(70), 193–199.

Coyle, J. T. (2012). NMDA receptor and schizophrenia: a brief history. *Schizophrenia Bulletin, 38*(5), 920–926. http://dx.doi.org/10.1093/schbul/sbs076.

Crumeyrolle-Arias, M., Jaglin, M., Bruneau, A., Vancassel, S., Cardona, A., Dauge, V., et al. (2014). Absence of the gut microbiota enhances anxiety-like behavior and

neuroendocrine response to acute stress in rats. *Psychoneuroendocrinology, 42*, 207–217. http://dx.doi.org/10.1016/j.psyneuen.2014.01.014.

Cryan, J. F., & Dinan, T. G. (2012). Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nature Reviews Neuroscience, 13*(10), 701–712. http://dx.doi.org/10.1038/nrn3346.

Cryan, J. F., & Dinan, T. G. (2013). Unraveling the longstanding scars of early neurodevelopmental stress. *Biological Psychiatry, 74*(11), 788–789. http://dx.doi.org/10.1016/j.biopsych.2013.10.004.

Cryan, J. F., & Dinan, T. G. (2015). More than a gut feeling: the microbiota regulates neurodevelopment and behavior. *Neuropsychopharmacology, 40*(1), 241–242. http://dx.doi.org/10.1038/npp.2014.224.

Cryan, J. F., & Leonard, B. E. (2000). 5-HT1A and beyond: the role of serotonin and its receptors in depression and the antidepressant response. *Human Psychopharmacology, 15*(2), 113–135. http://dx.doi.org/10.1002/(SICI)1099-1077(200003)15:2<113::AID-HUP150>3.0.CO;2-W.

Cryan, J. F., & O'Mahony, S. M. (2011). The microbiome-gut-brain axis: from bowel to behavior. *Neurogastroenterology & Motility, 23*(3), 187–192. http://dx.doi.org/10.1111/j.1365-2982.2010.01664.x.

Curran, E. A., O'Neill, S. M., Cryan, J. F., Kenny, L. C., Dinan, T. G., Khashan, A. S., et al. (2014). Research review: birth by caesarean section and development of autism spectrum disorder and attention-deficit/hyperactivity disorder: a systematic review and meta-analysis. *Journal of Child Psychology and Psychiatry*. http://dx.doi.org/10.1111/jcpp.12351.

Dai, C., Zheng, C. Q., Jiang, M., Ma, X. Y., & Jiang, L. J. (2013). Probiotics and irritable bowel syndrome. *World Journal of Gastroenterology, 19*(36), 5973–5980. http://dx.doi.org/10.3748/wjg.v19.i36.5973.

Davey, K. J., Cotter, P. D., O'Sullivan, O., Crispie, F., Dinan, T. G., Cryan, J. F., et al. (2013). Antipsychotics and the gut microbiome: olanzapine-induced metabolic dysfunction is attenuated by antibiotic administration in the rat. *Translational Psychiatry, 3*, e309. http://dx.doi.org/10.1038/tp.2013.83.

Davey, K. J., O'Mahony, S. M., Schellekens, H., Cotter, P. D., O'Sullivan, O., Dinan, T. G., et al. (2011). Olanzapine induced weight gain and associated metabolic effects: a possible role for gut microbiota. *European Neuropsychopharmacology, 21*, S511.

Davey, K. J., O'Mahony, S. M., Schellekens, H., O'Sullivan, O., Bienenstock, J., Cotter, P. D., et al. (2012). Gender-dependent consequences of chronic olanzapine in the rat: effects on body weight, inflammatory, metabolic and microbiota parameters. *Psychopharmacology (Berlin), 221*(1), 155–169. http://dx.doi.org/10.1007/s00213-011-2555-2.

David, L. A., Maurice, C. F., Carmody, R. N., Gootenberg, D. B., Button, J. E., Wolfe, B. E., et al. (2014). Diet rapidly and reproducibly alters the human gut microbiome. *Nature, 505*(7484). http://dx.doi.org/10.1038/Nature12820, 559–+.

Davies, J., & Davies, D. (2010). Origins and evolution of antibiotic resistance. *Microbiology and Molecular Biology Reviews, 74*(3), 417–433. http://dx.doi.org/10.1128/MMBR.00016-10.

Davis, K. D., Pope, G., Chen, J., Kwan, C. L., Crawley, A. P., & Diamant, N. E. (2008). Cortical thinning in IBS: implications for homeostatic, attention, and pain processing. *Neurology, 70*(2), 153–154. http://dx.doi.org/10.1212/01.wnl.0000295509.30630.10.

Delzenne, N. M., Neyrinck, A. M., Backhed, F., & Cani, P. D. (2011). Targeting gut microbiota in obesity: effects of prebiotics and probiotics. *Nature Reviews Endocrinology, 7*(11), 639–646. http://dx.doi.org/10.1038/nrendo.2011.126.

Dennison, U., McKernan, D., Cryan, J., & Dinan, T. (2012). Schizophrenia patients with a history of childhood trauma have a pro-inflammatory phenotype. *Psychological Medicine, 42*(9), 1865–1871. http://dx.doi.org/10.1017/S0033291712000074.

Desbonnet, L., Clarke, G., Shanahan, F., Dinan, T. G., & Cryan, J. F. (2014). Microbiota is essential for social development in the mouse. *Molecular Psychiatry, 19*(2), 146–148. http://dx.doi.org/10.1038/mp.2013.65.

Desbonnet, L., Garrett, L., Clarke, G., Bienenstock, J., & Dinan, T. G. (2008). The probiotic *Bifidobacteria infantis*: an assessment of potential antidepressant properties in the rat. *Journal of Psychiatric Research, 43*(2), 164–174. http://dx.doi.org/10.1016/j.jpsychires.2008.03.009.

Desbonnet, L., Garrett, L., Clarke, G., Kiely, B., Cryan, J. F., & Dinan, T. G. (2010). Effects of the probiotic *Bifidobacterium infantis* in the maternal separation model of depression. *Neuroscience, 170*(4), 1179–1188. http://dx.doi.org/10.1016/j.neuroscience.2010.08.005.

Deshmukh, H. S., Liu, Y., Menkiti, O. R., Mei, J., Dai, N., O'Leary, C. E., et al. (2014). The microbiota regulates neutrophil homeostasis and host resistance to *Escherichia coli* K1 sepsis in neonatal mice. *Nature Medicine, 20*(5), 524–530. http://dx.doi.org/10.1038/nm.3542.

Di Giancamillo, A., Vitari, F., Savoini, G., Bontempo, V., Bersani, C., Dell'Orto, V., et al. (2008). Effects of orally administered probiotic *Pediococcus acidilactici* on the small and large intestine of weaning piglets. A qualitative and quantitative micro-anatomical study. *Histology and Histopathology, 23*(6), 651–664.

Diaz Heijtz, R., Wang, S., Anuar, F., Qian, Y., Bjorkholm, B., Samuelsson, A., et al. (2011). Normal gut microbiota modulates brain development and behavior. *Proceedings of the National Academy of Sciences of the United States of America, 108*(7), 3047–3052. http://dx.doi.org/10.1073/pnas.1010529108.

Dickson, D. W., Fujishiro, H., Orr, C., DelleDonne, A., Josephs, K. A., Frigerio, R., et al. (2009). Neuropathology of non-motor features of Parkinson disease. *Parkinsonism & Related Disorders, 15*(Suppl. 3), S1–S5. http://dx.doi.org/10.1016/S1353-8020(09)70769-2.

Dinan, T. G., Borre, Y. E., & Cryan, J. F. (2014). Genomics of schizophrenia: time to consider the gut microbiome? *Molecular Psychiatry, 19*(12), 1252–1257. http://dx.doi.org/10.1038/mp.2014.93.

Dinan, T. G., & Cryan, J. F. (2012). Regulation of the stress response by the gut microbiota: implications for psychoneuroendocrinology. *Psychoneuroendocrinology, 37*(9), 1369–1378. http://dx.doi.org/10.1016/j.psyneuen.2012.03.007.

Dinan, T. G., & Cryan, J. F. (2013). Melancholic microbes: a link between gut microbiota and depression? *Neurogastroenterology & Motility, 25*(9), 713–719. http://dx.doi.org/10.1111/nmo.12198.

Dinan, T. G., Stanton, C., & Cryan, J. F. (2013). Psychobiotics: a novel class of psychotropic. *Biological Psychiatry, 74*(10), 720–726. http://dx.doi.org/10.1016/j.biopsych.2013.05.001.

Diop, L., Guillou, S., & Durand, H. (2008). Probiotic food supplement reduces stress-induced gastrointestinal symptoms in volunteers: a double-blind, placebo-controlled, randomized trial. *Nutrition Research, 28*(1), 1–5. http://dx.doi.org/10.1016/j.nutres.2007.10.001.

Douglas-Escobar, M., Elliott, E., & Neu, J. (2013). Effect of intestinal microbial ecology on the developing brain. *JAMA Pediatrics, 167*(4), 374–379. http://dx.doi.org/10.1001/jamapediatrics.2013.497.

Drakoularakou, A., Tzortzis, G., Rastall, R. A., & Gibson, G. R. (2010). A double-blind, placebo-controlled, randomized human study assessing the capacity of a novel galacto-oligosaccharide mixture in reducing travellers' diarrhoea. *European Journal of Clinical Nutrition, 64*(2), 146–152. http://dx.doi.org/10.1038/ejcn.2009.120.

Dupont, H. L. (2014). Review article: evidence for the role of gut microbiota in irritable bowel syndrome and its potential influence on therapeutic targets. *Alimentary Pharmacology & Therapeutics, 39*(10), 1033–1042. http://dx.doi.org/10.1111/apt.12728.

Durban, A., Abellan, J. J., Jimenez-Hernandez, N., Artacho, A., Garrigues, V., Ortiz, V., et al. (2013). Instability of the faecal microbiota in diarrhoea-predominant irritable bowel

syndrome. *FEMS Microbiology Ecology, 86*(3), 581–589. http://dx.doi.org/10.1111/1574-6941.12184.

Eckburg, P. B., Bik, E. M., Bernstein, C. N., Purdom, E., Dethlefsen, L., Sargent, M., et al. (2005). Diversity of the human intestinal microbial flora. *Science, 308*(5728), 1635–1638. http://dx.doi.org/10.1126/science.1110591.

van Eijk, R. V., Wolters, E. C., Tutuarima, J. A., Hische, E. A., Bos, J. D., van Trotsenburg, L., et al. (1987). Effect of early and late syphilis on central nervous system: cerebrospinal fluid changes and neurological deficit. *Genitourinary Medicine, 63*(2), 77–82.

El Karim, I. A., Linden, G. J., Orr, D. F., & Lundy, F. T. (2008). Antimicrobial activity of neuropeptides against a range of micro-organisms from skin, oral, respiratory and gastrointestinal tract sites. *Journal of Neuroimmunology, 200*(1–2), 11–16. http://dx.doi.org/10.1016/j.jneuroim.2008.05.014.

Emanuele, E., Orsi, P., Boso, M., Broglia, D., Brondino, N., Barale, F., et al. (2010). Low-grade endotoxemia in patients with severe autism. *Neuroscience Letters, 471*(3), 162–165. http://dx.doi.org/10.1016/j.neulet.2010.01.033.

Enck, P., Martens, U., & Klosterhalfen, S. (2007). The psyche and the gut. *World Journal of Gastroenterology, 13*(25), 3405–3408.

Evans, J. M., Morris, L. S., & Marchesi, J. R. (2013). The gut microbiome: the role of a virtual organ in the endocrinology of the host. *Journal of Endocrinology, 218*(3), R37–R47. http://dx.doi.org/10.1530/JOE-13-0131.

Farmer, A. D., Randall, H. A., & Aziz, Q. (2014). It's a gut feeling: how the gut microbiota affects the state of mind. *Journal of Physiology, 592*(Pt 14), 2981–2988. http://dx.doi.org/10.1113/jphysiol.2013.270389.

Felger, J. C., & Lotrich, F. E. (2013). Inflammatory cytokines in depression: neurobiological mechanisms and therapeutic implications. *Neuroscience, 246*, 199–229. http://dx.doi.org/10.1016/j.neuroscience.2013.04.060.

Finegold, S. M., Dowd, S. E., Gontcharova, V., Liu, C., Henley, K. E., Wolcott, R. D., et al. (2010). Pyrosequencing study of fecal microflora of autistic and control children. *Anaerobe, 16*(4), 444–453. http://dx.doi.org/10.1016/j.anaerobe.2010.06.008.

Finger, B. C., Dinan, T. G., & Cryan, J. F. (2010). Leptin-deficient mice retain normal appetitive spatial learning yet exhibit marked increases in anxiety-related behaviours. *Psychopharmacology (Berlin), 210*(4), 559–568. http://dx.doi.org/10.1007/s00213-010-1858-z.

Finger, B. C., Dinan, T. G., & Cryan, J. F. (2011). Behavioral satiety sequence in a genetic mouse model of obesity: effects of ghrelin receptor ligands. *Behavioural Pharmacology, 22*(7), 624–632. http://dx.doi.org/10.1097/FBP.0b013e32834afee6.

Fond, G., Boukouaci, W., Chevalier, G., Regnault, A., Eberl, G., Hamdani, N., et al. (2014). The "psychomicrobiotic": targeting microbiota in major psychiatric disorders: a systematic review. *Pathologie Biologie (Paris)*. http://dx.doi.org/10.1016/j.patbio.2014.10.003.

Ford, A. C., Quigley, E. M., Lacy, B. E., Lembo, A. J., Saito, Y. A., Schiller, L. R., et al. (2014). Efficacy of prebiotics, probiotics, and synbiotics in irritable bowel syndrome and chronic idiopathic constipation: systematic review and meta-analysis. *American Journal of Gastroenterology, 109*(10), 1547–1561. http://dx.doi.org/10.1038/ajg.2014.202. quiz 1546, 1562.

Forsythe, P., & Kunze, W. A. (2013). Voices from within: gut microbes and the CNS. *Cellular and Molecular Life Sciences, 70*(1), 55–69. http://dx.doi.org/10.1007/s00018-012-1028-z.

Foster, J. A., & McVey Neufeld, K. A. (2013). Gut-brain axis: how the microbiome influences anxiety and depression. *Trends in Neuroscience, 36*(5), 305–312. http://dx.doi.org/10.1016/j.tins.2013.01.005.

Fouhy, F., Guinane, C. M., Hussey, S., Wall, R., Ryan, C. A., Dempsey, E. M., et al. (2012). High-throughput sequencing reveals the incomplete, short-term recovery of infant gut microbiota following parenteral antibiotic treatment with ampicillin and gentamicin.

Antimicrobial Agents and Chemotherapy, 56(11), 5811–5820. http://dx.doi.org/10.1128/AAC.00789-12.

Fouhy, F., Ross, R. P., Fitzgerald, G. F., Stanton, C., & Cotter, P. D. (2012). Composition of the early intestinal microbiota: knowledge, knowledge gaps and the use of high-throughput sequencing to address these gaps. *Gut Microbes, 3*(3), 203–220. http://dx.doi.org/10.4161/gmic.20169.

Frank, D. N., & Pace, N. R. (2008). Gastrointestinal microbiology enters the metagenomics era. *Current Opinion in Gastroenterology, 24*(1), 4–10. http://dx.doi.org/10.1097/MOG.0b013e3282f2b0e8.

Frank, D. N., Robertson, C. E., Hamm, C. M., Kpadeh, Z., Zhang, T., Chen, H., et al. (2011). Disease phenotype and genotype are associated with shifts in intestinal-associated microbiota in inflammatory bowel diseases. *Inflammatory Bowel Diseases, 17*(1), 179–184. http://dx.doi.org/10.1002/ibd.21339.

Freestone, P. P., Williams, P. H., Haigh, R. D., Maggs, A. F., Neal, C. P., & Lyte, M. (2002). Growth stimulation of intestinal commensal *Escherichia coli* by catecholamines: a possible contributory factor in trauma-induced sepsis. *Shock, 18*(5), 465–470.

Galligan, J. J. (2002). Pharmacology of synaptic transmission in the enteric nervous system. *Current Opinion in Pharmacology, 2*(6), 623–629.

Ganal, S. C., Sanos, S. L., Kallfass, C., Oberle, K., Johner, C., Kirschning, C., et al. (2012). Priming of natural killer cells by nonmucosal mononuclear phagocytes requires instructive signals from commensal microbiota. *Immunity, 37*(1), 171–186. http://dx.doi.org/10.1016/j.immuni.2012.05.020.

Gao, Z., Yin, J., Zhang, J., Ward, R. E., Martin, R. J., Lefevre, M., et al. (2009). Butyrate improves insulin sensitivity and increases energy expenditure in mice. *Diabetes, 58*(7), 1509–1517. http://dx.doi.org/10.2337/db08-1637.

Gareau, M. G., Jury, J., MacQueen, G., Sherman, P. M., & Perdue, M. H. (2007). Probiotic treatment of rat pups normalises corticosterone release and ameliorates colonic dysfunction induced by maternal separation. *Gut, 56*(11), 1522–1528. http://dx.doi.org/10.1136/gut.2006.117176.

Gareau, M. G., Silva, M. A., & Perdue, M. H. (2008). Pathophysiological mechanisms of stress-induced intestinal damage. *Current Molecular Medicine, 8*(4), 274–281.

Gareau, M. G., Wine, E., Rodrigues, D. M., Cho, J. H., Whary, M. T., Philpott, D. J., et al. (2011). Bacterial infection causes stress-induced memory dysfunction in mice. *Gut, 60*(3), 307–317. http://dx.doi.org/10.1136/gut.2009.202515.

Gaykema, R. P., Goehler, L. E., & Lyte, M. (2004). Brain response to cecal infection with *Campylobacter jejuni*: analysis with Fos immunohistochemistry. *Brain, Behavior, and Immunity, 18*(3), 238–245. http://dx.doi.org/10.1016/j.bbi.2003.08.002.

Giordano, R., Pellegrino, M., Picu, A., Bonelli, L., Balbo, M., Berardelli, R., et al. (2006). Neuroregulation of the hypothalamus-pituitary-adrenal (HPA) axis in humans: effects of GABA-, mineralocorticoid-, and GH-secretagogue-receptor modulation. *Scientific World Journal, 6*, 1–11. http://dx.doi.org/10.1100/tsw.2006.09.

Goehler, L. E., Park, S. M., Opitz, N., Lyte, M., & Gaykema, R. P. (2008). Campylobacter jejuni infection increases anxiety-like behavior in the holeboard: possible anatomical substrates for viscerosensory modulation of exploratory behavior. *Brain, Behavior, and Immunity, 22*(3), 354–366. http://dx.doi.org/10.1016/j.bbi.2007.08.009.

Goldman, S. M., Kamel, F., Ross, G. W., Jewell, S. A., Marras, C., Hoppin, J. A., et al. (2014). Peptidoglycan recognition protein genes and risk of Parkinson's disease. *Movement Disorders, 29*(9), 1171–1180. http://dx.doi.org/10.1002/mds.25895.

Gondalia, S. V., Palombo, E. A., Knowles, S. R., Cox, S. B., Meyer, D., & Austin, D. W. (2012). Molecular characterisation of gastrointestinal microbiota of children with autism (with and without gastrointestinal dysfunction) and their neurotypical siblings. *Autism Research, 5*(6), 419–427. http://dx.doi.org/10.1002/aur.1253.

Gonzalez, A., Stombaugh, J., Lozupone, C., Turnbaugh, P. J., Gordon, J. I., & Knight, R. (2011). The mind-body-microbial continuum. *Dialogues in Clinical Neuroscience, 13*(1), 55–62.

Goodrich, J. K., Waters, J. L., Poole, A. C., Sutter, J. L., Koren, O., Blekhman, R., et al. (2014). Human genetics shape the gut microbiome. *Cell, 159*(4), 789–799. http://dx.doi.org/10.1016/j.cell.2014.09.053.

Govindarajan, N., Agis-Balboa, R. C., Walter, J., Sananbenesi, F., & Fischer, A. (2011). Sodium butyrate improves memory function in an Alzheimer's disease mouse model when administered at an advanced stage of disease progression. *Journal of Alzheimer's Disease, 26*(1), 187–197. http://dx.doi.org/10.3233/JAD-2011-110080.

Goyal, R. K., & Hirano, I. (1996). The enteric nervous system. *New England Journal of Medicine, 334*(17), 1106–1115. http://dx.doi.org/10.1056/NEJM199604253341707.

Grabrucker, A. M. (2012). Environmental factors in autism. *Frontiers in Psychiatry, 3*, 118. http://dx.doi.org/10.3389/fpsyt.2012.00118.

Graff, J., & Tsai, L. H. (2013). The potential of HDAC inhibitors as cognitive enhancers. *Annual Review of Pharmacology and Toxicology, 53*, 311–330. http://dx.doi.org/10.1146/annurev-pharmtox-011112-140216.

Grenham, S., Clarke, G., Cryan, J. F., & Dinan, T. G. (2011). Brain-gut-microbe communication in health and disease. *Frontiers in Physiology, 2*, 94. http://dx.doi.org/10.3389/fphys.2011.00094.

Grigoleit, J. S., Kullmann, J. S., Wolf, O. T., Hammes, F., Wegner, A., Jablonowski, S., et al. (2011). Dose-dependent effects of endotoxin on neurobehavioral functions in humans. *PLoS One, 6*(12), e28330. http://dx.doi.org/10.1371/journal.pone.0028330.

Grover, M., & Kashyap, P. C. (2014). Germ-free mice as a model to study effect of gut microbiota on host physiology. *Neurogastroenterology & Motility, 26*(6), 745–748. http://dx.doi.org/10.1111/nmo.12366.

Grundy, D., Al-Chaer, E. D., Aziz, Q., Collins, S. M., Ke, M., Tache, Y., et al. (2006). Fundamentals of neurogastroenterology: basic science. *Gastroenterology, 130*(5), 1391–1411. http://dx.doi.org/10.1053/j.gastro.2005.11.060.

Hamer, H. M., Jonkers, D., Venema, K., Vanhoutvin, S., Troost, F. J., & Brummer, R. J. (2008). Review article: the role of butyrate on colonic function. *Alimentary Pharmacology & Therapeutics, 27*(2), 104–119. http://dx.doi.org/10.1111/j.1365-2036.2007.03562.x.

Hansen, E. E., Lozupone, C. A., Rey, F. E., Wu, M., Guruge, J. L., Narra, A., et al. (2011). Pan-genome of the dominant human gut-associated archaeon, *Methanobrevibacter smithii*, studied in twins. *Proceedings of the National Academy of Sciences of the United States of America, 108*, 4599–4606. http://dx.doi.org/10.1073/pnas.1000071108.

Holzer, P. (2007). Role of visceral afferent neurons in mucosal inflammation and defense. *Current Opinion in Pharmacology, 7*(6), 563–569. http://dx.doi.org/10.1016/j.coph.2007.09.004.

Holzer, P., Reichmann, F., & Farzi, A. (2012). Neuropeptide Y, peptide YY and pancreatic polypeptide in the gut-brain axis. *Neuropeptides, 46*(6), 261–274. http://dx.doi.org/10.1016/j.npep.2012.08.005.

Hope, S., Ueland, T., Steen, N. E., Dieset, I., Lorentzen, S., Berg, A. O., et al. (2013). Interleukin 1 receptor antagonist and soluble tumor necrosis factor receptor 1 are associated with general severity and psychotic symptoms in schizophrenia and bipolar disorder. *Schizophrenia Research, 145*(1–3), 36–42. http://dx.doi.org/10.1016/j.schres.2012.12.023.

Hornig, M. (2013). The role of microbes and autoimmunity in the pathogenesis of neuropsychiatric illness. *Current Opinion in Rheumatology, 25*(4), 488–495. http://dx.doi.org/10.1097/Bor.0b013e32836208de.

Huang, Y., & Mucke, L. (2012). Alzheimer mechanisms and therapeutic strategies. *Cell, 148*(6), 1204–1222. http://dx.doi.org/10.1016/j.cell.2012.02.040.

Huttenhower, C., Gevers, D., Knight, R., Abubucker, S., Badger, J. H., Chinwalla, A. T., et al. (2012). Structure, function and diversity of the healthy human microbiome. *Nature, 486*(7402), 207–214. http://dx.doi.org/10.1038/Nature11234.

Hyland, N. P., O'Mahony, S. M., O'Malley, D., O'Mahony, C. M., Dinan, T. G., & Cryan, J. F. (2015). Early-life stress selectively affects gastrointestinal but not behavioral responses in a genetic model of brain-gut axis dysfunction. *Neurogastroenterology & Motility, 27*(1), 105–113. http://dx.doi.org/10.1111/nmo.12486.

Ichinohe, T., Pang, I. K., Kumamoto, Y., Peaper, D. R., Ho, J. H., Murray, T. S., et al. (2011). Microbiota regulates immune defense against respiratory tract influenza A virus infection. *Proceedings of the National Academy of Sciences of the United States of America, 108*(13), 5354–5359. http://dx.doi.org/10.1073/pnas.1019378108.

Jeffery, I. B., O'Toole, P. W., Ohman, L., Claesson, M. J., Deane, J., Quigley, E. M., et al. (2012). An irritable bowel syndrome subtype defined by species-specific alterations in faecal microbiota. *Gut, 61*(7), 997–1006. http://dx.doi.org/10.1136/gutjnl-2011-301501.

Jensen, J. B., Kumra, S., Leitten, W., Oberstar, J., Anjum, A., White, T., et al. (2008). A comparative pilot study of second-generation antipsychotics in children and adolescents with schizophrenia-spectrum disorders. *Journal of Child and Adolescent Psychopharmacology, 18*(4), 317–326. http://dx.doi.org/10.1089/cap.2007.0123.

Jiang, X., Zhang, D., Shi, J., Chen, Y., Zhang, P., & Mei, B. (2009). Increased inflammatory response both in brain and in periphery in presenilin 1 and presenilin 2 conditional double knock-out mice. *Journal of Alzheimer's Disease, 18*(3), 515–523. http://dx.doi.org/10.3233/JAD-2009-1164.

Jiang, P. F., Zhu, T., Gao, J. D., Gao, F., Mao, S. S., Zhao, W. T., et al. (2013). The effect of maternal infection on cognitive development and hippocampus neuronal apoptosis, proliferation and differentiation in the neonatal rats. *Neuroscience, 246*, 422–434. http://dx.doi.org/10.1016/j.neuroscience.2013.04.021.

Johnson, A. C., Greenwood-Van Meerveld, B., & McRorie, J. (2011). Effects of *Bifidobacterium infantis* 35624 on post-inflammatory visceral hypersensitivity in the rat. *Digestive Diseases and Sciences, 56*(11), 3179–3186. http://dx.doi.org/10.1007/s10620-011-1730-y.

Jones, M. P., Wessinger, S., & Crowell, M. D. (2006). Coping strategies and interpersonal support in patients with irritable bowel syndrome and inflammatory bowel disease. *Clinical Gastroenterology and Hepatology, 4*(4), 474–481. http://dx.doi.org/10.1016/j.cgh.2005.12.012.

Karlsson, F. H., Tremaroli, V., Nookaew, I., Bergstrom, G., Behre, C. J., Fagerberg, B., et al. (2013). Gut metagenome in European women with normal, impaired and diabetic glucose control. *Nature, 498*(7452), 99–103. http://dx.doi.org/10.1038/nature12198.

Kelley, K. W., & Dantzer, R. (2011). Alcoholism and inflammation: neuroimmunology of behavioral and mood disorders. *Brain, Behavior, and Immunity, 25*(Suppl. 1), S13–S20. http://dx.doi.org/10.1016/j.bbi.2010.12.013.

Kendler, K. S., Thornton, L. M., & Gardner, C. O. (2000). Stressful life events and previous episodes in the etiology of major depression in women: an evaluation of the "kindling" hypothesis. *American Journal of Psychiatry, 157*(8), 1243–1251.

Kennedy, P. J., Clarke, G., O'Neill, A., Groeger, J. A., Quigley, E. M., Shanahan, F., et al. (2014). Cognitive performance in irritable bowel syndrome: evidence of a stress-related impairment in visuospatial memory. *Psychological Medicine, 44*(7), 1553–1566. http://dx.doi.org/10.1017/S0033291713002171.

Kennedy, P. J., Clarke, G., Quigley, E. M., Groeger, J. A., Dinan, T. G., & Cryan, J. F. (2012). Gut memories: towards a cognitive neurobiology of irritable bowel syndrome. *Neuroscience & Biobehavioral Reviews, 36*(1), 310–340. http://dx.doi.org/10.1016/j.neubiorev.2011.07.001.

Keshavarzian, A., Farhadi, A., Forsyth, C. B., Rangan, J., Jakate, S., Shaikh, M., et al. (2009). Evidence that chronic alcohol exposure promotes intestinal oxidative stress, intestinal hyperpermeability and endotoxemia prior to development of alcoholic steatohepatitis in rats. *Journal of Hepatology, 50*(3), 538–547. http://dx.doi.org/10.1016/j.jhep.2008.10.028.

Khachatryan, Z. A., Ktsoyan, Z. A., Manukyan, G. P., Kelly, D., Ghazaryan, K. A., & Aminov, R. I. (2008). Predominant role of host genetics in controlling the composition of gut microbiota. *PLoS One, 3*(8), e3064. http://dx.doi.org/10.1371/journal.pone.0003064.

Kim, B. S., Jeon, Y. S., & Chun, J. (2013). Current status and future promise of the human microbiome. *Pediatric Gastroenterology, Hepatology & Nutrition, 16*(2), 71–79. http://dx.doi.org/10.5223/pghn.2013.16.2.71.

Kirchgessner, A. L. (2002). Orexins in the brain-gut axis. *Endocrine Reviews, 23*(1), 1–15. http://dx.doi.org/10.1210/edrv.23.1.0454.

Kirk, R. G. (2012). "Life in a germ-free world": isolating life from the laboratory animal to the bubble boy. *Bulletin of the History of Medicine, 86*(2), 237–275. http://dx.doi.org/10.1353/bhm.2012.0028.

Kirpich, I. A., Solovieva, N. V., Leikhter, S. N., Shidakova, N. A., Lebedeva, O. V., Sidorov, P. I., et al. (2008). Probiotics restore bowel flora and improve liver enzymes in human alcohol-induced liver injury: a pilot study. *Alcohol, 42*(8), 675–682. http://dx.doi.org/10.1016/j.alcohol.2008.08.006.

Knights, D., Ward, T. L., McKinlay, C. E., Miller, H., Gonzalez, A., McDonald, D., & Knight, R. (2014). Rethinking "enterotypes". *Cell Host Microbe, 16*(4), 433–437. http://dx.doi.org/10.1016/j.chom.2014.09.013.

Konieczna, P., Akdis, C. A., Quigley, E. M., Shanahan, F., & O'Mahony, L. (2012). Portrait of an immunoregulatory Bifidobacterium. *Gut Microbes, 3*(3), 261–266. http://dx.doi.org/10.4161/gmic.20358.

Konieczna, P., Groeger, D., Ziegler, M., Frei, R., Ferstl, R., Shanahan, F., et al. (2012). *Bifidobacterium infantis* 35624 administration induces Foxp3 T regulatory cells in human peripheral blood: potential role for myeloid and plasmacytoid dendritic cells. *Gut, 61*(3), 354–366. http://dx.doi.org/10.1136/gutjnl-2011-300936.

Koob, G. F., & Le Moal, M. (2005). Plasticity of reward neurocircuitry and the 'dark side' of drug addiction. *Nature Neuroscience, 8*(11), 1442–1444. http://dx.doi.org/10.1038/nn1105-1442.

Kotula, J. W., Kerns, S. J., Shaket, L. A., Siraj, L., Collins, J. J., Way, J. C., et al. (2014). Programmable bacteria detect and record an environmental signal in the mammalian gut. *Proceedings of the National Academy of Sciences of the United States of America, 111*(13), 4838–4843. http://dx.doi.org/10.1073/pnas.1321321111.

Kunze, W. A., Mao, Y. K., Wang, B., Huizinga, J. D., Ma, X., Forsythe, P., et al. (2009). *Lactobacillus reuteri* enhances excitability of colonic AH neurons by inhibiting calcium-dependent potassium channel opening. *Journal of Cellular and Molecular Medicine, 13*(8B), 2261–2270. http://dx.doi.org/10.1111/j.1582-4934.2009.00686.x.

Kushak, R. I., Lauwers, G. Y., Winter, H. S., & Buie, T. M. (2011). Intestinal disaccharidase activity in patients with autism: effect of age, gender, and intestinal inflammation. *Autism, 15*(3), 285–294. http://dx.doi.org/10.1177/1362361310369142.

Lahtinen, S. J., Boyle, R. J., Kivivuori, S., Oppedisano, F., Smith, K. R., Robins-Browne, R., et al. (2009). Prenatal probiotic administration can influence *Bifidobacterium* microbiota development in infants at high risk of allergy. *Journal of Allergy and Clinical Immunology, 123*(2), 499–501. http://dx.doi.org/10.1016/j.jaci.2008.11.034.

Lakhan, S. E., Caro, M., & Hadzimichalis, N. (2013). NMDA receptor activity in neuropsychiatric disorders. *Frontiers in Psychiatry, 4*, 52. http://dx.doi.org/10.3389/fpsyt.2013.00052.

Lakshminarayanan, B., Stanton, C., O'Toole, P. W., & Ross, R. P. (2014). Compositional dynamics of the human intestinal microbiota with aging: implications for health. *Journal of Nutrition Health and Aging, 18*(9), 773–786. http://dx.doi.org/10.1007/s12603-014-0513-5.

Latham, T., Mackay, L., Sproul, D., Karim, M., Culley, J., Harrison, D. J., et al. (2012). Lactate, a product of glycolytic metabolism, inhibits histone deacetylase activity and promotes changes in gene expression. *Nucleic Acids Research, 40*(11), 4794–4803. http://dx.doi.org/10.1093/nar/gks066.

Le Floc'h, N., Otten, W., & Merlot, E. (2011). Tryptophan metabolism, from nutrition to potential therapeutic applications. *Amino Acids, 41*(5), 1195–1205. http://dx.doi.org/10.1007/s00726-010-0752-7.

Leclercq, S., Cani, P. D., Neyrinck, A. M., Starkel, P., Jamar, F., Mikolajczak, M., et al. (2012). Role of intestinal permeability and inflammation in the biological and behavioral control of alcohol-dependent subjects. *Brain, Behavior, and Immunity, 26*(6), 911–918. http://dx.doi.org/10.1016/j.bbi.2012.04.001.

Leclercq, S., De Saeger, C., Delzenne, N., de Timary, P., & Starkel, P. (2014). Role of inflammatory pathways, blood mononuclear cells, and gut-derived bacterial products in alcohol dependence. *Biological Psychiatry, 76*(9), 725–733. http://dx.doi.org/10.1016/j.biopsych.2014.02.003.

Leclercq, S., Matamoros, S., Cani, P. D., Neyrinck, A. M., Jamar, F., Starkel, P., et al. (2014). Intestinal permeability, gut-bacterial dysbiosis, and behavioral markers of alcohol-dependence severity. *Proceedings of the National Academy of Sciences of the United States of America, 111*(42), E4485–E4493. http://dx.doi.org/10.1073/pnas.1415174111.

Lee, Y. K., Menezes, J. S., Umesaki, Y., & Mazmanian, S. K. (2011). Proinflammatory T-cell responses to gut microbiota promote experimental autoimmune encephalomyelitis. *Proceedings of the National Academy of Sciences of the United States of America, 108*(Suppl. 1), 4615–4622. http://dx.doi.org/10.1073/pnas.1000082107.

Lee, S., Sung, J., Lee, J., & Ko, G. (2011). Comparison of the gut microbiotas of healthy adult twins living in South Korea and the United States. *Applied and Environmental Microbiology, 77*(20), 7433–7437. http://dx.doi.org/10.1128/AEM.05490-11.

Lei, Y. M., Nair, L., & Alegre, M. L. (2014). The interplay between the intestinal microbiota and the immune system. *Clinics and Research in Hepatology and Gastroenterology*. http://dx.doi.org/10.1016/j.clinre.2014.10.008.

Lesniewska, V., Rowland, I., Cani, P. D., Neyrinck, A. M., Delzenne, N. M., & Naughton, P. J. (2006). Effect on components of the intestinal microflora and plasma neuropeptide levels of feeding *Lactobacillus delbrueckii*, *Bifidobacterium lactis*, and inulin to adult and elderly rats. *Applied and Environmental Microbiology, 72*(10), 6533–6538. http://dx.doi.org/10.1128/AEM.00915-06.

Levy, S. E., Mandell, D. S., & Schultz, R. T. (2009). Autism. *Lancet, 374*(9701), 1627–1638. http://dx.doi.org/10.1016/S0140-6736(09)61376-3.

Lewis, S., Burmeister, S., & Brazier, J. (2005). Effect of the prebiotic oligofructose on relapse of *Clostridium difficile*-associated diarrhea: a randomized, controlled study. *Clinical Gastroenterology and Hepatology, 3*(5), 442–448.

Lewis, S. J., & Heaton, K. W. (1997). Increasing butyrate concentration in the distal colon by accelerating intestinal transit. *Gut, 41*(2), 245–251.

Ley, R. E., Backhed, F., Turnbaugh, P., Lozupone, C. A., Knight, R. D., & Gordon, J. I. (2005). Obesity alters gut microbial ecology. *Proceedings of the National Academy of Sciences of the United States of America, 102*(31), 11070–11075. http://dx.doi.org/10.1073/pnas.0504978102.

Ley, R. E., Hamady, M., Lozupone, C., Turnbaugh, P. J., Ramey, R. R., Bircher, J. S., et al. (2008). Evolution of mammals and their gut microbes. *Science, 320*(5883), 1647–1651. http://dx.doi.org/10.1126/science.1155725.

Ley, R. E., Turnbaugh, P. J., Klein, S., & Gordon, J. I. (2006). Microbial ecology: human gut microbes associated with obesity. *Nature, 444*(7122), 1022–1023. http://dx.doi.org/10.1038/4441022a.

Li, M., Wang, M., & Donovan, S. M. (2014). Early development of the gut microbiome and immune-mediated childhood disorders. *Seminars in Reproductive Medicine, 32*(1), 74–86. http://dx.doi.org/10.1055/s-0033-1361825.

Longstreth, G. F., Thompson, W. G., Chey, W. D., Houghton, L. A., Mearin, F., & Spiller, R. C. (2006). Functional bowel disorders. *Gastroenterology, 130*(5), 1480–1491. http://dx.doi.org/10.1053/j.gastro.2005.11.061.

Louis, P. (2012). Does the human gut microbiota contribute to the etiology of autism spectrum disorders? *Digestive Diseases and Sciences, 57*(8), 1987–1989. http://dx.doi.org/10.1007/s10620-012-2286-1.

Lu, X. Y., Kim, C. S., Frazer, A., & Zhang, W. (2006). Leptin: a potential novel antidepressant. *Proceedings of the National Academy of Sciences of the United States of America, 103*(5), 1593–1598. http://dx.doi.org/10.1073/pnas.0508901103.

Luo, J., Wang, T., Liang, S., Hu, X., Li, W., & Jin, F. (2014). Ingestion of *Lactobacillus* strain reduces anxiety and improves cognitive function in the hyperammonemia rat. *Science China Life Sciences, 57*(3), 327–335. http://dx.doi.org/10.1007/s11427-014-4615-4.

Lupien, S. J., McEwen, B. S., Gunnar, M. R., & Heim, C. (2009). Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nature Reviews Neuroscience, 10*(6), 434–445. http://dx.doi.org/10.1038/nrn2639.

Lutter, M., Sakata, I., Osborne-Lawrence, S., Rovinsky, S. A., Anderson, J. G., Jung, S., et al. (2008). The orexigenic hormone ghrelin defends against depressive symptoms of chronic stress. *Nature Neuroscience, 11*(7), 752–753. http://dx.doi.org/10.1038/nn.2139.

Lyte, M. (2013). Microbial endocrinology in the microbiome-gut-brain axis: how bacterial production and utilization of neurochemicals influence behavior. *PLoS Pathogens, 9*(11), e1003726. http://dx.doi.org/10.1371/journal.ppat.1003726.

Lyte, M., Li, W., Opitz, N., Gaykema, R. P., & Goehler, L. E. (2006). Induction of anxiety-like behavior in mice during the initial stages of infection with the agent of murine colonic hyperplasia *Citrobacter rodentium*. *Physiology & Behavior, 89*(3), 350–357. http://dx.doi.org/10.1016/j.physbeh.2006.06.019.

Lyte, M., Varcoe, J. J., & Bailey, M. T. (1998). Anxiogenic effect of subclinical bacterial infection in mice in the absence of overt immune activation. *Physiology & Behavior, 65*(1), 63–68.

Macfabe, D. F. (2012). Short-chain fatty acid fermentation products of the gut microbiome: implications in autism spectrum disorders. *Microbial Ecology in Health and Disease, 23*. http://dx.doi.org/10.3402/mehd.v23i0.19260.

MacFabe, D. F., Cain, N. E., Boon, F., Ossenkopp, K. P., & Cain, D. P. (2011). Effects of the enteric bacterial metabolic product propionic acid on object-directed behavior, social behavior, cognition, and neuroinflammation in adolescent rats: relevance to autism spectrum disorder. *Behavioural Brain Research, 217*(1), 47–54. http://dx.doi.org/10.1016/j.bbr.2010.10.005.

Maes, M., Kubera, M., & Leunis, J. C. (2008). The gut-brain barrier in major depression: intestinal mucosal dysfunction with an increased translocation of LPS from gram negative enterobacteria (leaky gut) plays a role in the inflammatory pathophysiology of depression. *Neuro Endocrinology Letters, 29*(1), 117–124.

de Magistris, L., Familiari, V., Pascotto, A., Sapone, A., Frolli, A., Iardino, P., et al. (2010). Alterations of the intestinal barrier in patients with autism spectrum disorders and in their first-degree relatives. *Journal of Pediatric Gastroenterology and Nutrition, 51*(4), 418–424. http://dx.doi.org/10.1097/MPG.0b013e3181dcc4a5.

Major, G., & Spiller, R. (2014). Irritable bowel syndrome, inflammatory bowel disease and the microbiome. *Current Opinion in Endocrinology, Diabetes and Obesity, 21*(1), 15–21. http://dx.doi.org/10.1097/med.0000000000000032.

Maslowski, K. M., Vieira, A. T., Ng, A., Kranich, J., Sierro, F., Yu, D., et al. (2009). Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. *Nature, 461*(7268), 1282–1286. http://dx.doi.org/10.1038/nature08530.

Matthews, D. M., & Jenks, S. M. (2013). Ingestion of *Mycobacterium vaccae* decreases anxiety-related behavior and improves learning in mice. *Behavioural Processes, 96*, 27–35. http://dx.doi.org/10.1016/j.beproc.2013.02.007.

Maurer, M. H., Canis, M., Kuschinsky, W., & Duelli, R. (2004). Correlation between local monocarboxylate transporter 1 (MCT1) and glucose transporter 1 (GLUT1) densities in the adult rat brain. *Neuroscience Letters, 355*(1–2), 105–108.

Mayer, E. A., Knight, R., Mazmanian, S. K., Cryan, J. F., & Tillisch, K. (2014). Gut microbes and the brain: paradigm shift in neuroscience. *Journal of Neuroscience, 34*(46), 15490–15496. http://dx.doi.org/10.1523/JNEUROSCI.3299-14.2014.

Mayer, E. A., Padua, D., & Tillisch, K. (2014). Altered brain-gut axis in autism: comorbidity or causative mechanisms? *BioEssays, 36*(10), 933–939. http://dx.doi.org/10.1002/bies.201400075.

Mayer, E. A., Savidge, T., & Shulman, R. J. (2014). Brain-gut microbiome interactions and functional bowel disorders. *Gastroenterology, 146*(6), 1500–1512. http://dx.doi.org/10.1053/j.gastro.2014.02.037.

McKernan, D. P., Fitzgerald, P., Dinan, T. G., & Cryan, J. F. (2010). The probiotic *Bifidobacterium infantis* 35624 displays visceral antinociceptive effects in the rat. *Neurogastroenterology & Motility, 22*(9), 1029–1035. http://dx.doi.org/10.1111/j.1365-2982.2010.01520.x. e1268.

McKnite, A. M., Perez-Munoz, M. E., Lu, L., Williams, E. G., Brewer, S., Andreux, P. A., et al. (2012). Murine gut microbiota is defined by host genetics and modulates variation of metabolic traits. *PLoS One, 7*(6), e39191. http://dx.doi.org/10.1371/journal.pone.0039191.

McLean, P. G., Borman, R. A., & Lee, K. (2007). 5-HT in the enteric nervous system: gut function and neuropharmacology. *Trends in Neurosciences, 30*(1), 9–13. http://dx.doi.org/10.1016/j.tins.2006.11.002.

McVey Neufeld, K. A., Mao, Y. K., Bienenstock, J., Foster, J. A., & Kunze, W. A. (2013). The microbiome is essential for normal gut intrinsic primary afferent neuron excitability in the mouse. *Neurogastroenterology & Motility, 25*(2), 183–e188. http://dx.doi.org/10.1111/nmo.12049.

Mertz, H. (2002). Role of the brain and sensory pathways in gastrointestinal sensory disorders in humans. *Gut, 51*(Suppl. 1), i29–33.

Messaoudi, M., Lalonde, R., Violle, N., Javelot, H., Desor, D., Nejdi, A., et al. (2011). Assessment of psychotropic-like properties of a probiotic formulation (*Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175) in rats and human subjects. *British Journal of Nutrition, 105*(5), 755–764. http://dx.doi.org/10.1017/S0007114510004319.

Messaoudi, M., Violle, N., Bisson, J. F., Desor, D., Javelot, H., & Rougeot, C. (2011). Beneficial psychological effects of a probiotic formulation (*Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175) in healthy human volunteers. *Gut Microbes, 2*(4), 256–261. http://dx.doi.org/10.4161/gmic.2.4.16108.

Meyer, U., Feldon, J., & Fatemi, S. H. (2009). In-vivo rodent models for the experimental investigation of prenatal immune activation effects in neurodevelopmental brain disorders. *Neuroscience & Biobehavioral Reviews, 33*(7), 1061–1079. http://dx.doi.org/10.1016/j.neubiorev.2009.05.001.

Moayyedi, P., Ford, A. C., Talley, N. J., Cremonini, F., Foxx-Orenstein, A. E., Brandt, L. J., et al. (2010). The efficacy of probiotics in the treatment of irritable bowel syndrome: a systematic review. *Gut, 59*(3), 325–332. http://dx.doi.org/10.1136/gut.2008.167270.

Molloy, M. J., Grainger, J. R., Bouladoux, N., Hand, T. W., Koo, L. Y., Naik, S., et al. (2013). Intraluminal containment of commensal outgrowth in the gut during

infection-induced dysbiosis. *Cell Host Microbe, 14*(3), 318–328. http://dx.doi.org/10.1016/j.chom.2013.08.003.

Moloney, R. D., Desbonnet, L., Clarke, G., Dinan, T. G., & Cryan, J. F. (2014). The microbiome: stress, health and disease. *Mammalian Genome, 25*(1–2), 49–74. http://dx.doi.org/10.1007/s00335-013-9488-5.

Moloney, R. D., O'Leary, O. F., Felice, D., Bettler, B., Dinan, T. G., & Cryan, J. F. (2012). Early-life stress induces visceral hypersensitivity in mice. *Neuroscience Letters, 512*(2), 99–102. http://dx.doi.org/10.1016/j.neulet.2012.01.066.

de la Monte, S. M., & Wands, J. R. (2008). Alzheimer's disease is type 3 diabetes-evidence reviewed. *Journal of Diabetes Science and Technology, 2*(6), 1101–1113.

Morgan, A. P., Crowley, J. J., Nonneman, R. J., Quackenbush, C. R., Miller, C. N., Ryan, A. K., et al. (2014). The antipsychotic olanzapine interacts with the gut microbiome to cause weight gain in mouse. *PLoS One, 9*(12), e115225. http://dx.doi.org/10.1371/journal.pone.0115225.

Mueller, N. T., Bakacs, E., Combellick, J., Grigoryan, Z., & Dominguez-Bello, M. G. (2014). The infant microbiome development: mom matters. *Trends in Molecular Medicine*. http://dx.doi.org/10.1016/j.molmed.2014.12.002.

Muller, B., Assmus, J., Herlofson, K., Larsen, J. P., & Tysnes, O. B. (2013). Importance of motor vs. non-motor symptoms for health-related quality of life in early Parkinson's disease. *Parkinsonism & Related Disorders, 19*(11), 1027–1032. http://dx.doi.org/10.1016/j.parkreldis.2013.07.010.

Mulle, J. G., Sharp, W. G., & Cubells, J. F. (2013). The gut microbiome: a new frontier in autism research. *Current Psychiatry Reports, 15*(2), 337. http://dx.doi.org/10.1007/s11920-012-0337-0.

Musso, G., Gambino, R., & Cassader, M. (2010). Gut microbiota as a regulator of energy homeostasis and ectopic fat deposition: mechanisms and implications for metabolic disorders. *Current Opinion in Lipidology, 21*(1), 76–83. http://dx.doi.org/10.1097/MOL.0b013e3283347ebb.

Mutlu, E., Keshavarzian, A., Engen, P., Forsyth, C. B., Sikaroodi, M., & Gillevet, P. (2009). Intestinal dysbiosis: a possible mechanism of alcohol-induced endotoxemia and alcoholic steatohepatitis in rats. *Alcoholism Clinical and Experimental Research, 33*(10), 1836–1846. http://dx.doi.org/10.1111/j.1530-0277.2009.01022.x.

Myint, A. M., Bondy, B., Baghai, T. C., Eser, D., Nothdurfter, C., Schule, C., et al. (2013). Tryptophan metabolism and immunogenetics in major depression: a role for interferon-gamma gene. *Brain, Behavior, and Immunity, 31*, 128–133. http://dx.doi.org/10.1016/j.bbi.2013.04.003.

Naseer, M. I., Bibi, F., Alqahtani, M. H., Chaudhary, A. G., Azhar, E. I., Kamal, M. A., et al. (2014). Role of gut microbiota in obesity, type 2 diabetes and Alzheimer's disease. *CNS & Neurological Disorders Drug Targets, 13*(2), 305–311.

Naseribafrouei, A., Hestad, K., Avershina, E., Sekelja, M., Linlokken, A., Wilson, R., et al. (2014). Correlation between the human fecal microbiota and depression. *Neurogastroenterology & Motility, 26*(8), 1155–1162. http://dx.doi.org/10.1111/nmo.12378.

Naslund, J., Studer, E., Nilsson, K., Westberg, L., & Eriksson, E. (2013). Serotonin depletion counteracts sex differences in anxiety-related behaviour in rat. *Psychopharmacology (Berlin), 230*(1), 29–35. http://dx.doi.org/10.1007/s00213-013-3133-6.

Nemani, K., Hosseini Ghomi, R., McCormick, B., & Fan, X. (2014). Schizophrenia and the gut-brain axis. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 56C*, 155–160. http://dx.doi.org/10.1016/j.pnpbp.2014.08.018.

Nemani, K., Hosseini Ghomi, R., McCormick, B., & Fan, X. (2015). Schizophrenia and the gut-brain axis. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 56C*, 155–160. http://dx.doi.org/10.1016/j.pnpbp.2014.08.018.

Neufeld, K. M., Kang, N., Bienenstock, J., & Foster, J. A. (2011). Reduced anxiety-like behavior and central neurochemical change in germ-free mice. *Neurogastroenterology & Motility, 23*(3), 255–264. http://dx.doi.org/10.1111/j.1365-2982.2010.01620.x. e119.

Nguyen, T. L., Vieira-Silva, S., Liston, A., & Raes, J. (2015). How informative is the mouse for human gut microbiota research? *Disease Models & Mechanisms, 8*(1), 1–16. http://dx.doi.org/10.1242/dmm.017400.

Nieto, R., Kukuljan, M., & Silva, H. (2013). BDNF and schizophrenia: from neurodevelopment to neuronal plasticity, learning, and memory. *Frontiers in Psychiatry, 4*, 45. http://dx.doi.org/10.3389/fpsyt.2013.00045.

Nieuwdorp, M., Gilijamse, P. W., Pai, N., & Kaplan, L. M. (2014). Role of the microbiome in energy regulation and metabolism. *Gastroenterology, 146*(6), 1525–1533. http://dx.doi.org/10.1053/j.gastro.2014.02.008.

Noble, J. M., Scarmeas, N., Celenti, R. S., Elkind, M. S., Wright, C. B., Schupf, N., et al. (2014). Serum IgG antibody levels to periodontal microbiota are associated with incident Alzheimer disease. *PLoS One, 9*(12), e114959. http://dx.doi.org/10.1371/journal.pone.0114959.

van Nood, E., Vrieze, A., Nieuwdorp, M., Fuentes, S., Zoetendal, E. G., de Vos, W. M., et al. (2013). Duodenal infusion of donor feces for recurrent *Clostridium difficile*. *New England Journal of Medicine, 368*(5), 407–415. http://dx.doi.org/10.1056/NEJMoa1205037.

Nutt, D. J., & Malizia, A. L. (2004). Structural and functional brain changes in posttraumatic stress disorder. *Journal of Clinical Psychiatry, 65*, 11–17.

O'Connor, R. M., & Cryan, J. F. (2014). Adolescent brain vulnerability and psychopathology through the generations: role of diet and dopamine. *Biological Psychiatry, 75*(1), 4–6. http://dx.doi.org/10.1016/j.biopsych.2013.10.022.

O'Leary, O. F., & Cryan, J. F. (2014). A ventral view on antidepressant action: roles for adult hippocampal neurogenesis along the dorsoventral axis. *Trends in Pharmacological Sciences, 35*(12), 675–687. http://dx.doi.org/10.1016/j.tips.2014.09.011.

O'Mahony, S. M., Clarke, G., Borre, Y. E., Dinan, T. G., & Cryan, J. F. (2015). Serotonin, tryptophan metabolism and the brain-gut-microbiome axis. *Behavioural Brain Research, 277C*, 32–48. http://dx.doi.org/10.1016/j.bbr.2014.07.027.

O'Mahony, S. M., Felice, V. D., Nally, K., Savignac, H. M., Claesson, M. J., Scully, P., et al. (2014). Disturbance of the gut microbiota in early-life selectively affects visceral pain in adulthood without impacting cognitive or anxiety-related behaviors in male rats. *Neuroscience, 277*, 885–901. http://dx.doi.org/10.1016/j.neuroscience.2014.07.054.

O'Mahony, S. M., Hyland, N. P., Dinan, T. G., & Cryan, J. F. (2011). Maternal separation as a model of brain-gut axis dysfunction. *Psychopharmacology (Berlin), 214*(1), 71–88. http://dx.doi.org/10.1007/s00213-010-2010-9.

O'Mahony, S. M., Marchesi, J. R., Scully, P., Codling, C., Ceolho, A. M., Quigley, E. M., et al. (2009). Early life stress alters behavior, immunity, and microbiota in rats: implications for irritable bowel syndrome and psychiatric illnesses. *Biological Psychiatry, 65*(3), 263–267. http://dx.doi.org/10.1016/j.biopsych.2008.06.026.

O'Malley, D., Quigley, E. M., Dinan, T. G., & Cryan, J. F. (2011). Do interactions between stress and immune responses lead to symptom exacerbations in irritable bowel syndrome? *Brain, Behavior, and Immunity, 25*(7), 1333–1341. http://dx.doi.org/10.1016/j.bbi.2011.04.009.

Ogbonnaya, E. S., Clarke, G., Shanahan, F., Dinan, T. G., Cryan, J. F., & O'Leary, O. F. (2015). Adult hippocampal neurogenesis is regulated by the microbiome. *Biological Psychiatry, 78*.

Ohland, C. L., Kish, L., Bell, H., Thiesen, A., Hotte, N., Pankiv, E., et al. (2013). Effects of *Lactobacillus helveticus* on murine behavior are dependent on diet and genotype and correlate with alterations in the gut microbiome. *Psychoneuroendocrinology, 38*(9), 1738–1747. http://dx.doi.org/10.1016/j.psyneuen.2013.02.008.

Ohman, L., & Simren, M. (2013). Intestinal microbiota and its role in irritable bowel syndrome (IBS). *Current Gastroenterology Reports, 15*(5), 323. http://dx.doi.org/10.1007/s11894-013-0323-7.

Parent, A. (1996). *Carpenter's human neuroanatomy*. Baltimore: Williams & Wilkins.

Parkes, G. C., Sanderson, J. D., & Whelan, K. (2010). Treating irritable bowel syndrome with probiotics: the evidence. *Proceedings of the Nutrition Society, 69*(2), 187–194. http://dx.doi.org/10.1017/S002966511000011X.

Patterson, E., Cryan, J. F., Fitzgerald, G. F., Ross, R. P., Dinan, T. G., & Stanton, C. (2014). Gut microbiota, the pharmabiotics they produce and host health. *Proceedings of the Nutrition Society, 73*(4), 477–489. http://dx.doi.org/10.1017/S0029665114001426.

Patterson, E., Marques, T. M., O'Sullivan, O., Fitzgerald, P., Fitzgerald, G. F., Cotter, P. D., et al. (2015). Streptozotocin-induced type-1-diabetes disease onset in Sprague-Dawley rats is associated with an altered intestinal microbiota composition and decreased diversity. *Microbiology, 161*(Pt 1), 182–193. http://dx.doi.org/10.1099/mic.0.082610-0.

Peleg, S., Sananbenesi, F., Zovoilis, A., Burkhardt, S., Bahari-Javan, S., Agis-Balboa, R. C., et al. (2010). Altered histone acetylation is associated with age-dependent memory impairment in mice. *Science, 328*(5979), 753–756. http://dx.doi.org/10.1126/science.1186088.

Pellerin, L. (2005). How astrocytes feed hungry neurons. *Molecular Neurobiology, 32*(1), 59–72. http://dx.doi.org/10.1385/MN:32:1:059.

Perez-Burgos, A., Mao, Y. K., Bienenstock, J., & Kunze, W. A. (2014). The gut-brain axis rewired: adding a functional vagal nicotinic "sensory synapse". *FASEB Journal, 28*(7), 3064–3074. http://dx.doi.org/10.1096/fj.13-245282.

Perez-Burgos, A., Wang, B., Mao, Y. K., Mistry, B., McVey Neufeld, K. A., Bienenstock, J., et al. (2013). Psychoactive bacteria *Lactobacillus rhamnosus* (JB-1) elicits rapid frequency facilitation in vagal afferents. *American Journal of Physiology-Gastrointestinal and Liver Physiology, 304*(2), G211–G220. http://dx.doi.org/10.1152/ajpgi.00128.2012.

Perry, V. H., & Holmes, C. (2014). Microglial priming in neurodegenerative disease. *Nature Reviews Neurology, 10*(4), 217–224. http://dx.doi.org/10.1038/nrneurol.2014.38.

Petrof, E. O., Gloor, G. B., Vanner, S. J., Weese, S. J., Carter, D., Daigneault, M. C., et al. (2013). Stool substitute transplant therapy for the eradication of *Clostridium difficile* infection: 'RePOOPulating' the gut. *Microbiome, 1*(1), 3. http://dx.doi.org/10.1186/2049-2618-1-3.

Picchioni, M. M., & Murray, R. M. (2007). Schizophrenia. *BMJ, 335*(7610), 91–95. http://dx.doi.org/10.1136/bmj.39227.616447.BE.

Pierre, K., & Pellerin, L. (2005). Monocarboxylate transporters in the central nervous system: distribution, regulation and function. *Journal of Neurochemistry, 94*(1), 1–14. http://dx.doi.org/10.1111/j.1471-4159.2005.03168.x.

Pinn, D. M., Aroniadis, O. C., & Brandt, L. J. (2014). Is fecal microbiota transplantation the answer for irritable bowel syndrome? A single-center experience. *American Journal of Gastroenterology, 109*(11), 1831–1832. http://dx.doi.org/10.1038/ajg.2014.295.

Pomare, E. W., Branch, W. J., & Cummings, J. H. (1985). Carbohydrate fermentation in the human colon and its relation to acetate concentrations in venous blood. *Journal of Clinical Investigation, 75*(5), 1448–1454. http://dx.doi.org/10.1172/JCI111847.

Power, S. E., O'Toole, P. W., Stanton, C., Ross, R. P., & Fitzgerald, G. F. (2014). Intestinal microbiota, diet and health. *British Journal of Nutrition, 111*(3), 387–402. http://dx.doi.org/10.1017/S0007114513002560.

Prenderville, J. A., Kennedy, P. J., Dinan, T. G., & Cryan, J. F. (2015). Adding fuel to the fire: the impact of stress on the ageing brain. *Trends in Neurosciences, 38*(1), 13–25. http://dx.doi.org/10.1016/j.tins.2014.11.001.

de Preter, V., Vanhoutte, T., Huys, G., Swings, J., Rutgeerts, P., & Verbeke, K. (2008). Baseline microbiota activity and initial bifidobacteria counts influence responses to

prebiotic dosing in healthy subjects. *Alimentary Pharmacology & Therapeutics, 27*(6), 504–513. http://dx.doi.org/10.1111/j.1365-2036.2007.03588.x.

Pyndt Jorgensen, B., Krych, L., Pedersen, T. B., Plath, N., Redrobe, J. P., Hansen, A. K., et al. (2014). Investigating the long-term effect of subchronic phencyclidine-treatment on novel object recognition and the association between the gut microbiota and behavior in this animal model of schizophrenia. *Physiology & Behavior.* http://dx.doi.org/10.1016/j.physbeh.2014.12.042.

Qiao, M., Thornton, A. M., & Shevach, E. M. (2007). $CD4^+$ $CD25^+$ [corrected] regulatory T cells render naive $CD4^+$ $CD25^-$ T cells anergic and suppressive. *Immunology, 120*(4), 447–455. http://dx.doi.org/10.1111/j.1365-2567.2007.02544.x.

Qin, J., Li, Y., Cai, Z., Li, S., Zhu, J., Zhang, F., et al. (2012). A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature, 490*(7418), 55–60. http://dx.doi.org/10.1038/nature11450.

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K. S., Manichanh, C., et al. (2010). A human gut microbial gene catalogue established by metagenomic sequencing. *Nature, 464*(7285), 59–65. http://dx.doi.org/10.1038/nature08821.

Querfurth, H. W., & LaFerla, F. M. (2010). Alzheimer's disease. *New England Journal of Medicine, 362*(4), 329–344. http://dx.doi.org/10.1056/NEJMra0909142.

Rafiki, A., Boulland, J. L., Halestrap, A. P., Ottersen, O. P., & Bergersen, L. (2003). Highly differential expression of the monocarboxylate transporters MCT2 and MCT4 in the developing rat brain. *Neuroscience, 122*(3), 677–688.

Rajilic-Stojanovic, M., Smidt, H., & de Vos, W. M. (2007). Diversity of the human gastrointestinal tract microbiota revisited. *Environmental Microbiology, 9*(9), 2125–2136. http://dx.doi.org/10.1111/j.1462-2920.2007.01369.x.

Rastmanesh, R. (2011). High polyphenol, low probiotic diet for weight loss because of intestinal microbiota interaction. *Chemico-Biological Interactions, 189*(1–2), 1–8. http://dx.doi.org/10.1016/j.cbi.2010.10.002.

Rausch, P., Rehman, A., Kunzel, S., Hasler, R., Ott, S. J., Schreiber, S., et al. (2011). Colonic mucosa-associated microbiota is influenced by an interaction of Crohn disease and FUT2 (Secretor) genotype. *Proceedings of the National Academy of Sciences of the United States of America, 108*(47), 19030–19035. http://dx.doi.org/10.1073/pnas.1106408108.

Rehman, A., Sina, C., Gavrilova, O., Hasler, R., Ott, S., Baines, J. F., et al. (2011). Nod2 is essential for temporal development of intestinal microbial communities. *Gut, 60*(10), 1354–1362. http://dx.doi.org/10.1136/gut.2010.216259.

Rhee, S. H., Pothoulakis, C., & Mayer, E. A. (2009). Principles and clinical implications of the brain-gut-enteric microbiota axis. *Nature Reviews Gastroenterology & Hepatology, 6*(5), 306–314. http://dx.doi.org/10.1038/nrgastro.2009.35.

Rogers, J., Viding, E., Blair, R. J., Frith, U., & Happe, F. (2006). Autism spectrum disorder and psychopathy: shared cognitive underpinnings or double hit? *Psychological Medicine, 36*(12), 1789–1798. http://dx.doi.org/10.1017/S0033291706008853.

Rousseaux, C., Thuru, X., Gelot, A., Barnich, N., Neut, C., Dubuquoy, L., et al. (2007). *Lactobacillus acidophilus* modulates intestinal pain and induces opioid and cannabinoid receptors. *Nature Medicine, 13*(1), 35–37. http://dx.doi.org/10.1038/nm1521.

Royet, J., Gupta, D., & Dziarski, R. (2011). Peptidoglycan recognition proteins: modulators of the microbiome and inflammation. *Nature Reviews Immunology, 11*(12), 837–851. http://dx.doi.org/10.1038/nri3089.

Ruddick, J. P., Evans, A. K., Nutt, D. J., Lightman, S. L., Rook, G. A., & Lowry, C. A. (2006). Tryptophan metabolism in the central nervous system: medical implications. *Expert Reviews in Molecular Medicine, 8*(20), 1–27. http://dx.doi.org/10.1017/S1462399406000068.

Rustay, N. R., Wrenn, C. C., Kinney, J. W., Holmes, A., Bailey, K. R., Sullivan, T. L., et al. (2005). Galanin impairs performance on learning and memory tasks: findings from

galanin transgenic and GAL-R1 knockout mice. *Neuropeptides, 39*(3), 239–243. http://dx.doi.org/10.1016/j.npep.2004.12.026.

Saadi, M., & McCallum, R. W. (2013). Rifaximin in irritable bowel syndrome: rationale, evidence and clinical use. *Therapeutic Advances in Chronic Disease, 4*(2), 71–75. http://dx.doi.org/10.1177/2040622312472008.

Salonen, A., de Vos, W. M., & Palva, A. (2010). Gastrointestinal microbiota in irritable bowel syndrome: present state and perspectives. *Microbiology, 156*(Pt 11), 3205–3215. http://dx.doi.org/10.1099/mic.0.043257-0.

Sam, A. H., Troke, R. C., Tan, T. M., & Bewick, G. A. (2012). The role of the gut/brain axis in modulating food intake. *Neuropharmacology, 63*(1), 46–56. http://dx.doi.org/10.1016/j.neuropharm.2011.10.008.

Sandler, R. H., Finegold, S. M., Bolte, E. R., Buchanan, C. P., Maxwell, A. P., Vaisanen, M. L., et al. (2000). Short-term benefit from oral vancomycin treatment of regressive-onset autism. *Journal of Child Neurology, 15*(7), 429–435.

Saulnier, D. M., Ringel, Y., Heyman, M. B., Foster, J. A., Bercik, P., Shulman, R. J., et al. (2013). The intestinal microbiome, probiotics and prebiotics in neurogastroenterology. *Gut Microbes, 4*(1), 17–27. http://dx.doi.org/10.4161/gmic.22973.

Savignac, H. M., Corona, G., Mills, H., Chen, L., Spencer, J. P., Tzortzis, G., et al. (2013). Prebiotic feeding elevates central brain derived neurotrophic factor, N-methyl-D-aspartate receptor subunits and D-serine. *Neurochemistry International, 63*(8), 756–764. http://dx.doi.org/10.1016/j.neuint.2013.10.006.

Savignac, H. M., Kiely, B., Dinan, T. G., & Cryan, J. F. (2014). Bifidobacteria exert strain-specific effects on stress-related behavior and physiology in BALB/c mice. *Neurogastroenterology & Motility, 26*(11), 1615–1627. http://dx.doi.org/10.1111/nmo.12427.

Schele, E., Grahnemo, L., Anesten, F., Hallen, A., Backhed, F., & Jansson, J. O. (2013). The gut microbiota reduces leptin sensitivity and the expression of the obesity-suppressing neuropeptides proglucagon (Gcg) and brain-derived neurotrophic factor (Bdnf) in the central nervous system. *Endocrinology, 154*(10), 3643–3651. http://dx.doi.org/10.1210/en.2012-2151.

Schellekens, H., Dinan, T. G., & Cryan, J. F. (2013). Taking two to tango: a role for ghrelin receptor heterodimerization in stress and reward. *Frontiers in Neuroscience,* 7, 148. http://dx.doi.org/10.3389/fnins.2013.00148.

Schellekens, H., Finger, B. C., Dinan, T. G., & Cryan, J. F. (2012). Ghrelin signalling and obesity: at the interface of stress, mood and food reward. *Pharmacology & Therapeutics, 135*(3), 316–326. http://dx.doi.org/10.1016/j.pharmthera.2012.06.004.

Scheperjans, F., Aho, V., Pereira, P. A., Koskinen, K., Paulin, L., Pekkonen, E., et al. (2014). Gut microbiota are related to Parkinson's disease and clinical phenotype. *Movement Disorders*. http://dx.doi.org/10.1002/mds.26069.

Schizophrenia Working Group of the Psychiatric Genomics, C. (2014). Biological insights from 108 schizophrenia-associated genetic loci. *Nature, 511*(7510), 421–427. http://dx.doi.org/10.1038/nature13595.

Schmidt, K., Cowen, P. J., Harmer, C. J., Tzortzis, G., Errington, S., & Burnet, P. W. (2014). Prebiotic intake reduces the waking cortisol response and alters emotional bias in healthy volunteers. *Psychopharmacology (Berlin)*. http://dx.doi.org/10.1007/s00213-014-3810-0.

Schroeder, F. A., Lin, C. L., Crusio, W. E., & Akbarian, S. (2007). Antidepressant-like effects of the histone deacetylase inhibitor, sodium butyrate, in the mouse. *Biological Psychiatry, 62*(1), 55–64. http://dx.doi.org/10.1016/j.biopsych.2006.06.036.

Schultz, S. T., Klonoff-Cohen, H. S., Wingard, D. L., Akshoomoff, N. A., Macera, C. A., Ji, M., et al. (2006). Breastfeeding, infant formula supplementation, and Autistic disorder: the results of a parent survey. *International Breastfeeding Journal, 1*, 16. http://dx.doi.org/10.1186/1746-4358-1-16.

Schwiertz, A., Taras, D., Schafer, K., Beijer, S., Bos, N. A., Donus, C., et al. (2010). Microbiota and SCFA in lean and overweight healthy subjects. *Obesity (Silver Spring), 18*(1), 190–195. http://dx.doi.org/10.1038/oby.2009.167.

Serino, M., Luche, E., Gres, S., Baylac, A., Berge, M., Cenac, C., et al. (2012). Metabolic adaptation to a high-fat diet is associated with a change in the gut microbiota. *Gut, 61*(4), 543–553. http://dx.doi.org/10.1136/gutjnl-2011-301012.

Shanahan, F. (2012). The gut microbiota-a clinical perspective on lessons learned. *Nature Reviews Gastroenterology & Hepatology, 9*(10), 609–614. http://dx.doi.org/10.1038/nrgastro.2012.145.

Shanahan, F., & Quigley, E. M. (2014). Manipulation of the microbiota for treatment of IBS and IBD-challenges and controversies. *Gastroenterology, 146*(6), 1554–1563. http://dx.doi.org/10.1053/j.gastro.2014.01.050.

Sherman, M. P., Zaghouani, H., & Niklas, V. (2014). Gut microbiota, the immune system, and diet influence the neonatal gut-brain axis. *Pediatric Research*. http://dx.doi.org/10.1038/pr.2014.161.

Singh, S., Kumar, A., Agarwal, S., Phadke, S. R., & Jaiswal, Y. (2014). Genetic insight of schizophrenia: past and future perspectives. *Gene, 535*(2), 97–100. http://dx.doi.org/10.1016/j.gene.2013.09.110.

Sleeth, M. L., Thompson, E. L., Ford, H. E., Zac-Varghese, S. E., & Frost, G. (2010). Free fatty acid receptor 2 and nutrient sensing: a proposed role for fibre, fermentable carbohydrates and short-chain fatty acids in appetite regulation. *Nutrition Research Reviews, 23*(1), 135–145. http://dx.doi.org/10.1017/S0954422410000089.

Smith, R. S. (1991). Is schizophrenia caused by excessive production of interleukin-2 and interleukin-2 receptors by gastrointestinal lymphocytes? *Medical Hypotheses, 34*(3), 225–229.

Smith, R. S. (1992). A comprehensive macrophage-T-lymphocyte theory of schizophrenia. *Medical Hypotheses, 39*(3), 248–257.

Smith, P. M., Howitt, M. R., Panikov, N., Michaud, M., Gallini, C. A., Bohlooly, Y. M., et al. (2013). The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science, 341*(6145), 569–573. http://dx.doi.org/10.1126/science.1241165.

Smits, L. P., Bouter, K. E., de Vos, W. M., Borody, T. J., & Nieuwdorp, M. (2013). Therapeutic potential of fecal microbiota transplantation. *Gastroenterology, 145*(5), 946–953. http://dx.doi.org/10.1053/j.gastro.2013.08.058.

Sommer, F., & Backhed, F. (2013). The gut microbiota–masters of host development and physiology. *Nature Reviews Microbiology, 11*(4), 227–238. http://dx.doi.org/10.1038/nrmicro2974.

Song, X., Fan, X., Song, X., Zhang, J., Zhang, W., Li, X., et al. (2013). Elevated levels of adiponectin and other cytokines in drug naive, first episode schizophrenia patients with normal weight. *Schizophrenia Research, 150*(1), 269–273. http://dx.doi.org/10.1016/j.schres.2013.07.044.

Song, Y., Liu, C., & Finegold, S. M. (2004). Real-time PCR quantitation of clostridia in feces of autistic children. *Applied and Environmental Microbiology, 70*(11), 6459–6465. http://dx.doi.org/10.1128/AEM.70.11.6459-6465.2004.

Spiller, R., & Garsed, K. (2009). Postinfectious irritable bowel syndrome. *Gastroenterology, 136*(6), 1979–1988. http://dx.doi.org/10.1053/j.gastro.2009.02.074.

Spor, A., Koren, O., & Ley, R. (2011). Unravelling the effects of the environment and host genotype on the gut microbiome. *Nature Reviews Microbiology, 9*(4), 279–290. http://dx.doi.org/10.1038/nrmicro2540.

Steere, A. C., Pachner, A. R., & Malawista, S. E. (1983). Neurologic abnormalities of Lyme disease: successful treatment with high-dose intravenous penicillin. *Annals of Internal Medicine, 99*(6), 767–772.

Stefansson, H., Ophoff, R. A., Steinberg, S., Andreassen, O. A., Cichon, S., Rujescu, D., et al. (2009). Common variants conferring risk of schizophrenia. *Nature, 460*(7256), 744–747. http://dx.doi.org/10.1038/nature08186.

Stilling, R. M., Bordenstein, S. R., Dinan, T. G., & Cryan, J. F. (2014). Friends with social benefits: host-microbe interactions as a driver of brain evolution and development? *Frontiers in Cellular and Infection Microbiology, 4*, 147. http://dx.doi.org/10.3389/fcimb.2014.00147.

Stilling, R. M., Dinan, T. G., & Cryan, J. F. (2014). Microbial genes, brain & behaviour - epigenetic regulation of the gut-brain axis. *Genes, Brain and Behavior, 13*(1), 69–86. http://dx.doi.org/10.1111/gbb.12109.

Sudo, N., Chida, Y., Aiba, Y., Sonoda, J., Oyama, N., Yu, X. N., et al. (2004). Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. *Journal of Physiology, 558*(Pt 1), 263–275. http://dx.doi.org/10.1113/jphysiol.2004.063388.

Sundin, J., Rangel, I., Fuentes, S., Heikamp-de Jong, I., Hultgren-Hornquist, E., de Vos, W. M., et al. (2014). Altered faecal and mucosal microbial composition in post-infectious irritable bowel syndrome patients correlates with mucosal lymphocyte phenotypes and psychological distress. *Alimentary Pharmacology & Therapeutics*. http://dx.doi.org/10.1111/apt.13055.

Tack, J., Broekaert, D., Fischler, B., Van Oudenhove, L., Gevers, A. M., & Janssens, J. (2006). A controlled crossover study of the selective serotonin reuptake inhibitor citalopram in irritable bowel syndrome. *Gut, 55*(8), 1095–1103. http://dx.doi.org/10.1136/gut.2005.077503.

Tana, C., Umesaki, Y., Imaoka, A., Handa, T., Kanazawa, M., & Fukudo, S. (2010). Altered profiles of intestinal microbiota and organic acids may be the origin of symptoms in irritable bowel syndrome. *Neurogastroenterology & Motility, 22*(5), 512–519. http://dx.doi.org/10.1111/j.1365-2982.2009.01427.x. e114–e515.

Taur, Y., & Pamer, E. G. (2013). The intestinal microbiota and susceptibility to infection in immunocompromised patients. *Current Opinion in Infectious Diseases, 26*(4), 332–337. http://dx.doi.org/10.1097/QCO.0b013e3283630dd3.

Taylor, M. W., & Feng, G. S. (1991). Relationship between interferon-gamma, indoleamine 2,3-dioxygenase, and tryptophan catabolism. *FASEB Journal, 5*(11), 2516–2522.

de Theije, C. G., Wu, J., da Silva, S. L., Kamphuis, P. J., Garssen, J., Korte, S. M., et al. (2011). Pathways underlying the gut-to-brain connection in autism spectrum disorders as future targets for disease management. *European Journal of Pharmacology, 668*(Suppl. 1), S70–S80. http://dx.doi.org/10.1016/j.ejphar.2011.07.013.

Theodorou, V., Ait Belgnaoui, A., Agostini, S., & Eutamene, H. (2014). Effect of commensals and probiotics on visceral sensitivity and pain in irritable bowel syndrome. *Gut Microbes, 5*(3), 430–436. http://dx.doi.org/10.4161/gmic.29796.

Thomas, R. H., Meeking, M. M., Mepham, J. R., Tichenoff, L., Possmayer, F., Liu, S., et al. (2012). The enteric bacterial metabolite propionic acid alters brain and plasma phospholipid molecular species: further development of a rodent model of autism spectrum disorders. *Journal of Neuroinflammation, 9*, 153. http://dx.doi.org/10.1186/1742-2094-9-153.

Tilg, H. (2010). Obesity, metabolic syndrome, and microbiota: multiple interactions. *Journal of Clinical Gastroenterology, 44*(Suppl. 1), S16–S18. http://dx.doi.org/10.1097/MCG.0b013e3181dd8b64.

Tillisch, K., Labus, J., Kilpatrick, L., Jiang, Z., Stains, J., Ebrat, B., et al. (2013). Consumption of fermented milk product with probiotic modulates brain activity. *Gastroenterology, 144*(7), 1394–1401. http://dx.doi.org/10.1053/j.gastro.2013.02.043, 1401 e1391–e1394.

de Timary, P., Cordovil de Sousa Uva, M., Denoel, C., Hebborn, L., Derely, M., Desseilles, M., et al. (2013). The associations between self-consciousness, depressive state

and craving to drink among alcohol dependent patients undergoing protracted withdrawal. *PLoS One, 8*(8), e71560. http://dx.doi.org/10.1371/journal.pone.0071560.

Tims, S., Derom, C., Jonkers, D. M., Vlietinck, R., Saris, W. H., Kleerebezem, M., et al. (2013). Microbiota conservation and BMI signatures in adult monozygotic twins. *ISME Journal, 7*(4), 707–717. http://dx.doi.org/10.1038/ismej.2012.146.

Torrente, F., Ashwood, P., Day, R., Machado, N., Furlano, R. I., Anthony, A., et al. (2002). Small intestinal enteropathy with epithelial IgG and complement deposition in children with regressive autism. *Molecular Psychiatry, 7*(4), 375–382. http://dx.doi.org/10.1038/sj.mp.4001077, 334.

Torres-Fuentes, C., Schellekens, H., Dinan, T. G., & Cryan, J. F. (2014). A natural solution for obesity: bioactives for the prevention and treatment of weight gain. A review. *Nutritional Neuroscience*. http://dx.doi.org/10.1179/1476830513Y.0000000099.

Tremaroli, V., & Backhed, F. (2012). Functional interactions between the gut microbiota and host metabolism. *Nature, 489*(7415), 242–249. http://dx.doi.org/10.1038/nature11552.

Turnbaugh, P. J., Ley, R. E., Mahowald, M. A., Magrini, V., Mardis, E. R., & Gordon, J. I. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature, 444*(7122), 1027–1031. http://dx.doi.org/10.1038/nature05414.

Udayappan, S. D., Hartstra, A. V., Dallinga-Thie, G. M., & Nieuwdorp, M. (2014). Intestinal microbiota and faecal transplantation as treatment modality for insulin resistance and type 2 diabetes mellitus. *Clinical & Experimental Immunology, 177*(1), 24–29. http://dx.doi.org/10.1111/cei.12293.

Verdu, E. F., Bercik, P., Verma-Gandhu, M., Huang, X. X., Blennerhassett, P., Jackson, W., et al. (2006). Specific probiotic therapy attenuates antibiotic induced visceral hypersensitivity in mice. *Gut, 55*(2), 182–190. http://dx.doi.org/10.1136/gut.2005.066100.

van Vlies, N., Hogenkamp, A., Thijssen, S., Dingjan, G. M., Knipping, K., Garssen, J., et al. (2012). Effects of short-chain galacto- and long-chain fructo-oligosaccharides on systemic and local immune status during pregnancy. *Journal of Reproductive Immunology, 94*(2), 161–168. http://dx.doi.org/10.1016/j.jri.2012.02.007.

de Vos, W. M., & de Vos, E. A. (2012). Role of the intestinal microbiome in health and disease: from correlation to causation. *Nutrition Reviews, 70*(Suppl. 1), S45–S56. http://dx.doi.org/10.1111/j.1753-4887.2012.00505.x.

Vrieze, A., Van Nood, E., Holleman, F., Salojarvi, J., Kootte, R. S., Bartelsman, J. F., et al. (2012). Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology, 143*(4). http://dx.doi.org/10.1053/j.gastro.2012.06.031, 913–916 e917.

Vulevic, J., Drakoularakou, A., Yaqoob, P., Tzortzis, G., & Gibson, G. R. (2008). Modulation of the fecal microflora profile and immune function by a novel trans-galactooligosaccharide mixture (B-GOS) in healthy elderly volunteers. *American Journal of Clinical Nutrition, 88*(5), 1438–1446.

Wacklin, P., Makivuokko, H., Alakulppi, N., Nikkila, J., Tenkanen, H., Rabina, J., et al. (2011). Secretor genotype (FUT2 gene) is strongly associated with the composition of Bifidobacteria in the human intestine. *PLoS One, 6*(5), e20113. http://dx.doi.org/10.1371/journal.pone.0020113.

Waldecker, M., Kautenburger, T., Daumann, H., Busch, C., & Schrenk, D. (2008). Inhibition of histone-deacetylase activity by short-chain fatty acids and some polyphenol metabolites formed in the colon. *Journal of Nutritional, 19*(9), 587–593. http://dx.doi.org/10.1016/j.jnutbio.2007.08.002.

Wang, L., Christophersen, C. T., Sorich, M. J., Gerber, J. P., Angley, M. T., & Conlon, M. A. (2012). Elevated fecal short chain fatty acid and ammonia concentrations in children with autism spectrum disorder. *Digestive Diseases and Sciences, 57*(8), 2096–2102. http://dx.doi.org/10.1007/s10620-012-2167-7.

Wang, X., Wang, B. R., Zhang, X. J., Xu, Z., Ding, Y. Q., & Ju, G. (2002). Evidences for vagus nerve in maintenance of immune balance and transmission of immune information from gut to brain in STM-infected rats. *World Journal of Gastroenterology, 8*(3), 540–545.

Weilburg, J. B. (2004). An overview of SSRI and SNRI therapies for depression. *Managed Care, 13*(6 Suppl. Depression), 25–33.

Williams, B. L., Hornig, M., Buie, T., Bauman, M. L., Cho Paik, M., Wick, I., et al. (2011). Impaired carbohydrate digestion and transport and mucosal dysbiosis in the intestines of children with autism and gastrointestinal disturbances. *PLoS One, 6*(9), e24585. http://dx.doi.org/10.1371/journal.pone.0024585.

Williams, B. L., Hornig, M., Parekh, T., & Lipkin, W. I. (2012). Application of novel PCR-based methods for detection, quantitation, and phylogenetic characterization of *Sutterella* species in intestinal biopsy samples from children with autism and gastrointestinal disturbances. *mBio, 3*(1). http://dx.doi.org/10.1128/mBio.00261-11.

Wood, J. D., Alpers, D. H., & Andrews, P. L. (1999). Fundamentals of neurogastroenterology. *Gut, 45*(Suppl. 2), II6–II16.

Wopereis, H., Oozeer, R., Knipping, K., Belzer, C., & Knol, J. (2014). The first thousand days - intestinal microbiology of early life: establishing a symbiosis. *Pediatric Allergy and Immunology, 25*(5), 428–438. http://dx.doi.org/10.1111/pai.12232.

Wren, A. M., & Bloom, S. R. (2007). Gut hormones and appetite control. *Gastroenterology, 132*(6), 2116–2130. http://dx.doi.org/10.1053/j.gastro.2007.03.048.

Wrenn, C. C., Turchi, J. N., Schlosser, S., Dreiling, J. L., Stephenson, D. A., & Crawley, J. N. (2006). Performance of galanin transgenic mice in the 5-choice serial reaction time attentional task. *Pharmacology Biochemistry & Behavior, 83*(3), 428–440. http://dx.doi.org/10.1016/j.pbb.2006.03.003.

Wu, G. D., Bewtra, M., Hoffmann, C., Chen, Y. Y., Keilbaugh, S. A., Bittinger, K., et al. (2011). Controlled feeding experiments demonstrate the impact of diet on the composition of the human gut microbiome. *Gastroenterology, 140*(5), S47.

Yan, A. W., Fouts, D. E., Brandl, J., Starkel, P., Torralba, M., Schott, E., et al. (2011). Enteric dysbiosis associated with a mouse model of alcoholic liver disease. *Hepatology, 53*(1), 96–105. http://dx.doi.org/10.1002/hep.24018.

Yap, I. K., Angley, M., Veselkov, K. A., Holmes, E., Lindon, J. C., & Nicholson, J. K. (2010). Urinary metabolic phenotyping differentiates children with autism from their unaffected siblings and age-matched controls. *Journal of Proteome Research, 9*(6), 2996–3004. http://dx.doi.org/10.1021/pr901188e.

Yatsunenko, T., Rey, F. E., Manary, M. J., Trehan, I., Dominguez-Bello, M. G., Contreras, M., et al. (2012). Human gut microbiome viewed across age and geography. *Nature, 486*(7402), 222–227. http://dx.doi.org/10.1038/nature11053.

Yolken, R. H., Jones-Brando, L., Dunigan, D. D., Kannan, G., Dickerson, F., Severance, E., et al. (2014). Chlorovirus ATCV-1 is part of the human oropharyngeal virome and is associated with changes in cognitive functions in humans and mice. *Proceedings of the National Academy of Sciences of the United States of America, 111*(45), 16106–16111. http://dx.doi.org/10.1073/pnas.1418895111.

Yoon, J. S., Sohn, W., Lee, O. Y., Lee, S. P., Lee, K. N., Jun, D. W., et al. (2014). Effect of multispecies probiotics on irritable bowel syndrome: a randomized, double-blind, placebo-controlled trial. *Journal of Gastroenterology and Hepatology, 29*(1), 52–59. http://dx.doi.org/10.1111/jgh.12322.

Youngster, I., Russell, G. H., Pindar, C., Ziv-Baran, T., Sauk, J., & Hohmann, E. L. (2014). Oral, capsulized, frozen fecal microbiota transplantation for relapsing *Clostridium difficile* infection. *JAMA, 312*(17), 1772–1778. http://dx.doi.org/10.1001/jama.2014.13875.

Zhao, C., Sun, R., Cao, B., Gu, S., Zhao, J., Liu, L., et al. (2014). An in vitro metabolic system of gut flora and the metabolism of ginsenoside Rg3 and cholic acid. *European Journal*

of Drug Metabolism and Pharmacokinetics, 39(2), 129–137. http://dx.doi.org/10.1007/s13318-013-0143-z.

Zhu, B., Wang, X., & Li, L. (2010). Human gut microbiome: the second genome of human body. *Protein & Cell, 1*(8), 718–725. http://dx.doi.org/10.1007/s13238-010-0093-z.

Zoetendal, E. G., Akkermans, A. D., & De Vos, W. M. (1998). Temperature gradient gel electrophoresis analysis of 16S rRNA from human fecal samples reveals stable and host-specific communities of active bacteria. *Applied and Environmental Microbiology, 64*(10), 3854–3859.

CHAPTER TWO

Aromatic Metabolism of Filamentous Fungi in Relation to the Presence of Aromatic Compounds in Plant Biomass

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Contents

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Abstract

The biological conversion of plant lignocellulose plays an essential role not only in carbon cycling in terrestrial ecosystems but also is an important part of the production of second generation biofuels and biochemicals. The presence of the recalcitrant aromatic polymer lignin is one of the major obstacles in the biofuel/biochemical production process and therefore microbial degradation of lignin is receiving a great deal of attention. Fungi are the main degraders of plant biomass, and in particular the basidiomycete white rot fungi are of major importance in converting plant aromatics due to their ability to degrade lignin. However, the aromatic monomers that are released from lignin and other aromatic compounds of plant biomass are toxic for most fungi already at low levels, and therefore conversion of these compounds to less toxic metabolites is essential for fungi. Although the release of aromatic compounds from plant biomass by fungi has been studied extensively, relatively little attention has been given to the metabolic pathways that convert the resulting aromatic monomers. In this review we provide an overview of the aromatic components of plant biomass, and their release and conversion by fungi. Finally, we will summarize the applications of fungal systems related to plant aromatics.

1. INTRODUCTION

The biological conversion of plant lignocellulose plays an essential role not only in carbon cycling in terrestrial ecosystems but also is an important part of the production of second generation biofuels (Naik, Goud, Rout, & Dalai, 2010). Lignocellulosic biomass as a renewable resource from forestry and agriculture has been estimated to provide about 25% of global energy requirements (Briens, Piskorz, & Berruti, 2008). In addition, biomass can potentially be converted into different high value products including value-added fine chemicals (Asgher, Ahmad, & Iqbal, 2013; Isroi et al., 2011). Fungi are the main degraders of lignocellulosic biomass. Most species mainly focus on the polysaccharide fractions of the biomass, which releases only small amounts of aromatic compounds, but the basidiomycete white-rot fungi also degrade lignin, releasing substantial amounts of aromatic compounds (Mäkelä, Hildén, & de Vries, 2014).

Lignocellulosic biomass is a complex matrix of three main types of carbon-based polymers—cellulose, hemicelluloses, and lignin—together with proteins and a small amount of extractives (McCann et al., 2001). Forty to fifty percent of plant biomass consists of cellulose, which is the most abundant organic compound on Earth. Cellulose is a polymer of glucose, composed of linear chains of several hundred to over ten thousand β-1,4 linked D-glucose units. These chains are noncovalently organized into packed microfibrils, which contain highly ordered crystalline regions and more amorphous regions. Hemicelluloses are heterogeneous polysaccharides of various sizes, and can be divided into xylans, galactomannans, and xyloglucans. They are named after their main-chain components (xylose, mannose, and glucose, respectively) and are decorated with monomeric or small oligomeric side-chains consisting of a variety of sugars, and uronic, acetyl, and aromatic acids. Hemicelluloses are closely associated with cellulose microfibrils and chemically linked with lignin via ester linkages through ferulic acid or (4-*O*-methyl-)glucuronic acid. Hemicelluloses comprise 20–40% of the plant biomass. Depending on its origin, xylan can contain various aromatic residues linked to arabinose side-chains, such as ferulic acid, *p*-coumaric acid, and caffeic acid. Pectin is a heteropolysaccharide with four defined substructures: homogalacturonan (HG), xylogalacturonan (XG), and rhamnogalacturonan (RG) I and II. RG I has a backbone of alternating galacturonic acid and rhamnose residues, whereas the other three substructures have a galacturonic acid backbone. Side chains of RG I consist of

arabinose (arabinan) or galactose (galactan) or both (arabinogalactan) and these can contain terminal ferulic acid and coumaric acid residues. Lignin is a complex, aromatic heteropolymer, composed of phenylpropanoid units which are linked together via a variety of ether and C—C bonds (more details below). Lignin gives strength and rigidity to a plant and constitutes 15—30% of the dry matter of woody plants (Sjöström, 1993). All cell wall components interact with each other forming the intricate structure that provides strength and rigidity to the plant cell, as well as defense against pathogens. In addition to the aromatic components of plant polysaccharides and the aromatic polymer lignin, other aromatic compounds are present in plant biomass such as flavonoids and monoterpenoids.

2. PLANT BIOMASS DEGRADING FUNGI

Fungi are highly efficient degraders of plant biomass. They degrade plant biomass mainly by enzymatic attack, although nonenzymatic approaches such as Fenton chemistry (Hatakka & Hammel, 2010; Wood, 1994) also participate in the overall decay process. Fungi produce a wide range of enzymes that enables them to attack the various linkages that are present in plant biomass (de Vries & Visser, 2001; Mäkelä et al., 2014; Rytioja et al., 2014). Genomic studies of fungi have revealed large differences in their sets of plant biomass active enzymes that can to some extent be related to their biotope, their preferred substrate or their life style. Most of the studies into the plant biomass degrading strategies of fungi have addressed ascomycete and basidiomycete fungi, whereas relatively little attention has been given to fungi earlier classified the zygomycetes and other early lineages of fungi. Significant differences exist between basidiomycetes and ascomycetes, but they also share many features of this process. A more detailed description of basidiomycete and ascomycete approaches to plant biomass degradation is described below.

2.1 Basidiomycetes

2.1.1 Wood-Decaying Fungi

Wood-decaying basidiomycetes have been traditionally classified into white-rot and brown-rot fungi according to the visually distinguishable type of decay they cause. In addition, white- and brown-rot fungi typically possess different enzyme sets for lignocellulose depolymerization. However, a more nuanced classification of wood-rotting fungi was recently suggested

due to the more diverse mechanisms of wood decay revealed by the basidiomycete genomes (Riley et al., 2014).

White-rot fungi (e.g., *Phanerochaete chrysosporium*, *Phlebia radiata*, *Dichomitus squalens*, *Trametes versicolor*) are the best studied wood-rotting organisms because of their ability to degrade all the major components of plant cell wall including cellulose, hemicelluloses, and lignin, leaving cellulose-enriched white material (Hatakka & Hammel, 2010). The characteristic feature of the white-rot fungi which differentiates them from other wood-decaying fungi is the production of an array of lignin-modifying peroxidases (see Section 5.1.2) together with various H_2O_2-generating enzymes (Mäkelä et al., 2014). Compared to white-rot fungi, brown-rot fungi (e.g., *Gloeophyllum trabeum*, *Postia placenta*, *Piptoporus betulinus*) exhibit a different strategy of wood decay. They can degrade most of the cellulose and hemicellulose, leaving behind demethoxylated lignin (Dey, Maiti, & Bhattacharyya, 1994). In contrast to white-rot fungi, brown-rot fungi initiate cellulose depolymerization nonenzymatically using highly reactive hydroxyl radicals generated by Fenton reaction ($Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + OH^{\cdot} + OH^{-}$) (Kerem & Hammel, 1999; Wood, 1994). In wood-rotting basidiomycete fungi, the metabolism of aromatic compounds has been mainly studied in the connection of lignin modification and degradation (Ander, Hatakka, & Eriksson, 1980; Eriksson, Blanchette, & Ander, 1990).

2.1.2 Litter-Decomposing Basidiomycete Fungi

Basidiomycete litter-decomposing fungi are saprobes that colonize grasslands and grand litter layers of soil and participate in the decomposition of organic material to CO_2 and humus (Dix & Webster, 1995). The plant biomass decomposing enzymatic machinery of litter-decomposing fungi resembles that of white-rot fungi enabling them to decompose plant-derived lignin-rich polymers (Hatakka & Hammel, 2010; Steffen, Hofrichter, & Hatakka, 2000). Although litter-decomposing and wood-rotting species are physiologically closely related, litter-decomposing fungi differ from wood-rotters with respect to their growth substrate, forest litter and soil, that is characterized by a higher C:N ratio and microbiological activity (Baldrian & Šnajdr, 2006).

2.1.3 Plant Pathogens

Plant pathogenic fungi are heterotrophic organisms that secrete a complex array of enzymes for the digestion of plant host tissues during infection. Recently, the genome sequence of *Armillaria mellea* revealed a rich reservoir of nine putative lignin-modifying peroxidases and six *sensu stricto* laccases

(Collins et al., 2013). *Ustilago maydis*, another basidiomycete plant pathogen causing corn smut disease, produces laccase (Desentis-Mendoza et al., 2006). Basidiomycete yeast *Cryptococcus neoformans*, a plant and animal pathogen, also produced a laccase (Williamson, 1994) capable of oxidizing phenols and aminophenols, but not tyrosine (De Jesus, Nicola, Rodrigues, Janbon, & Casadevall, 2009), which also contributes to the resistance to fungicides (Ikeda, Sugita, Jacobson, & Shinoda, 2003). The most severe tree pathogen in boreal forests, *Heterobasidion irregulare* (syn. *Heterobasidion annosum*) has full capacity to grow saprotrophically and degrade lignin with its eight manganese peroxidases and five laccase gene models (Olson et al., 2012).

2.2 Ascomycetes

Ascomycete fungi mainly degrade nonwoody biomass and are not known for a general ability to degrade lignin. They participate in wood decay through a life style called soft-rot decay that is not as well understood as white-rot and brown-rot (Blanchette, Held, & Farrell, 2002). Soft-rot fungi show preference for cellulose and hemicellulose, and they do not appear to degrade lignin within the middle lamella of plant cell wall. For instance, soft-rot fungi are incapable of advancing to the heartwood in dead tree trunks but may decompose cellulose and hemicelluloses of wounded or cut wood surfaces and timber (Lundell, Mäkelä, de Vries, & Hildén, 2014). Soft-rot fungi typically degrade wood in wet environments and also cause characteristic decay patterns. Type I attack produces cavities within the secondary walls of the wood cells following the microfibrillar orientation of cellulose. In the type II attack, the secondary cell wall is completely eroded and the middle lamella is left intact (Blanchette, 2003; Eriksson et al., 1990). Ligninolytic ability has been described for some ascomycete fungi (e.g., *Xylaria* spp. and *Coccomyces* spp.), which results in selective delignification, but relatively little is known about their enzymatic system (Koide, Osono, & Takeda, 2005; Liers, Bobeth, Pecyna, Ullrich, & Hofrichter, 2010; Osono & Takeda, 2001).

Another group of ascomycete fungi that colonize wood are the blue or sap stain fungi. This group of fungi contains several genera (e.g., *Ophiostoma* and *Grosmannia*) and is named after their ability to produce dark-colored melanins on their ascocarps (peritechia) and their hyphal cell walls that protect them, for example, against light and drought. Blue stain fungi grow in phloem or ray parenchyma cells or in resin ducts of conifers where they degrade wood resins and waxes without decomposing the main lignocellulose components (Ballard, Walsh, & Cole, 1984; DiGuistini et al., 2011).

During the decay of nonwoody plant biomass ascomycetes also encounter aromatic compounds, such as lignin (e.g., in wheat straw), and aromatic components of plant polysaccharides, such as ferulic acid attached to xylan and pectin. However, the enzyme systems of most ascomycetes do not contain the typical lignin-modifying enzymes, with the exception of laccases, suggesting that they leave lignin largely intact. However, they produce feruloyl esterases that remove ferulic acid and other cinnamic acids from the polysaccharides (see Section 5.2.1) (Benoit, Danchin, Bleichrodt, & de Vries, 2008), indicating that they are exposed to monomeric aromatic compounds.

2.3 Ectomycorrhizal Fungi

An ectomycorrhiza is a mutualistic symbiosis between a fungus and a plant, which is primarily formed by the species of the class agaricomycetes. Numerous other examples exist within ascomycetes (Rinaldi, Comandini, & Kuyper, 2008; Tedersoo, May, & Smith, 2010). As root symbionts, ectomycorrhizal (ECM) fungi have limited ability to degrade plant cell wall polymers (Martin et al., 2008; Nagendran, Hallen-Adams, Paper, Aslam, & Walton, 2009). Phylogenetic reconstructions show that the capacity to form ectomycorrhizas has appeared independently several times in the course of evolution from saprobic ancestor (Hibbett, Gilbert, & Donoghue, 2000). Although the genome of basidiomycete ECM *Laccaria bicolor* lacks lignin-modifying peroxidases (Martin et al., 2008), the occurrence of gene sequences with similarity to fungal class II peroxidases has been reported from ECM species (Bödeker, Nygren, Taylor, Olson, & Lindahl, 2009; Bödeker et al., 2014). In addition, *Paxillus involutus* was shown to be able to significantly modify organic matter using a free-radical-based mechanism, similar to brown-rot fungi (Rineau et al., 2012). Additionally, chemical modifications of organic matter occurring in the presence of glucose were correlated with the expression of a laccase, cytochrome P450 monooxygenase, and unspecific peroxygenase (Lundell, Mäkelä, & Hildén, 2010). Furthermore, laccases were upregulated in ECM root tips of both *L. bicolor* and ascomycete species *Tuber melanosporum*, suggesting that laccases may facilitate root colonization (Veneault-Fourrey, Plett, & Martin, 2013).

3. AROMATIC COMPOUNDS IN PLANT BIOMASS

Various types of aromatic compounds are found in plants, but the most common ones are bound phenolics that are present in various forms, such as those that are linked to lignin (see Section 3.1) or polysaccharides

(see Section 3.2), and soluble aromatic compounds (Kaisoon, Siriamornpun, Weerapreeyakul, & Meeso, 2011). However, other aromatic compounds are also present (see Section 3.3). These aromatic compounds result in different monomers that can be converted through the various aromatic pathways in fungal cells (see Section 7). Several studies have been performed to analyze the aromatic profile of plants, resulting in a wide variation of compounds. A study using six herbs and spices resulted in the identification of 51 aromatic compounds (Vallverdu-Queralt et al., 2014), whereas several free aromatic acids and flavonoids were also detected in 12 edible flowers (Kaisoon et al., 2011). Studies involving larger sets of plants confirm this variety of aromatic compounds covering both bound and soluble aromatic compounds as well as flavonoids, volatile oils, and other compounds (Cai, Luo, Sun, & Corke, 2004; Cai, Sun, Xing, & Corke, 2004; Surveswaran, Cai, Corke, & Sun, 2007; Wojdylo, Oszmianski, & Czemerys, 2007).

3.1 Lignin

After cellulose, lignin is the second most abundant renewable biopolymer on Earth. This complex aromatic heteropolymer accounts typically for 26–32% and 20–25% of dry weight in softwoods and hardwoods, respectively (Sjöström, 1993). The content of lignin in gramineous plants varies substantially and depends on the plant species. For example, corn stover and wheat straw, which are the two most abundant agricultural residues, comprise of 15–21% and 5–17% of lignin as dry weight, respectively (Buranov & Mazza, 2008). Evolutionarily, lignin was introduced to the cell walls when plants colonized land. Lignin is tightly cross-linked with other cell wall components, covalently bound to hemicellulose but not to cellulose, and thus can be considered "cellular glue" providing strength to plant tissues and fibers and stiffness to cell walls (Rubin, 2008). It aids in water transportation by physically attaching the xylem cells together and protects the more easily degradable cellulose and hemicellulose polymers from microbial attack (Moura, Bonine, de Oliveira Fernandes Viana, Dornelas, & Mazzafera, 2010).

Lignin consists of three phenylpropane monomer units (monolignols), namely sinapyl, coniferyl, and *p*-coumaryl alcohol (Argyropoulos & Menachem, 1997; Kuhad, Singh, & Eriksson, 1997; Sjöström, 1993). Monolignols are synthesized by the general phenylpropanoid pathway where aromatic amino acid phenylalanine is converted to cinnamic acids such as *p*-coumaric, caffeic, ferulic, 5-hydroxyferulic, and sinapic acid, and further to *p*-coumaryl CoA. The formation of monolignols requires enzyme-mediated reactions including the hydroxylation of the aromatic ring, methylation of hydroxyl

groups, and the stepwise reduction of monolignol side chains from carboxylic acids to alcohols (Boerjan, Ralph, & Baucher, 2003). The monomeric lignin precursors (Figure 1(a)) are translocated to the plant cell wall and oxidized to monolignol radicals, which are polymerized to *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) type of phenylpropanoid units (Boerjan et al., 2003; Campbell & Sederoff, 1996; Chakar & Ragauskas, 2004; Freudenberg & Neish, 1968; Higuchi, 2006; Ralph et al., 2004). Oxidative enzymes such as class III plant peroxidases, and laccases and other phenol oxidases have been proposed to catalyze the polymerization of the lignin monomers by the random radical coupling of hydroxycinnamyl subunits resulting with a complex branched polymer (Fagerstedt, Kukkola, Koistinen, Takahashi, & Marjamaa, 2010). The biosynthesis of lignin results in an almost random occurrence of the different linkage types, and therefore, the lignin polymer has no single repeating bond between these subunits, which makes the polymeric structure amorphic (Figure 1(b)).

The nonhydrolyzable intermonomeric linkages account for the rigidity of lignin. This is in contrast with other native polymers which are generally fully hydrolyzed to monomeric units (Higuchi, 2006). Lignin comprises many kinds of C−C and C−O bonds with β-*O*-4 aryl ether linkage being the most abundant type accounting for 30−40% of total linkages in softwood and 40−50% in hardwood lignin (Brunow & Lundquist, 2010). Other abundant bonding patterns in native lignin include β-5 phenyl coumaran, β-β′ pinoresinol, 5-5′ biphenyl, β-1 diaryl propane, and 4-*O*-5′ diaryl ether type linkages, as well as dibenzodioxocin structures (DBDO; 5-5′-α, β-*O*-4′). Both the DBDO and 4-*O*-5′ motif could constitute branching points in the lignin polymer (Boerjan et al., 2003; Brunow & Lundquist, 2010). Although lignin is traditionally seen as highly complex cross-linked, branched polymer, newer findings on milled wood lignin suggest that lignin exists as 6−12 phenolic units long linear oligomers, which strongly interact in such a manner that traditional analyses of the molecular mass are biased and thus suggest higher molecular mass polymeric units (Crestini, Melone, Sette, & Saladino, 2011). The functional groups such as methoxyl, phenolic and aliphatic hydroxyl, benzyl alcohol, noncyclic benzyl ether, and carbonyl groups have major influence on the reactivity of lignin. Moreover, the compact structure that is insoluble in water and other common solvents as well as already mentioned abundance of different linkages make lignin biodegradation a challenge. Thus biological lignin degradation, caused by oxidative enzymes and/or small molecular weight mediators or factors such as radicals, is unspecific (Hatakka & Hammel, 2010).

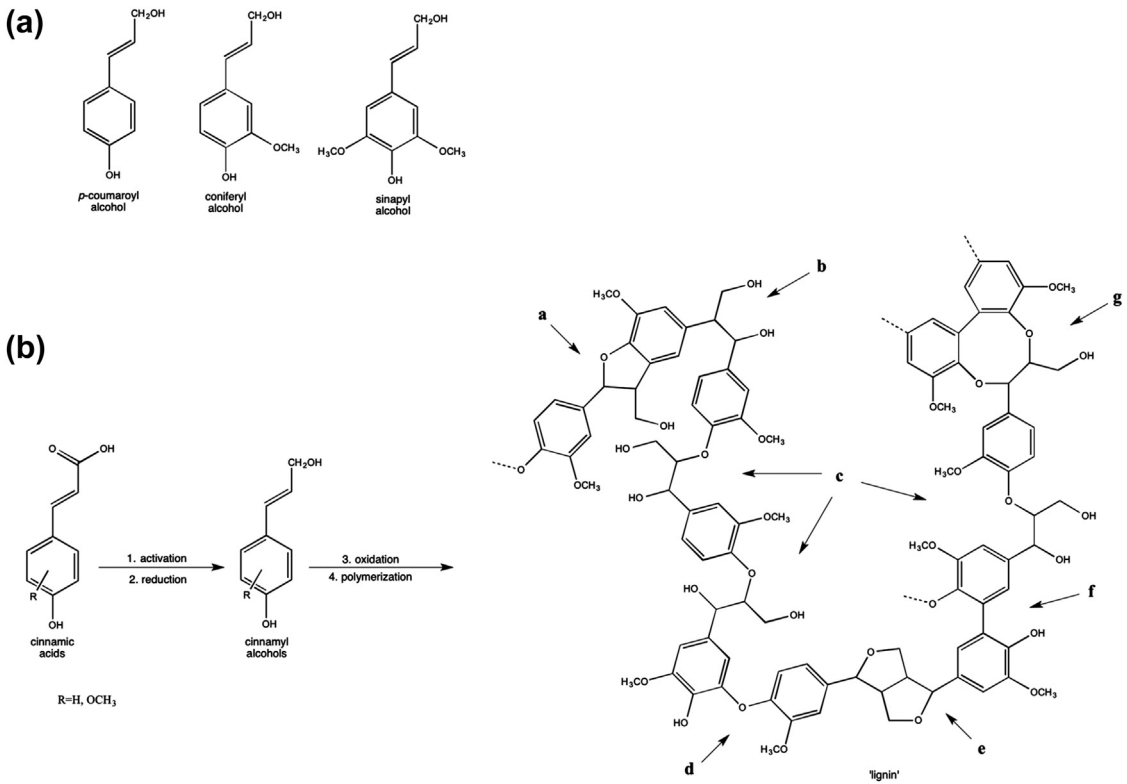


Figure 1 Schematic representation of (a) monolignols and (b) lignin synthesis and combination of abundant linkages in lignin macromolecule. a) β-5, b) β-1, c) β-O-4, d) 5-O-4′, e) β-β′, f) 5-5′, g) 5-5′-O-4; R=H, OCH_3. *Modified based on Önnerud, Zhang, Gellerstedt, and Henriksson (2002).*

The precise chemical composition of lignin is difficult to determine due to its complex nature and lignin–polysaccharide–protein structures (Amthor, 2003). The knowledge of the chemical structure of diverse plant lignins is still incomplete, although several lignin models have been presented. Recently, a novel unusual lignin polymer consisting of caffeyl alcohol was identified in seed coats of both monocots and dicots (Chen, Tobimatsu, Havkin-Frenkel, Dixon, & Ralph, 2012). The amount and composition of lignin vary among taxa, cell types, location in secondary wall of individual cells, and between walls of different cells in the same tissue and organs of the same plant (Agarwal & Atalla, 1986; Campbell & Sederoff, 1996). Softwoods have higher contents of lignin than hardwoods and grasses (Sjöström, 1993). Softwood lignin mainly contains guaiacyl units with low levels of *p*-hydroxyphenyl units, whereas hardwood lignin predominantly consists of guaiacyl and syringyl units with traces of *p*-hydroxyphenyl units. Lignin from hardwood, therefore, contains higher methoxyl content than softwood (Brunow, 2006). Lignin from grasses incorporate comparable amounts of all three units (Billa & Monties, 1995; Obst, 1982; Vanholme, Demedts, Morreel, Ralph, & Boerjan, 2010).

3.2 Aromatic Components of Plant Polysaccharides

Several plant polysaccharides can contain aromatic residues attached to their side chains. Ferulic acid and *p*-coumaric acid can be ester linked to the C(O)5 residue of L-arabinose in xylan, and ferulic acid has also been detected ester linked to the C(O)6 residue of D-galactose and the C(O)2 or C(O)3 residue of L-arabinose in pectin (Fry, 1982; Fry, 1983; Ishii, 1994; Ishii, 1997; Ishii & Hiroi, 1990a,b; Ishii, Hiroi, & Thomas, 1990; Ishii & Tabita, 1993; Kato & Nevin, 1985; Rombouts & Thibault, 1986; Saulnier, Vigouroux, & Thibault, 1995; Schooneveld-Bergmans, Hopman, Beldman, & Voragen, 1998; Smith & Harris, 2001). In addition, ferulic acid can be ester-linked to the C(O)4 residue of D-xylose side chains of xyloglucan (Ishii et al., 1990). The linkages vary depending on the polysaccharide (Thibault et al., 1998) (Figure 2). The most commonly isolated oligosaccharides containing aromatic acids are water soluble *O*-[5-*O*-(*trans*-*p*-coumaroyl)-α-L-arabinofuranosyl]-1,3-*O*-β-D-xylopyranosyl-1,4-D-xylopyranose (PAXX) and *O*-[5-*O*-(*trans*-feruloyl)-α-L-arabinofuranosyl]-1,3-*O*-β-D-xylopyranosyl-1,4-D-xylopyranose (FAXX) (Akin, 2008; Romero, Ferreira, Martinez, & Martinez, 2009). Ferulic acid and *p*-coumaric acid have also been identified as being linked to cutins and suberins, indicating their common presence in a variety of plant polysaccharides (Micard, Grabber, Ralph,

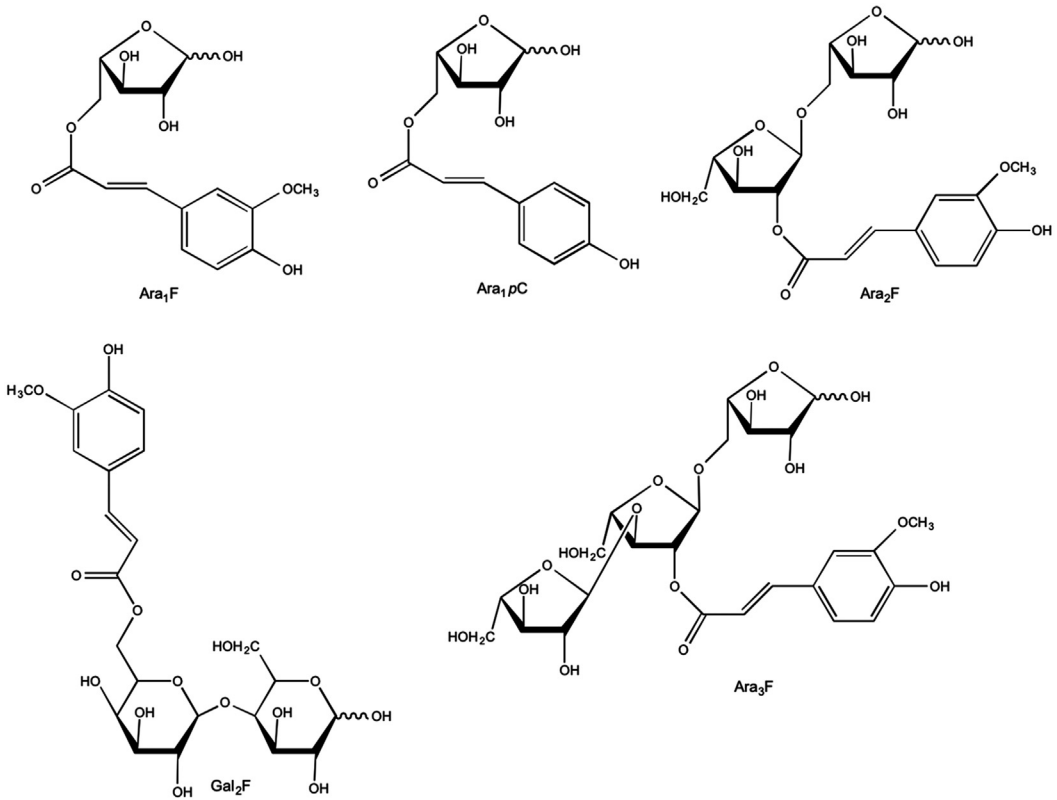


Figure 2 Linkages of some ferulic acid and *p*-coumaric acid derived mono- and oligosaccharides isolated from sugar beet pulp and wheat bran. Ara_1F = ferulic acid linked to L-arabinose; Ara_2F = ferulic acid linked to arabinobiose; Ara_3F = ferulic acid linked to arabinotriose; Ara_1pC = *p*-coumaric acid linked to L-arabinose; Gal_2F = ferulic acid linked arabinobiose. *Redrawn partially based on Thibault et al. (1998) with permission from Elsevier.*

Renard, & Thibault, 1997). The release of these acids from the polysaccharides will result in the monomeric aromatic compounds that enter fungal aromatic metabolism.

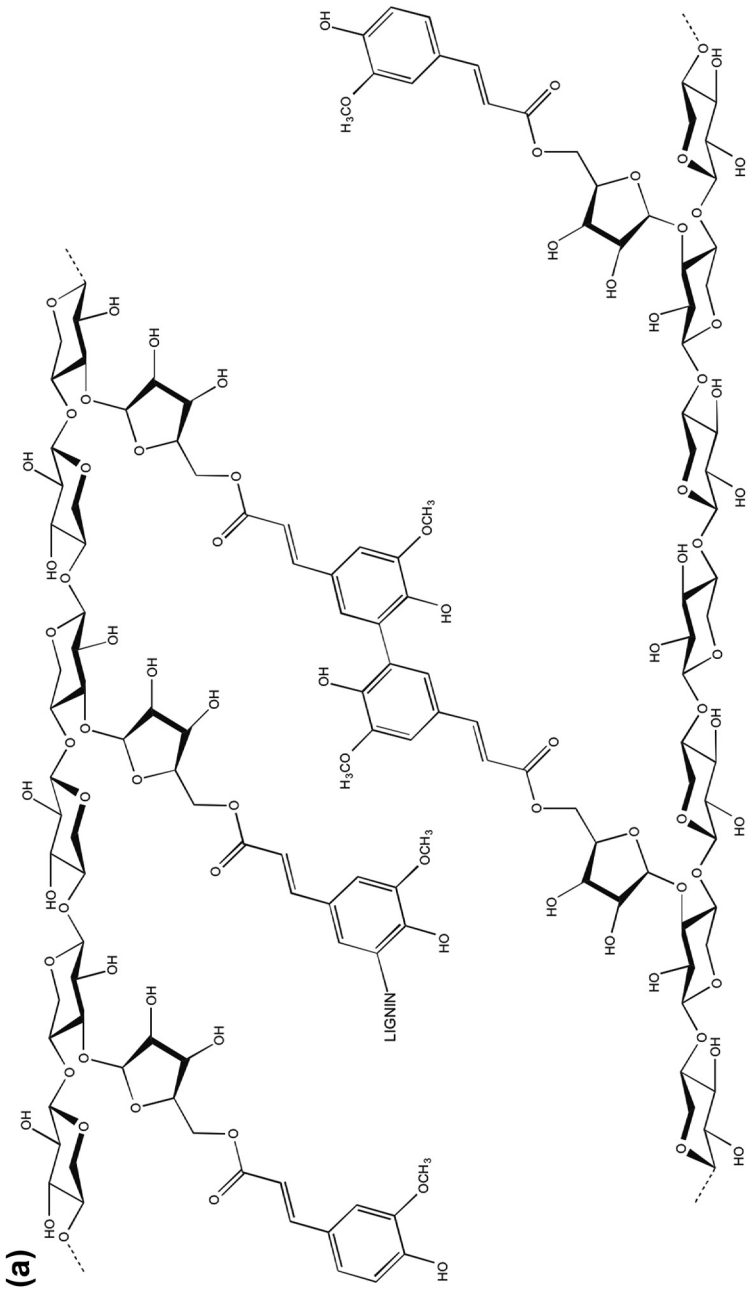
Ferulic acid can form dimers that enable the linkage of lignin to plant cell wall polysaccharides and polysaccharides to each other (Figure 3), and these dimers can be released by feruloyl esterases (see Section 5.2.1) (Faulds, Sancho, & Bartolome, 2002; Faulds, Mandalari, LoCurto, Bisignano, Waldron, 2004; Ishii, 1991; Kroon, Garcia-Conesa, Fillingham, Hazlewood, & Williamson, 1999). Different types of diferulic acid linkages have been identified (Eraso & Hartley, 1990; Fry, 1986; Ishii, 1991; Kato & Nevin, 1985; Micard et al., 1997; Ralph, Grabber, & Hatfield, 1995; Shibuya & Nakane, 1984) demonstrating the diversity of ferulic acid dimerization in plant cell walls (Figure 4).

3.3 Other Aromatic Plant Compounds

Plants can also contain free or nonesterified aromatic compounds, such as ferulic acid, *p*-coumaric acid, vanillic acid, and *p*-hydroxybenzoic acid in water extracts of alfalfa (Newby, Sablon, Synge, Casteele, & van Sumere, 1980), and ferulic acid, *p*-coumaric acid, and caffeic acid in various grasses and legumes (Cherney, Anliker, Albrecht, & Wood, 1989) (Figure 5). In addition, β-glucosides of *o*-coumaric acid, coumarinic acid and melilotic acid have been identified in plants as well as their metabolic product, coumarin (Kosuge & Conn, 1959; Kosuge & Conn, 1961) (Figure 5).

Another class of aromatic compounds are the flavonoids, which contain a structure that consists of two aromatic rings connected by three carbon atoms (Figure 6) (Dykes & Rooney, 2007). Several main groups of flavonoids can be recognized (Figure 6) and each group consists of a variety of compounds that differ in the groups (e.g., OH, OCH_3) attached to the aromatic rings (Corradini et al., 2011; Dykes & Rooney, 2007; Kumar & Pandey, 2013).

Tannins are widespread in the plant kingdom and they form the second most abundant group of plant phenolics after lignin (McLeod, 1974). Tannins are found in leaves, roots, bark, and wood and can accumulate in large amounts in particular organs or tissues of the plant (Haslam, 2007). Tannins are polymeric compounds that are formed mainly of gallic acid. Tannins can reduce the digestibility of other plant components, such as carbohydrates and proteins, by binding to them, and also provide a specific odor and taste to these plants, making them nonpreferred by insects and birds (Dykes & Rooney, 2007). Their antioxidant activity is higher than that of monomeric aromatic compounds (Hagerman et al., 1998) and have been shown to have various beneficial effects on human health (Dykes & Rooney, 2007; Prior &


(a)
LIGNIN
OCH3
HO
OH
H3CO
O

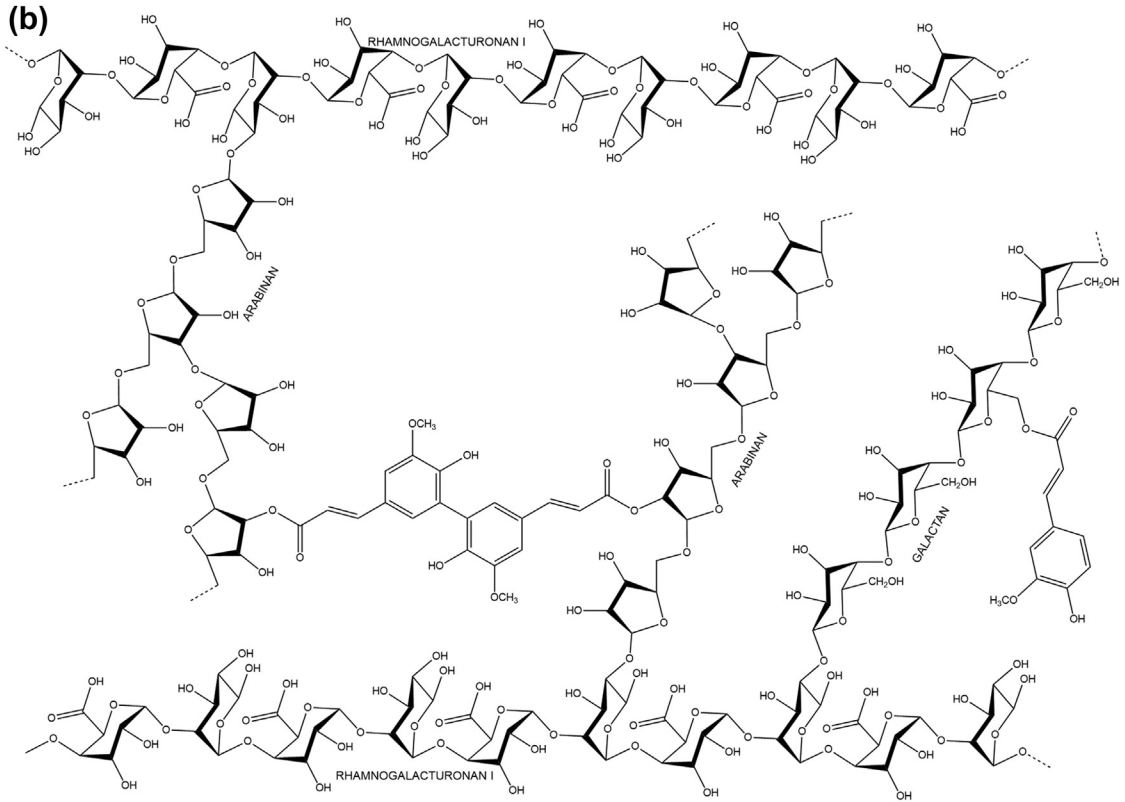


Figure 3 Diferulic acid cross-links in plant cell walls. (a) Cross-linked xylan and lignin. (b) Cross-linked pectin. *Redrawn based on Wong (2006) with kind permission from Springer Science and Business Media.*

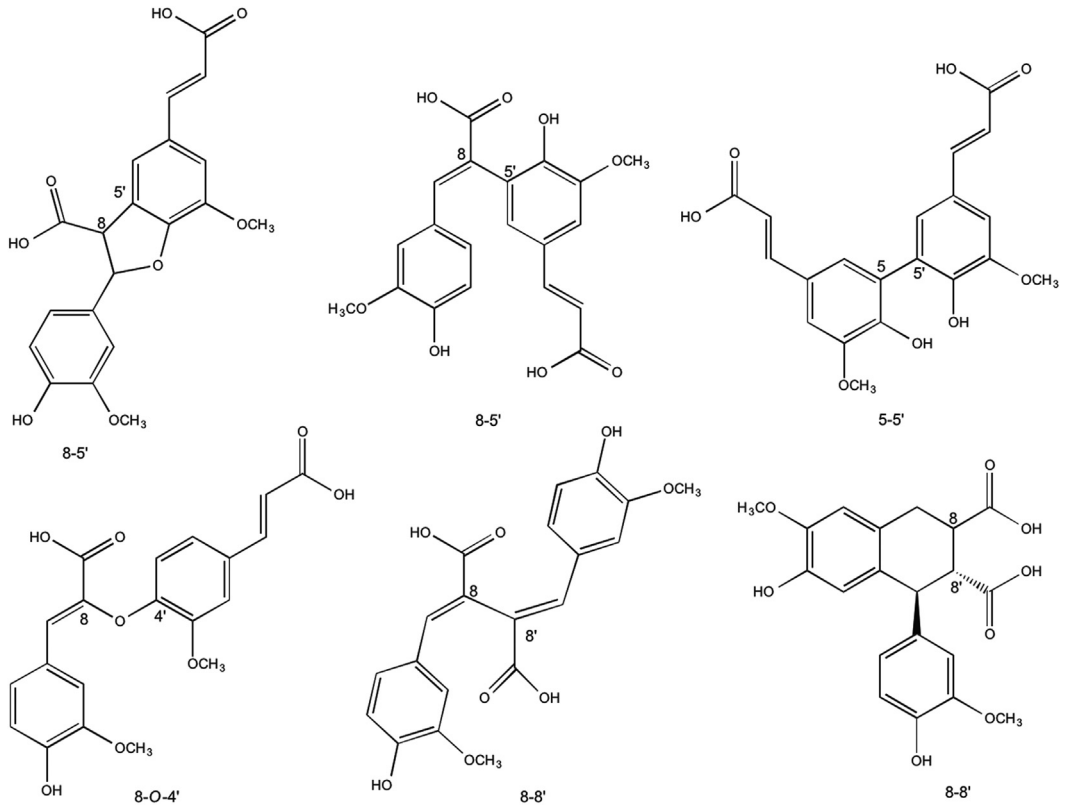


Figure 4 Diferulic acid structures identified in plant cell walls. *Redrawn based on Micard et al. (1997) with permission from Elsevier.*

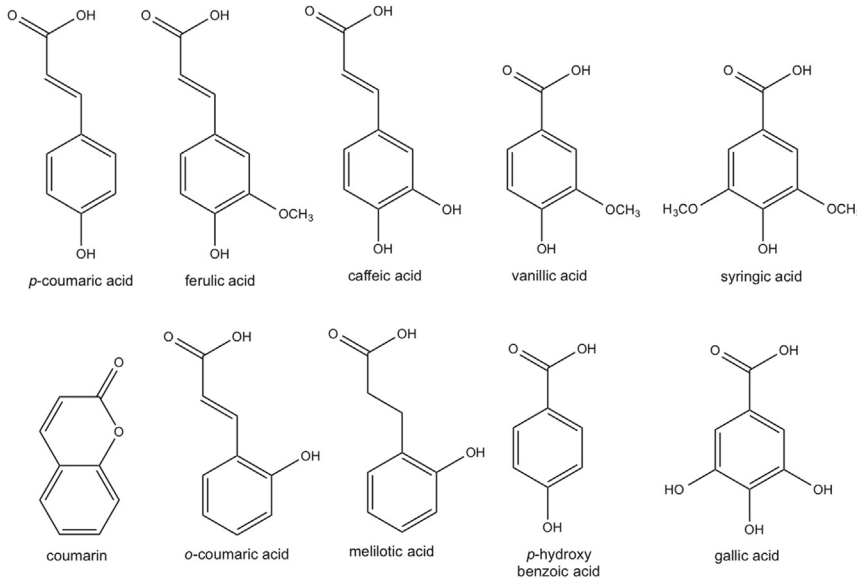


Figure 5 Free aromatic acids and related compounds extracted from plants.

Gu, 2005). The structure of tannins varies depending on the plant species (Bentley & Payne, 2013; Dykes & Rooney, 2007) and two main groups are recognized: flavonoid-based condensed tannins (syn. proanthocyanidins together with phloroglucinol) and hydrolyzable tannins consisting of esters of gallic acid with a central polyol (Gross, 2008) (Figure 7). The degradation of these structures results in flavonoids (see above) or gallic acid that can be further metabolized (see Section 7.5).

Finally, plants also contain stilbenoids, which are compounds containing two aromatic rings and a variety of side chains (Figure 8) (Pawlus et al., 2013; Ververidis et al., 2007). A separate side branch of the flavonoid biosynthetic pathway forms these compounds and they are of major importance to plant disease resistance, for instance in *Vitis* (Pawlus et al., 2013). Flavonoids possess diverse biological activities and potential as new drugs with, e.g., antitumor, antimicrobial, and antioxidant effects and have therefore major economic importance.

4. SENSITIVITY OF FUNGI TO AROMATIC COMPOUNDS

During the growth of fungi on plant biomass (some of) the aromatic compounds are released and fungi are therefore exposed to them. Free (non-polymeric) aromatic compounds are toxic for most fungi at relatively low

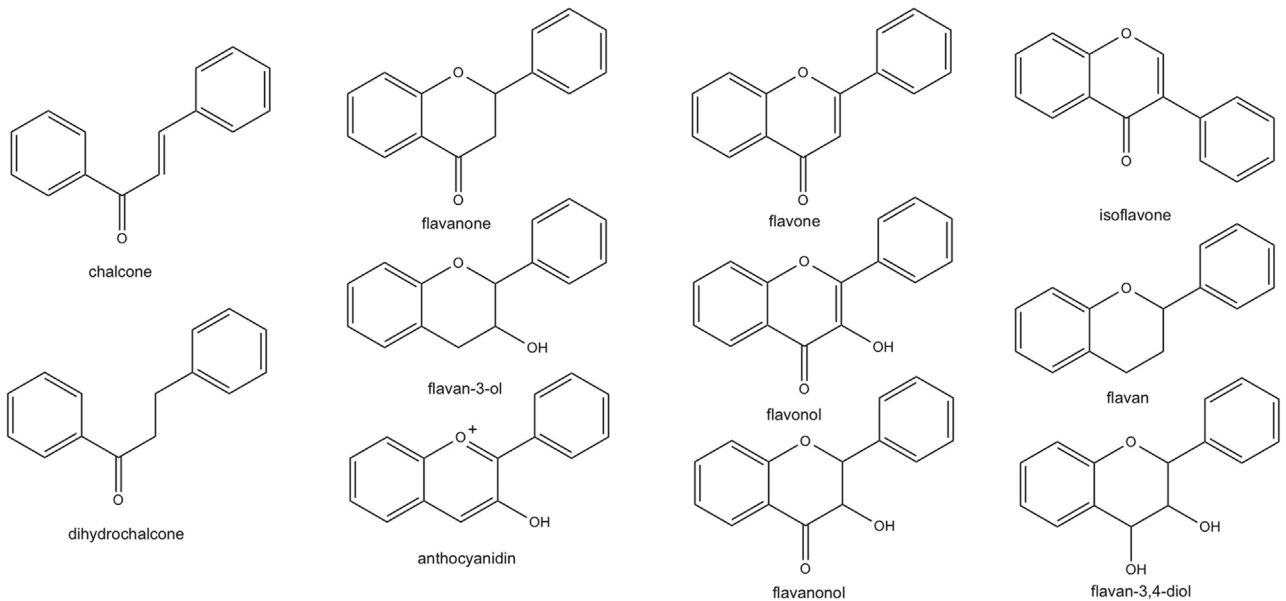


Figure 6 The major flavonoid backbones present in plants. *Based on (Corradini et al., 2011; Dykes & Rooney, 2007; Kumar & Pandey, 2013).*

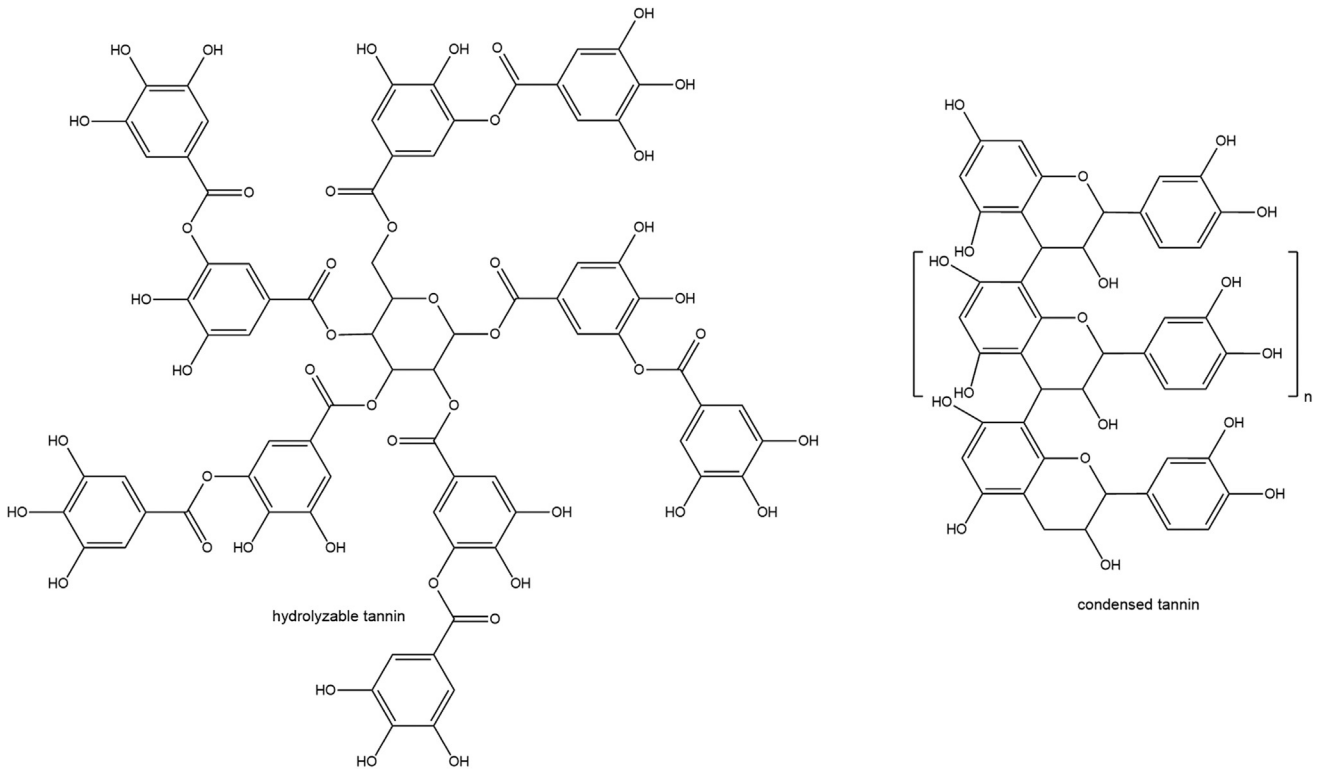


Figure 7 Models for the structure of hydrolyzable and condensed tannins. *Adapted and used by permission of Bentley and Payne (2013), Dykes and Rooney (2007).*

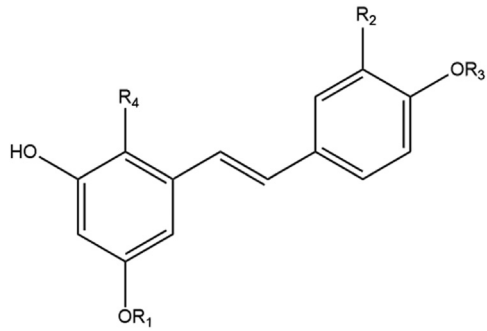


Figure 8 Schematic presentation of the stilbenoid backbone. R_1—R_4 can be a variety of side chains such as OH, OCH_3 and glucose. *Based on Pawlus et al. (2013), Ververidis et al. (2007).*

concentrations. This poses various challenges to the fungi which are exposed to these compounds while using the plant biomass (mainly polysaccharides) as a carbon source. Wood-rotting fungi cannot grow on aromatic compounds as the sole carbon source, and some of them, e.g., vanillin is toxic to them in higher concentrations (Ander et al., 1980). Several studies into the effects of the presence of aromatic compounds have been performed which are summarized here. The presence of cinnamic acid, ferulic acid, and cinnamic aldehyde inhibited the growth of *Neurospora crassa* at 250 μg/mL, but did not affect morphology, whereas caffeic acid and *p*-coumaric acid changed the morphology of the fungus without inhibiting growth (Mendonça Neves, Kawano, & Said, 2005). A larger set of lignin-related aromatic compounds and their unsubstituted and 4-*O*-methylated derivatives and 4-hydroxyl substituted benzaldehydes was tested for their inhibition of growth of eight white-rot fungi. The significant reduction of growth was observed at 10 mM concentrations for most compounds, whereas some reduced fungal growth already at 5 and 1 mM concentrations (Buswell & Eriksson, 1994). Similarly, 50% or more of the growth of the white-rot fungus *Phanerochaete chrysosporium* was inhibited by phenethyl alcohol and several other phenolic compounds at 10 mM or lower concentrations (Hage, Schoemaker, Wever, Zennaro, & Heipieper, 2001).

A larger set of fungi (88) was analyzed for their sensitivity to eight lignin-related phenolic compounds (Guiraud, Steiman, Seigle-Murandi, & Benoit-Guyod, 1995). This revealed strong differences between the species and for most groups no common features could be observed with respect to pigment formation or growth inhibition. However, three groups of fungi, that were previously classified as groups of the fungi imperfecti,

Zygomycetes, Hypocreales, and Melanconiales, were highly resistant to phenolic compounds.

The growth of two white-rot fungi, *Lentinus edodes* and *Pleurotus sajor-caju*, was enhanced by several phenols up to 5 mM concentrations, whereas vanillic acid, *p*-coumaric acid, caffeic acid, and vanillin had the same effect on *L. edodes*, but not on *P. sajor-caju* (Cai, Buswell, & Chang, 1993). The growth of straw decomposing species *Volvariella volvacea* was only stimulated by low concentrations of vanillic acid and caffeic acid. In contrast, other compounds, such as 4-hydroxybenzoic acid, syringic acid, and 4-hydroxybenzaldehyde, reduced growth of these fungi (Cai et al., 1993). The most toxic aromatic compounds for white-rot species *Ceriporiopsis subvermispora* and the dung-dwelling basidiomycete *Cyathus stercoreus* are benzaldehyde derivatives (Sethuraman, Akin, Eisele, & Eriksson, 1998). The growth was enhanced up to 5 mM after which it was either retarded or stopped. Lignin-modifying enzymes of white-rot fungi were required for the detoxification of exogenous derivatives of benzoic acid, cinnamic acid, and benzaldehyde. It has been proposed that a higher degree of methoxylation produces less toxic derivatives (Gupta, Hamp, Buswell, & Eriksson, 1981; Li, Xu, & Eriksson, 1999).

These data demonstrate that fungi that grow in biotopes where monomeric aromatic compounds are released need to develop methods to reduce the concentrations of these compounds. Many fungi are capable of aromatic conversions that ultimately result in nonaromatic compounds that are less or nontoxic to the fungi. The current knowledge on the release and conversion of aromatic compounds by fungi is described below.

5. ENZYMATIC RELEASE OF AROMATICS COMPOUNDS FROM PLANT BIOMASS BY FUNGI

Fungi produce a variety of extracellular enzymes involved in the conversion and degradation of aromatic compounds in plant biomass. These enzymes are required to degrade large aromatic compounds to smaller subunits which can then be metabolized in fungal cells. Although fungal manganese peroxidases are able to mineralize polymeric lignin to carbon dioxide in vitro ((Hatakka, 2001; Kapich, Hofrichter, Vares, & Hatakka, 1999) and references therein), it is likely that at least part of aromatic degradation products are taken inside the fungal cell and metabolized by intracellular enzymes. However, almost nothing is known about the transport of aromatic compounds from the growth medium to fungal cell (Shary et al., 2008). The best characterized of these enzymes are oxidoreductases such as class II

heme-containing peroxidases from the CAZy family AA2 and laccases (CAZy family AA1_1) (Martinez, Ruiz-Dueñas, Guillen, & Martinez, 1996; Mester et al., 2001; Youn, Hah, & Kang, 1995). These enzymes are responsible for generating highly reactive free radicals that attack lignin bonds unspecifically. In addition, with a growing number of sequenced basidiomycete genomes, new superfamilies of fungal secretory peroxidases have been designated, i.e., dye-decolorizing peroxidases (DyPs) and heme-thiolate peroxidases (HTPs). In addition to oxidative enzymes, hydrolytic enzymes such as feruloyl esterases are involved in the release of plant aromatic compounds.

5.1 Oxidoreductases

5.1.1 Laccase

Laccases classified to CAZy family AA1_1 (EC 1.10.3.2, *p*-diphenol:oxygen oxidoreductases) are metalloenzymes that belong to the diverse superfamily of multicopper oxidases (Baldrian, 2006; Levasseur, Drula, Lombard, Coutinho, & Henrissat, 2013; Thurston, 1994). They are widely distributed in nature and in addition to fungi, similar types of multicopper oxidases exist in plants, insects, bacteria, and archaea (Alexandre & Zhulin, 2000; Claus, 2004; Martins et al., 2002; Mayer & Staples, 2002; Uthandi, Saad, Humbard, & Maupin-Furlow, 2010). Fungal laccases have been suggested to participate in the detoxification of byproducts of lignin degradation and also in morphogenesis, fungal plant pathogen interaction, and stress defense (reviewed by Baldrian (2006), Thurston (1994)).

Laccases catalyze the oxidation of a variety of phenolic compounds, such as *o*- and *p*-diphenols, methoxy-substituted phenols, polyphenols, aromatic amines, benzenethiols, hydroxindols, 1-naphthol, and syringaldazine, with the subsequent reduction of molecular oxygen to water (Call & Mücke, 1997; Thurston, 1994). In the presence of small molecular weight charge transfer mediator molecules, laccases are also able to oxidize nonphenolic compounds, and some phenolic lignin precursors and degradation products have been proposed to act as laccase mediators in nature (Camarero, Ibarra, Martinez, & Martinez, 2005).

5.1.2 Class II Lignin-Modifying Peroxidases

CAZy family AA2 includes class II lignin-modifying peroxidases (Levasseur et al., 2013), namely manganese peroxidases (MnPs), lignin peroxidases (LiPs), and versatile peroxidases (VPs), which have been only found in the white-rot and litter-decomposing basidiomycete genomes (Floudas et al., 2012; Riley et al., 2014). They are extracellular heme-containing enzymes

that use hydrogen peroxide or organic peroxides as electron acceptors to catalyze a number of oxidative reactions (Hofrichter, Ullrich, Pecyna, Liers, & Lundell, 2010). Two electrons derived from substrate molecules reduce the enzyme followed by a concomitant release of two water molecules.

MnPs (EC 1.11.1.13) catalyze the specific oxidation of Mn(II) to Mn(III) in the presence of H_2O_2 (Hammel & Cullen, 2008; Lundell et al., 2010). These highly reactive Mn(III) ions are chelated into a stable form by fungal secreted organic acids, such as oxalate, malonate, and fumarate. Chelated Mn(III) ions act as diffusible oxidants which are suggested to be capable of penetrating small molecular pores between cellulose microfibrils in plant cell wall and attack phenolic lignin substructures (Blanchette, Krueger, Haight, Akhtar, & Akin, 1997). As a result unstable free radicals are formed which disintegrate spontaneously (Hofrichter, 2002). Although, nonphenolic lignin substructures, which comprise approximately 90% of lignin subunits in wood, are not normally oxidized by MnPs, they have been shown to be slowly co-oxidized by MnP-mediated lipid peroxidation reactions (Jensen, Bao, Kawai, Srebotnik, & Hammel, 1996; Kapich et al., 1999). In addition, straw lignin and synthetic lignin, humic substances, dyes, and xenobiotic compounds are converted and even mineralized to carbon dioxide by MnP (Hofrichter & Fritsche, 1997; Hofrichter, Scheibner, Schneegaß, & Fritsche, 1998; Kawai, Umezawa, Shimada, & Higuchi, 1988).

LiP (EC 1.11.1.14) is the first enzyme which was connected to the oxidative breakdown of lignin. In a H_2O_2-dependent reaction, LiP catalyzes the initial one-electron oxidation of both phenolic and nonphenolic aromatic compounds, including the substructures of lignin, and several other substrates like veratryl alcohol (Hammel et al., 1993; Tien & Kirk, 1983). LiP possesses the aromatic substrate-binding tryptophan residue (Trp-171 in *Phanerochaete chrysosporium* LiP H8), which is exposed on the enzyme surface. This catalytically active tryptophan is thought to participate in the so-called long-range electron transfer from bulky aromatic substrates that cannot directly contact the oxidized heme in the active centre of LiP (Doyle, Blodig, Veitch, Piontek, & Smith, 1998). LiP oxidizes nonphenolic β-O-4 and diarylpropane lignin model compounds (see Section 6) followed by side-chain cleavage, demethylation, intramolecular addition, and rearrangements (Baciocchi, Bietti, Gerini, Lanzalunga, & Mancinelli, 2001; Hammel, Tien, Kalyanaraman, & Kirk, 1985; Miki, Renganathan, & Gold, 1986; Miki, Renganathan, Mayfield, Gold, 1987; Umezawa & Higuchi, 1989). Phenolic substrates are converted to phenoxy radicals and in the presence of oxygen these phenoxy radicals can form ring-cleavage products or lead

to coupling and polymerization (Harvey, Gilardi, Goble, & Palmer, 1993; Schoemaker, Harvey, Bowen, & Palmer, 1985).

The third type of class II lignin-modifying peroxidases is VP (EC 1.11.1.16.), which comprises both LiP and MnP activities (Martínez, 2002; Ruiz-Dueñas et al., 2009). Therefore, VP is capable of degrading a wider range of substrates than nonhybrid enzymes (Hammel et al., 1993). Similarly to MnP, VP has shown to efficiently oxidize Mn(II) (Ruiz-Dueñas et al., 2009). In addition, VP performs long-range electron transfer from aromatic donors, thus resembling LiP and consequently enables the enzyme to oxidize nonphenolic structures in the absence of Mn ions (Perez-Boada et al., 2005).

5.1.3 Dye-Decolorizing Peroxidases

Dye-decolorizing peroxidases (DyP-type peroxidases; EC 1.11.1.19) are named after their ability to oxidize a wide range of dye compounds, in particular, anthraquinone dyes, which are poorly oxidized by other peroxidases (Kim & Shoda, 1999; Passardi, Cosio, Penel, & Dunand, 2005; Sugano, 2009). It has been suggested that DyPs have oxygenase or hydrolase activities as well as peroxidase catalytic activities. Typical peroxidase substrates degraded by DyPs are, for example, 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulphonate and phenolic compounds. DyPs have also been reported to cleave β-carotene and other carotenoids as well as to oxidize methoxylated aromatics such as veratryl alcohol and nonphenolic β-O-4 lignin model compounds (Liers et al., 2010; van Bloois et al., 2009; Zelena, Hardebusch, Hülsdau, Berger, & Zorn, 2009). However, their physiological function still remains unclear.

5.1.4 Heme-Thiolate Peroxidases

Heme-thiolate peroxidases (HTPs) are one superfamily of peroxidases including unspecific peroxygenases (UPOs; formerly aromatic peroxygenases) and chloroperoxidases, which can incorporate oxygen into their substrate molecules. UPOs (EC 1.11.2.1) catalyze a wide range of reactions including oxidations of different aliphatic and aromatic compounds (Gutiérrez et al., 2011; Ullrich & Hofrichter, 2005). Depending on the particular substrate and reaction conditions, UPOs catalyze various reactions, such as *N*-oxidations, aromatic oxygenations, alkyl hydroxylations, and epoxidation (Hofrichter & Ullrich, 2014). Thus, UPOs combine unique features of cytochrome P450s (oxygen transfer) and peroxidases (phenol oxidation, halide oxidation). The physiological function of UPOs is still unclear, but their extracellular location and the catalytic

diversity suggest that they have a role in the conversion and detoxification of organic compounds in fungal natural habitats. It has been reported that UPOs are able to cleave nonphenolic β-*O*-4 lignin model dimers, which refers to the involvement of peroxygenases in the degradation of methoxylated compounds derived from lignin and other aromatic compounds in plants (Kinne et al., 2011).

5.2 Hydrolases

5.2.1 Feruloyl Esterases

Feruloyl esterases (EC 3.1.1.73), also known as ferulic acid esterases, together with *p*-coumaroyl esterases and cinnamoyl esterases remove hydroxycinnamic acids from plant cell wall polysaccharides. They also hydrolyze the linkages between uronic acid moieties of xylan and the lignin polymer (Li & Helm, 1995). Feruloyl esterases form a heterogeneous group of enzymes and the distribution of the different feruloyl esterase subgroups in the fungal kingdom varies strongly (Benoit et al., 2008). The difference in the enzymatic properties of the subgroups is exemplified for the two characterized feruloyl esterases from *Aspergillus niger*, FaeA and FaeB (de Vries et al., 2002). Although both enzymes act on xylan and pectin, the activity of FaeA is highest on xylan, and the activity of FaeB is highest on pectin (de Vries et al., 2002). They also differ with respect to the residue from which they can release ferulic acid. FaeB releases ferulic acid only from L-arabinose in xylan and pectin and FaeA releases ferulic acid from $C(O)_5$ of L-arabinose in xylan and from $C(O)_6$ of D-galactose in pectin (Ralet, Faulds, Williamson, & Thibault, 1994). Using methyl esters of aromatic acids, it was shown that the presence of methoxy group on the aromatic ring favors the activity of FaeA, whereas the presence of hydroxyl groups favors the activity of FaeB (Kroon, Faulds, Brezillon, & Williamson, 1997). Interestingly, the induction of the corresponding genes by a set of aromatic acids mirrored the substrate specificity with respect to the substitutions on the aromatic ring (de Vries et al., 2002; de Vries and Visser, 1999). A subset of the feruloyl esterases is also able to release diferulic acid from plant biomass. The 5,5′ dimer of ferulic acid (Figure 4) was released by several feruloyl esterases (Bartolome et al., 1997; Crepin, Faulds, & Connerton, 2004a; Crepin, Faulds, & Connerton, 2004b; Faulds et al., 2002; Faulds et al., 2003; Kroon et al., 1999; Topakas, Christakopoulos, & Faulds, 2005), whereas only two enzymes were shown to release the 8-*O*-4′-dimer (Kroon et al., 1999) and only an *Aspergillus oryzae* tannase efficiently releases the 8,5′ dimer (Garcia-Conesa, Ostergaard, Kauppinen, & Williamson, 2001).

5.2.2 Tannase

Tannase (tannin acyl hydrolase, EC 3.1.1.20) catalyzes the hydrolysis of ester bonds from gallotannins, which are also called complex or hydrolysable tannins (see Figure 7), producing gallic acid and glucose (Aguilar et al., 2007). Fungal tannases have been mainly produced and studied from the species of the genus *Aspergillus* and *Penicillium* that tolerate even up to 20% tannin (de Paiva et al., 2013; Murray et al., 2008; Suseela & Nandy, 1983; van Diepeningen et al., 2004). The production of tannase can be induced by phenolic compounds (e.g., gallic acid, tannic acid, pyrogallol, methyl gallate) (Mori et al., 1999). However, gallic acid has also been reported to repress the production of tannase (Aguilar, Augur, Favela-Torres, & Viniegra-Gonzalez, 2001; Bradoo, Gupta, & Saxena, 1997).

6. CONVERSION OF LIGNIN MODEL COMPOUNDS

Monomeric aromatic compounds are sometimes considered as lignin model compounds, but so-called dimeric aromatic compounds are the most commonly used compounds for this. Lignin model compounds are an important tool in testing the activity of enzymes involved in lignin degradation. Although the structure of natural lignin is not fully known, several of the linkages in lignin have been identified (see Section 3.1) and model compounds for these linkages have been synthesized. It should be noted though that the degradation of polymeric lignin and simple aromatic compounds may require totally different decay mechanisms. Polymeric high molecular weight lignins are large and cannot be taken into the fungal cell and thus they must be oxidized outside the cell, while the metabolism of small molecular weight aromatic compounds mostly occurs intracellularly (Hatakka, 2001), even though the role of extracellular enzymes in the initial cleavage of dimeric model compounds has been clearly described (see below).

These model compounds also provide an indication as to which monomeric aromatics could be the result of lignin degradation by fungi and which would likely be starting compounds of intracellular aromatic metabolism. Here we provide an overview of the enzymatic conversion of lignin model compounds and indicate the monomeric aromatic compounds that are discussed in the next section on fungal aromatic metabolism.

6.1 Arylglycerol-β-Aryl Ether Substructures

Arylglycerol-β-aryl ether (*β*-O-4) linkages account for approximately 35—60% and 50—70% of linkages in softwood and hardwood lignins,

respectively (Adler, 1977; Henriksson, 2009). For that reason its cleavage has been considered as the most important process in lignin degradation. A large variety of model compounds containing this linkage have been synthesized and these have been used to study the ability of lignin-modifying enzymes, such as MnP, LiP, DyP (Liers et al., 2010), UPO (Kinne et al., 2011) and laccases to cleave this linkage. In the latter case most of the products are not monomeric aromatic compounds that could enter the metabolic pathways described in this review (Kawai, Nakagawa, & Ohashi, 2002; Kawai et al., 2004; Srebotnik & Hammel, 2000). For MnP and LiP some examples of the monomeric aromatic compounds that could enter fungal aromatic metabolism are given below.

6.1.1 Action of Lignin Peroxidase

Studies using LiP from *P. chrysosporium* and *P. radiata* using a range of (β-O-4) model compounds revealed the variety of products that can be obtained after the action of this enzyme (Figures 9, 10) (Lundell et al., 1993; Miki et al., 1986; Srebotnik, Jensen, & Hammel, 1994; Umezawa, Shimada, Higuchi, & Kusai, 1986), which are then likely imported into the cell and converted further, if not repolymerized by laccase which is produced by most white-rot fungi (Hatakka, 1985). The oxidative cleavage of a dimeric β-O-4 lignin model compound, 1-(3,4-dimethoxypheny1)-2-phenylethanediol (dimethoxyhydrobenzoin) by LiP of *P. chrysosporium* resulted in the production of benzaldehyde, propylphenol, phenylglycerol, and catechol (Hammel et al., 1985). Benzaldehyde and propylphenol arise through C_α—C_β oxidative cleavage of this dimer via a pathway similar to that found for the oxidation of diarylpropanes by LiP (Renganathan, Miki, & Gold, 1986; Schoemaker et al., 1985). Phenylglycerol is formed through β-O-4 ether bond hydrolysis (Enoki, Goldsby, & Gold, 1981) whereas catechol is formed through demethoxylation of β-O-4 dimers (Miki et al., 1986).

6.1.2 Action of Manganese Peroxidase

Several (β-O-4) model compounds were also used to examine the action of manganese peroxidase (MnP) of *P. chrysosporium* and *Ceriporiopsis subvermispora* resulting in a range of monomeric aromatic compounds (Figure 11) (Bao, Fukushima, Jensen, Moen, & Hammel, 1994; Hofrichter, 2002; Jensen et al., 1996; Tuor, Wariishi, Schoemaker, & Gold, 1992). The oxidation of phenolic β-aryl ether structures involves the formation of reactive phenoxy radical intermediates which undergo C_α—C_β oxidative cleavage, alkyl phenyl cleavage, and C_α oxidation, yielding methoxylated

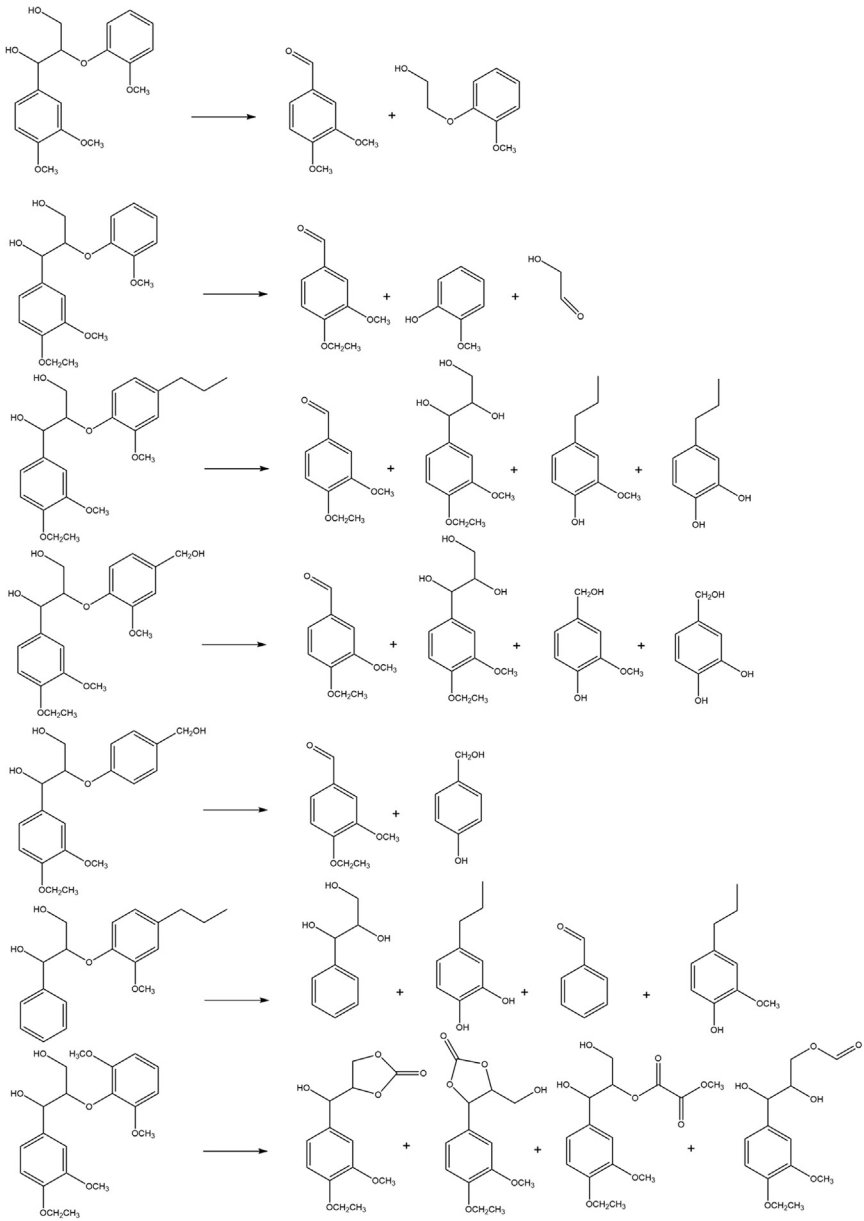


Figure 9 Aromatic monomers resulting from the action of LiP on β-O-4 model compounds. *Based on Lundell et al. (1993), Miki et al. (1986), Srebotnik et al. (1994), Umezawa et al. (1986).*

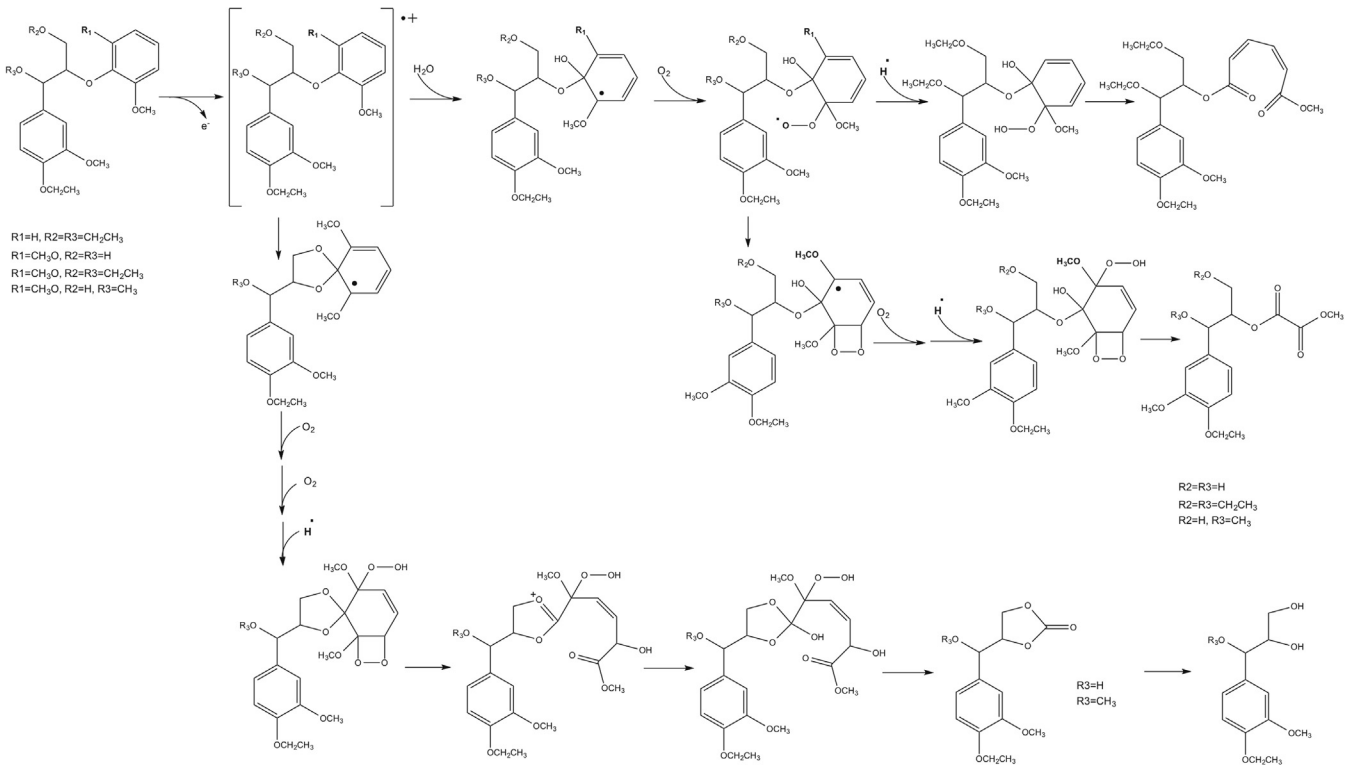


Figure 10 Mechanism for the degradation of β-O-4 model compounds by LiP with end-product hydrolysis further to triole. *Redrawn based on Umezawa and Higuchi (1985), Umezawa and Higuchi (1987) with permission from Elsevier.*

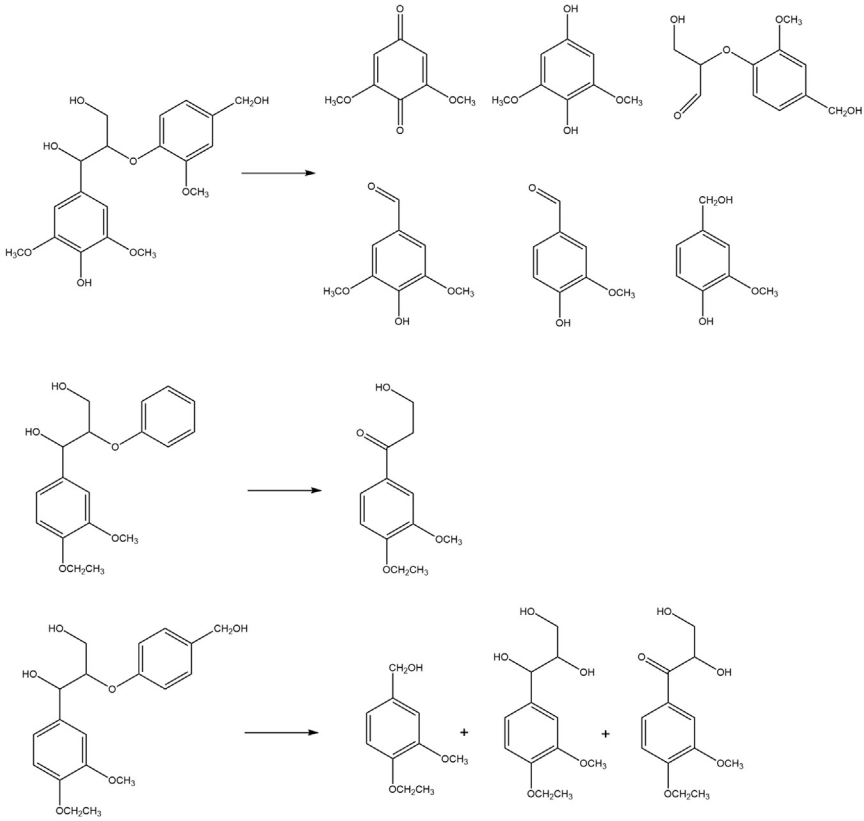


Figure 11 Aromatic monomers resulting from the degradation of β-O-4 model compounds by MnP. *Based on Bao et al. (1994), Hofrichter (2002), Jensen et al. (1996), Tuor et al. (1992).*

benzoquinone, phenoxylated hydroxypropane, and methoxylated catechol (Tuor et al., 1992). The cleavage of nonphenolic β-O-4 substrates by MnP under lipid peroxidation conditions with linoleic acid involves hydrogen abstraction from the benzylic carbon (C_α) followed by O_2 addition to form a peroxy radical, and subsequent oxidative cleavage and nonenzymatic degradation (Kapich, Steffen, Hofrichter, & Hatakka, 2005).

6.2 Conversion of Biphenyl Compounds

The biphenyl (5-5′) type of lignin substructures are one of the main linkage motifs in lignin, commonly occurring between two guaiacyl units and comprising 5−10% of the total linkages depending on the wood type

(Henriksson, 2009). However, studies involving biphenyl lignin model compounds are scarce. The breakdown of biphenyl lignin model compounds was shown to be enzymatically catalyzed by laccases of the white-rot fungus *T. versicolor* (Figure 12) (Katayama, Nishida, Morohoshi, & Kuroda, 1989). This reaction results in ring fission but the exact mechanism and intermediates are still elusive. However, when the model compound contained benzylic hydrogens, the formation of only benzylic oxidation products and tertrameric products such as dibenzodioxepin-type structures were reported (Crestini & Argyropoulos, 1998; Lahtinen, Kruus, Heinonen, & Sipilä, 2009).

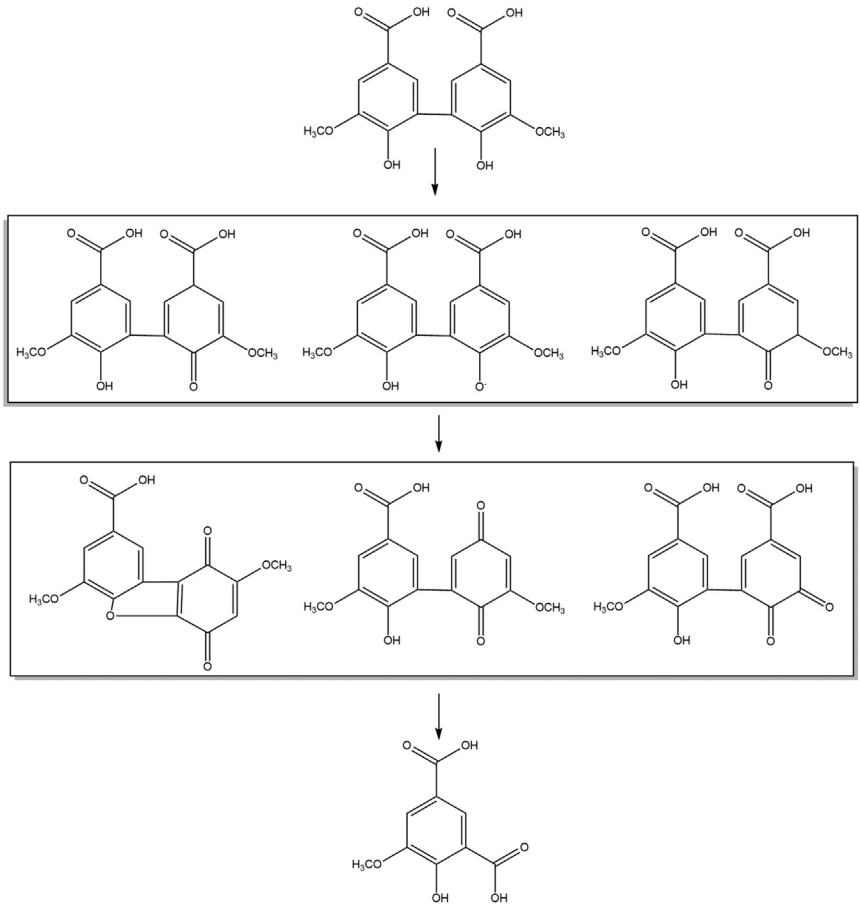


Figure 12 Conversion of a biphenyl (5-5′) lignin model compound by *T. versicolor*. *Redrawn based on Katayama et al. (1989) with permission of John Wiley & Sons, Inc.*

6.3 Conversion of Diarylpropane Compounds

Diarylpropane (β-1) linkages comprise 1−2% of total phenylpropane linkages in wood lignin (Henriksson, 2009). The degradation of diarylpropane lignin model compounds in which the C_α−C_β linkage gets cleaved has been well studied and proceeds through an aryl cation radical intermediate (Figure 13(a) and (b)) (Hammel et al., 1985; Renganathan et al., 1986). This reaction results in the aromatic aldehyde products anisyl aldehyde and

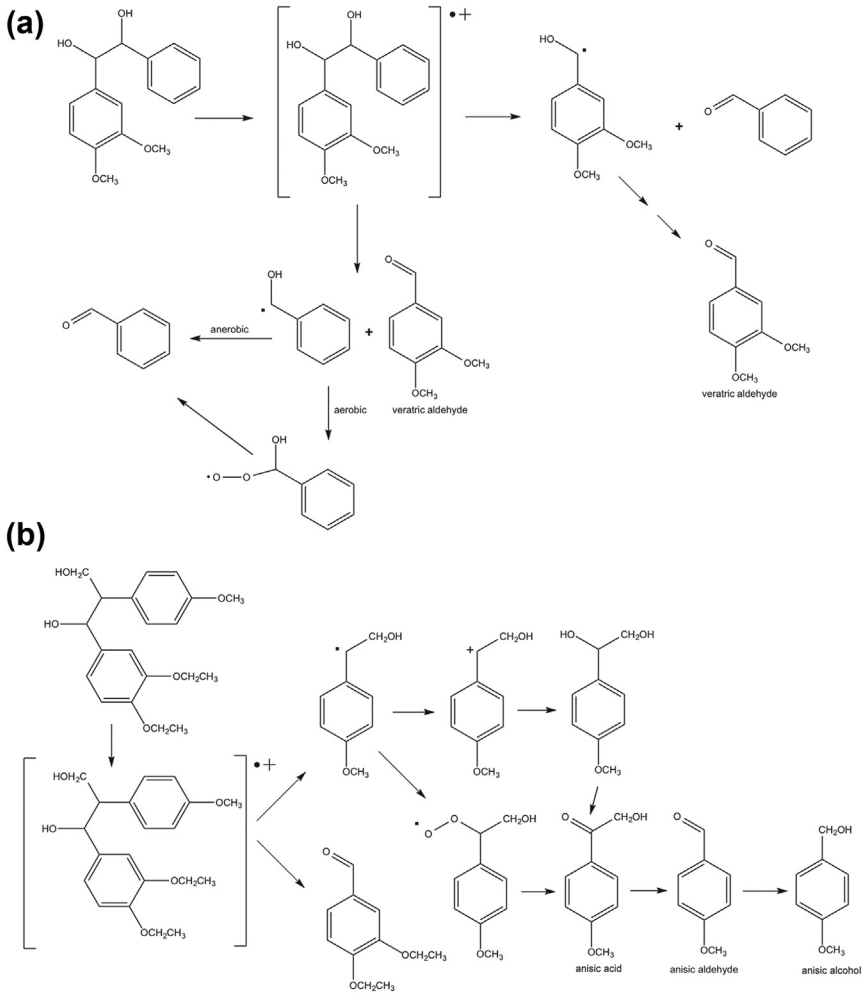


Figure 13 Conversion of C_α−C_β bond in diarylpropane (β-1) lignin model structures. The conversion depends on the structure of the dimer, in particular the presence of an OH (a) or CH_2OH (b) group at the link between the aromatic rings. *Based on Enoki and Gold (1982), Hammel et al. (1985), Renganathan et al. (1986).*

veratryl aldehyde (Enoki & Gold, 1982; Hammel et al., 1985). A different version of this model compound (other groups attached to the aromatic rings) can be converted to vanillin, using a similar reaction (Kirk & Farrell, 1987). The cleavage of C_α—C_β linkages by LiP was also observed for trimeric lignin model compounds (Mester et al., 2001) and a tetrameric lignin model compound (Hilden et al., 2000).

6.4 Conversion of Phenylcoumarane Compounds

Lignins contain 4—12% phenylcoumarane (β-5) linkages depending on the wood type (Henriksson, 2009). The metabolism of phenylcoumarane substructure has been investigated using a nonphenolic lignin model compound diarylpropane methyl dehydrodiconiferyl alcohol in the cultures of *P. chrysosporium* (Nakatsubo, Kirk, Shimada, & Higuchi, 1981). The degradation of this nonphenolic alkylated phenylcoumarane is initialized by oxidation in its side chain via a glycerol intermediate yielding phenylcoumarane-α'-aldehyde. The aldehyde is then oxidized to a phenylcoumarone and converted to a benzoic acid derivate (Figure 14(a)) (Nakatsubo et al., 1981). In contrast, in laccase-catalyzed degradation of phenolic model compound, the phenylcoumarane-α'-aldehyde was first hydroxylated or dehydrogenated in its coumarane ring to give the propiosyringone derivative and two coumarones (Figure 14(b)) (Umezawa, Nakatsubo, & Higuchi, 1982). This propiosyringone derivate was further converted to corresponding benzoquinone, syringic acid, and carboxyvanillic acid.

6.5 Conversion of Resinol Compounds

Resinol (β-β')-linked structures form only 2—4% of different lignins (Henriksson, 2009), and very little is known about their degradation pathway. Breakdown of a β-β' linked lignin model compound by an unidentified phenol oxidizing enzyme was reported for the plant pathogenic ascomycete *Fusarium solani* M-13-1 (Kamaya, Nakatsubo, Higuchi, & Iwahara, 1981). The model compound was first oxidized at the α-position of the side chain to give a hemiketal, an α-hydroxylated compound, which was then transformed to ketoalcohol (Figure 15). Subsequent aryl—alkyl oxidation yielded a carboxylic acid product and the corresponding lactone. When pinoresinol structures were degraded by the white-rot fungus *P. chrysosporium* under ligninolytic conditions, i.e., under low nutrient nitrogen with glucose as carbon source, the alkyl—aryl cleavage was also in this case the major reaction (Kamaya & Higuchi, 1984a).

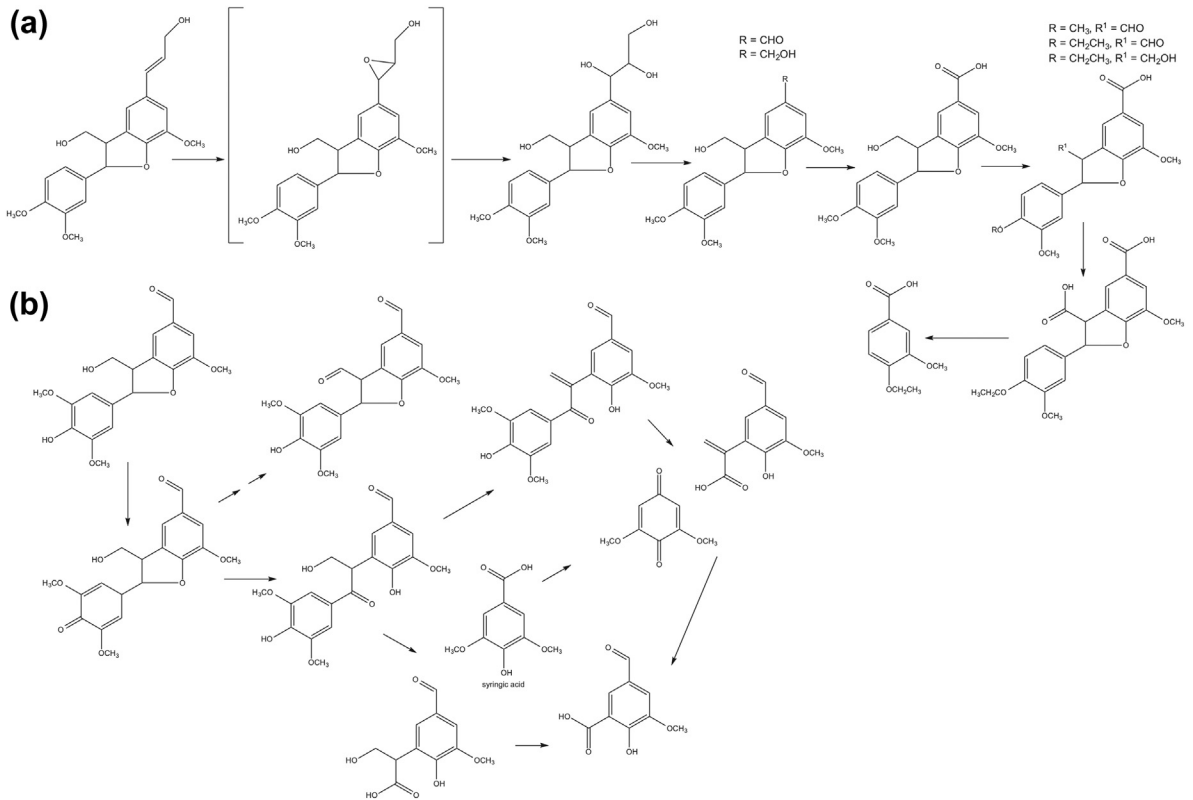


Figure 14 Conversion of two phenylcoumaran lignin model compounds by *P. chrysosporium*. The mechanism depends on the aliphatic group attached to the second aromatic ring, being either a C_3H_4OH (a) or a CHO (b) group. *Based on Nakatsubo et al. (1981), Umezawa et al. (1982).*

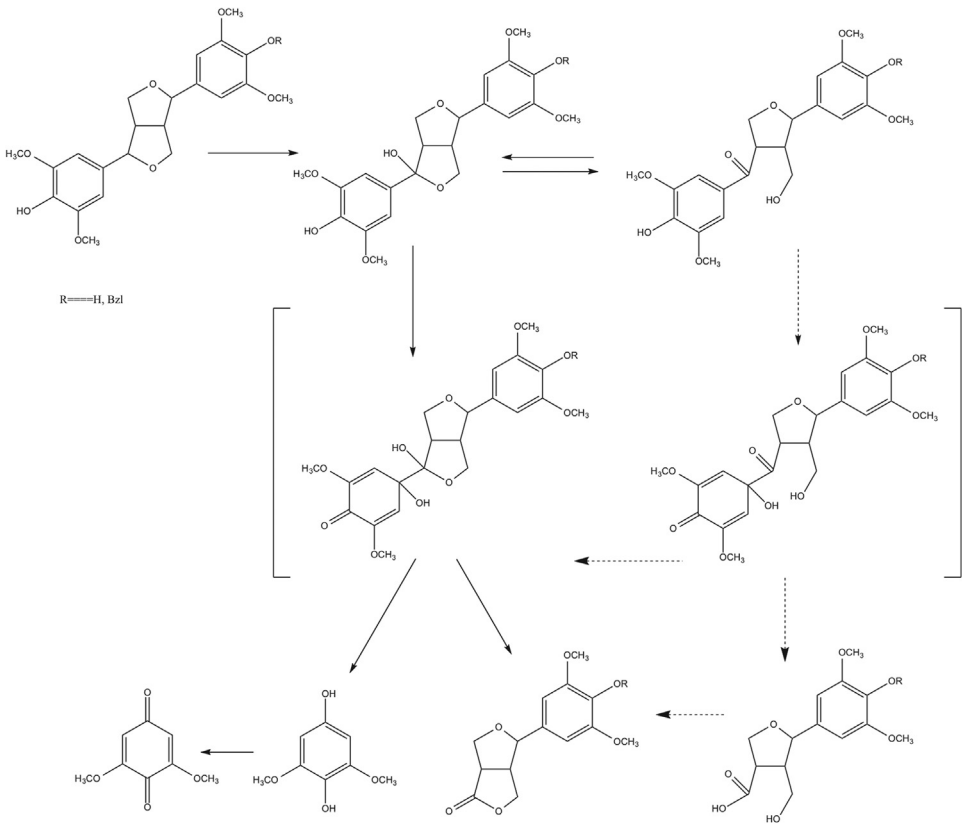


Figure 15 Conversion of a *d*,l-syringaresinol, a β-β' lignin model compound, by ascomycete *F. solani*. Bzl means that one of the phenolic OHs has been protected as its benzyl ether. *Redrawn based on Kamaya et al. (1981) with kind permission from Springer Science and Business Media.*

6.6 Conversion of a Lignin-Polysaccharide Model Compound by LiP

Although most lignin model compounds consist only of phenolic or nonphenolic structures related to lignin, in nature lignin is linked to hemicellulose. The synthesis of veratrylchitosan by covalently attaching 3(3,4-dimethoxybenzyloxy)propionic acid to chitosan provided a substrate to study the oxidizing abilities of LiP on a polysaccharide-linked lignin model compound. The analysis of the reaction demonstrated that LiP of *P. chrysosporium* was able to oxidize this substrate in a radical-mediated reaction (Figure 16) (Srebotnik et al., 1994).

7. AROMATIC METABOLISM IN FUNGI

Fungal aromatic metabolism has been studied mainly in the 1940s to 1990s, but has received little attention in recent years. Many of these studies focused on the analysis of the compounds that were formed by fungi from commonly found aromatic compounds such as ferulic acid and cinnamic acid. Wood-rotting basidiomycetes also produce *de novo* aromatic compounds from glucose such as veratryl alcohol (Harper, Buswell, Kennedy, & Hamilton, 1990; Lundquist & Kirk, 1978) and also other aromatic compounds (Hatakka, Lundell, Tervilä-Wilo, & Brunow, 1991; Rogalski, Niemenmaa, Uusi-Rauva, & Hatakka, 1996). Actually wood-rotting basidiomycetes have a great capacity to synthesize and produce different, often volatile aromatics such as vanillin, benzaldehyde, phenylacetaldehyde, 1-phenylethanone, and methyl benzoate and terpenoids. All these compounds have potential as flavoring compounds (Lomascolo et al., 1999). Although these studies were often focused on a specific part of aromatic metabolism and performed using a variety of fungi, together they enable the construction of a putative map for the main conversions of fungal aromatic metabolism (Figure 17). This scheme is initially based on a study of Milstein et al. with the ascomycete *Aspergillus japonicus* (Milstein et al., 1983). In this study metabolic conversions were depicted with a range of aromatic compounds providing the most first comprehensive aromatic metabolic map in fungi. This map has been combined with data from other fungi to result in the scheme presented in Figure 17. The evidence for the different conversions is shown in Figure 17 as well as some alternative/additional pathways are described in some detail below.

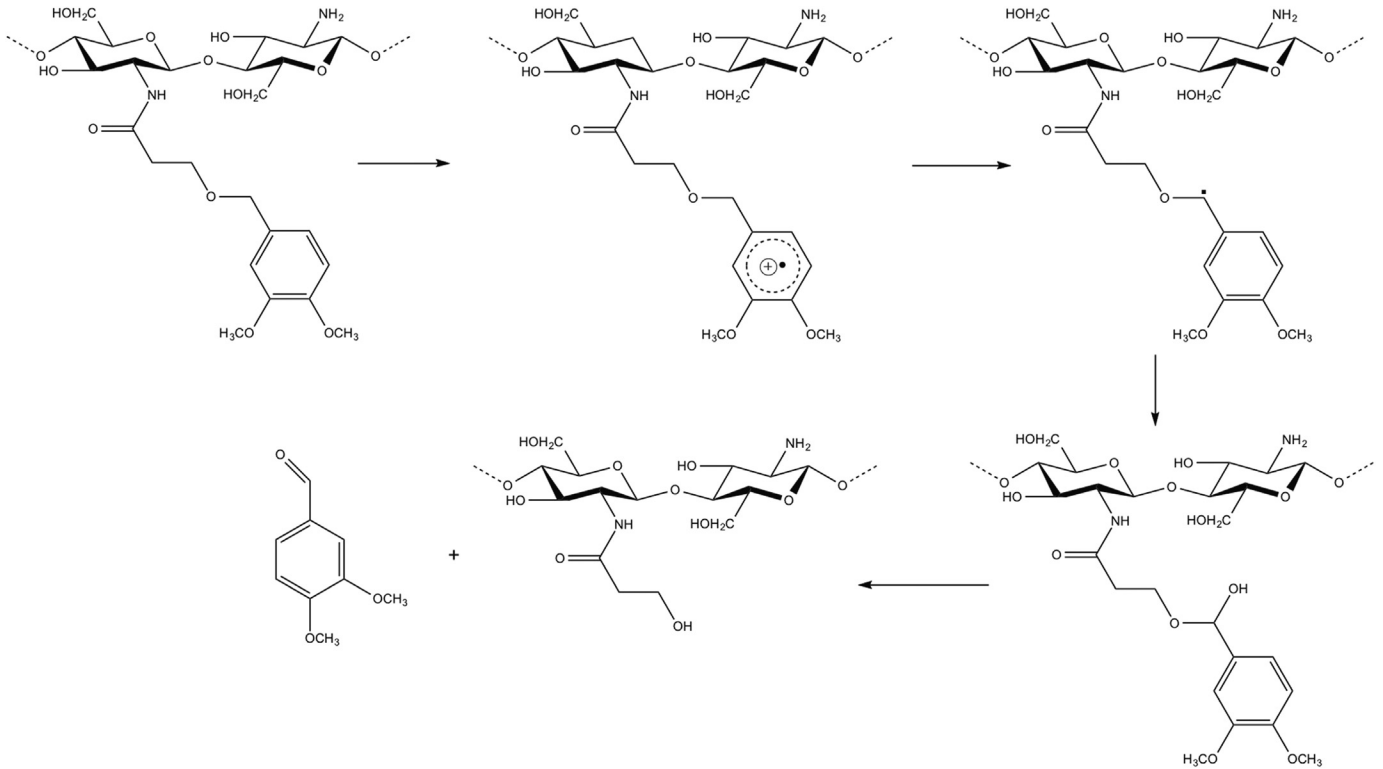


Figure 16 Oxidation of veratrylchitosan by *P. chrysosporium* LiP. *Based on D'Acuno and Lanzalunga (2004). Copyright (1994) National Academy of Sciences, USA.*

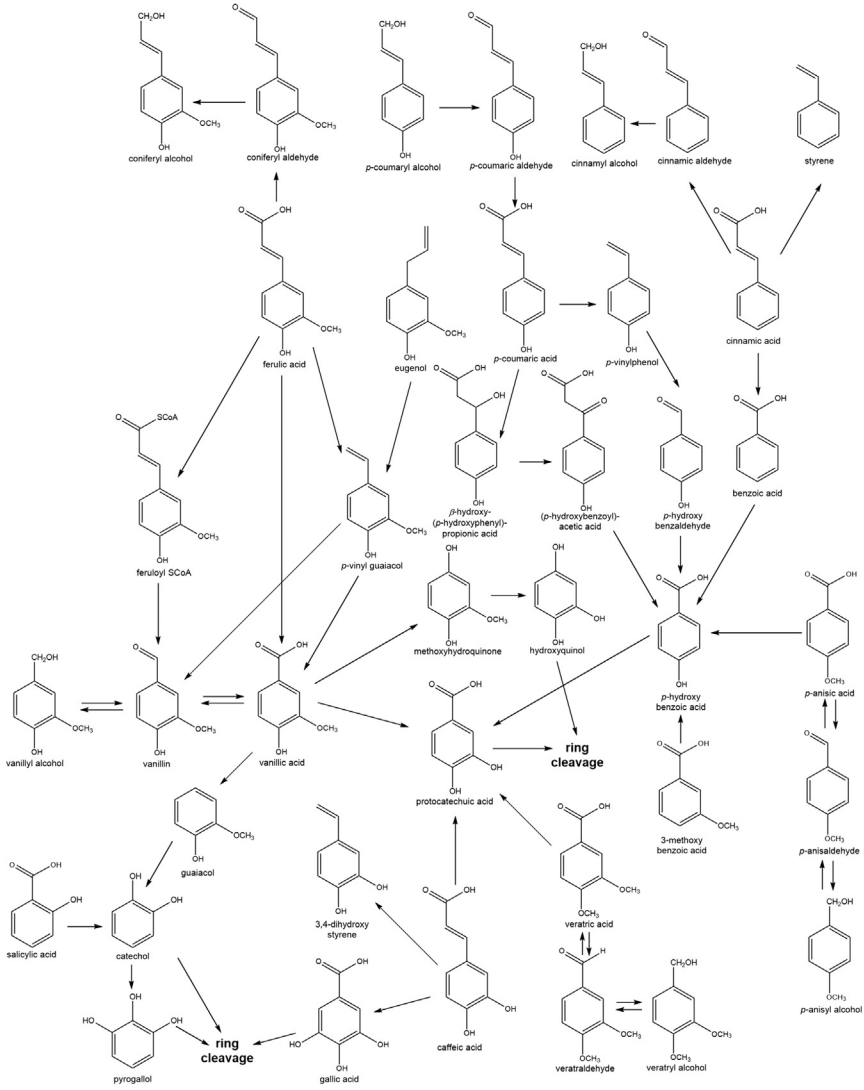


Figure 17 Aromatic metabolism in fungi. *Modified from Milstein et al. (1983) by including additional conversions that are described below.*

7.1 Ferulic Acid, Vanillic Acid and Vanillin and Their Conversion Products

Ferulic acid can reversibly be reduced to coniferyl aldehyde in the basidiomycete *Pycnoporus cinnabarinus*, which can be further converted into coniferyl alcohol (Figure 18) (Falconnier et al., 1994). This pathway was also

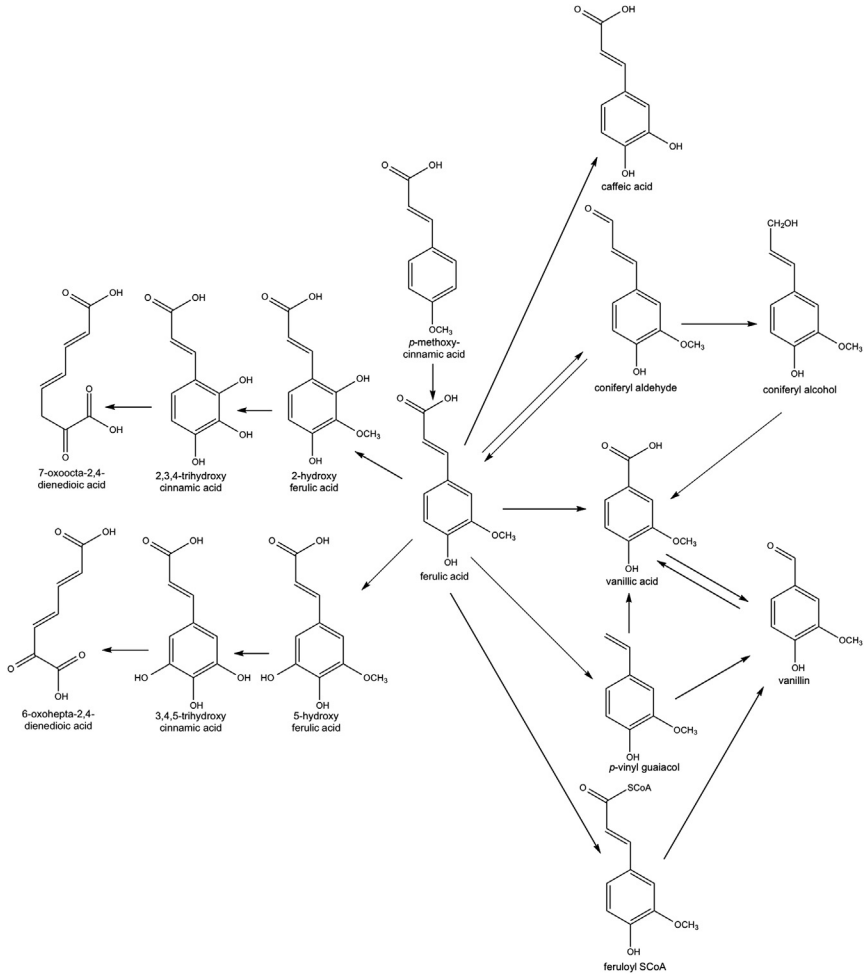


Figure 18 Fungal conversion of ferulic acid. *Combined from the references mentioned in the text of Section 7.1.*

observed in another basidiomycete, *Trametes* sp., in which coniferyl alcohol was further converted into vanillic acid, vanillyl alcohol, and methoxyhydroquinone (Nishida & Fukuzumi, 1978). In addition, the propenoic side chain of ferulic acid can be oxidized to form vanillic acid in *P. cinnabarinus*, which is then converted to vanillin (see below) (Falconnier et al., 1994). Both pathways were later confirmed by following the conversion of 5-^{2}H-labeled compounds (Krings, Pilawa, Theobald, & Berger, 2001). The conversion of ferulic acid into vanillic acid was also observed for the

ascomycete fungi *A. niger* (Lesage-Meessen et al., 1996) and *Botrytis*, *Cephalosporium*, *Penicillium*, *Trichoderma*, and *Verticillium* species (Henderson & Farmer, 1955), indicating that this metabolic step is present in both fungal phyla. An alternative pathway in which ferulic acid was decarboxylated into 4-vinyl guaiacol, followed by oxidation of this compound to vanillin and vanillic acid, was described in the basidiomycete *Schizophyllum commune* (Ghosh, Sachan, & Mitra, 2005; Tsujiyama & Ueno, 2008) and the ascomycetes *Paecilomyces variotii* (Rahouti, Seigle-Murandi, Steiman, & Eriksson, 1989) and *F. solani* (Nazareth & Mavinkurve, 1986). This pathway was also described for the ascomycete *Sporotrichum thermophile* (*Myceliophthora thermophila*), although it was suggested to convert 4-vinyl guaiacol into vanillic acid (Topakas, Kalogeris, Kekos, Macris, & Christakopoulos, 2003). In the ascomycete yeast *Debaromyces hansenii* the pathway from ferulic acid via 4-vinyl guaiacol to vanillin was also described, involving the enzyme ferulic acid decarboxylase for the first step and an oxidative two carbon fragmentation for the second step (Mathew, Abraham, & Sudheesh, 2007). This study also suggested a second metabolic pathway from ferulic acid to vanillin in this yeast, with feruloyl SCoA as an intermediate. In contrast, only trace amounts of vanillic acid were found for the white-rot basidiomycete *Sporotrichum pulverulentum*, an anamorph of *P. chrysosporium*, in the presence of ferulic acid (Gupta et al., 1981). The dominant pathway in this fungus appears to be toward coniferyl alcohol, whereas a demethylation pathway of ferulic acid to caffeic acid was described for a *Penicillium* species (Tillett & Walker, 1990).

Finally two different pathways for ferulic acid conversion were described for the edible white-rot fungus *L. edodes* (Crestini & Sermani, 1994). In these pathways, the hydroxylation of the aromatic ring occurred followed by ring cleavage, without the modification of the aliphatic side chain (Figure 18).

Vanillic acid is a naturally occurring aromatic acid, which is widely reported to be present in the extracts of wood that has undergone a various degree of microbial degradation (Ishikawa, Schubert, & Nord, 1963). It is found to be a major intermediate of hardwood lignin degradation (Chen & Chang, 1985). Vanillic acid is a common product when spruce lignin preparations are chemically oxidized (Adler, 1977). It was believed to be an intermediate in lignin degradation by fungi and bacteria (Ander et al., 1980), but it is possible that at least part of the lignin can be mineralized by ligninolytic peroxidases to carbon dioxide and water outside the fungal cell (Hatakka, 2001). Catabolism of vanillic acid may proceed via several routes: (1) demethylation of vanillic acid to protocatechuate which is followed by aromatic ring cleavage

(Cartwright & Buswell, 1967; Milstein et al., 1983), (2) nonoxidative decarboxylation to guaiacol (Crawford & Perkins Olson, 1978), (3) oxidative decarboxylation to methoxy-*p*-hydroquinone (MHQ) (Buswell, Ander, Pettersson, & Eriksson, 1979; Yajima, Enoki, Mayfield, & Gold, 1979), and (4) reduction to vanillin and vanillyl alcohol (Ander et al., 1980).

The demethylation of vanillic acid to protocatechuate has been detected in *A. japonicus* (Milstein et al., 1983). Nonoxidative decarboxylation to guaiacol has also been reported in ascomycetes, such as *S. thermophile* (Topakas et al., 2003), *P. variotii* (Rahouti et al., 1989), and some *Aspergilli* (Guiraud, Steiman, Seigle-Murandi, & Benoit-Guyod, 1992; Huang, Dostal, & Rosazza, 1993). As a result of nonoxidative decarboxylation, guaiacol is converted to catechol and pyrogallol before ring cleavage takes place (Figure 19). However, this appears to be a rarer pathway because so far it has only been reported in few ascomycetes species. In contrast, vanillic acid catabolism via MHQ seems to be widely distributed among white- and brown-rot fungi. Oxidative decarboxylation by the concomitant hydroxylation of vanillic acid is catalyzed by intracellular enzyme vanillate hydroxylase which has been described from the white-rot fungus *P. chrysosporium* and the activity has been detected in many white- and brown-rot species (Buswell &

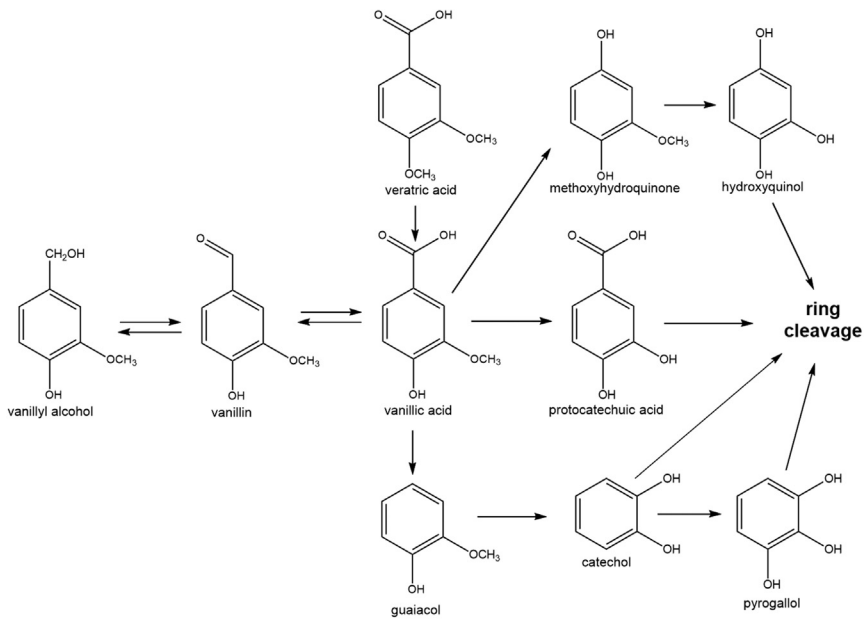


Figure 19 Conversion of vanillin and related compounds. *Based on references in the text above.*

Eriksson, 1988; Buswell, Eriksson, Gupta, Hamp, & Nordh, 1982; Yajima et al., 1979). However, in *S. pulverulentum* (*P. chrysosporium*) it was shown that MHQ needs to be hydroxylated before ring cleavage will take place, whereas demethoxylation is less important (Ander et al., 1980). Most of the studied white- and brown-rot fungi also had a dioxygenase activity that catalyzed the ring cleavage of hydroxyquinol (1,2,4-trihydroxybenzene) to maleylacetate (Buswell et al., 1982). When the production of $^{14}CO_2$ from ^{14}C-labeled methoxyl labeled vanillic acid or from a nonphenolic lignin model, β-O-4 dimer, [O^{14}CH$_3$]-labeled at position 4 in the A ring, was followed by white-rot (*P. radiata*) and brown-rot fungi (*Gloeophyllum trabeum* and *Poria* (*Postia*) *placenta*), it was found that *P. radiata* and *G. trabeum* readily mineralized the methoxyl group but *P. placenta* demethylated the dimer only poorly (Niemenmaa, Uusi-Rauva, & Hatakka, 2006; Niemenmaa, Uusi-Rauva, & Hatakka, 2008).

Vanillate hydroxylase has a wide substrate specificity including phenolic acids, such as 4-hydroxybenzoate, protocatechuate, and vanillate. Vanillic acid can also be decarboxylated to methoxy-*p*-quinone by the action of some other enzymes such as laccases and peroxidases. The product can further be reduced to MHQ either by intracellular NAD(P)H:quinone oxidoreductases (Buswell et al., 1979) or extracellularly by cellobiose:quinone oxidoreductase (Westermark & Eriksson, 1974). The latter enzyme is now referred to as cellobiose dehydrogenase (EC 1.1.99.18), which is classified in CAZy database to Auxiliary Activities family 3 and 8 (AA3_1, AA8).

No vanillate hydroxylase or hydroxyquinol 1,2-dioxygenase activity was detected for the a set of soft-rot (ascomycete) species, whereas protocatechuate 3,4-dioxygenase was detected, suggesting that vanillic acid conversion in these fungi occurs through the demethylation of the aromatic ring (Figure 19) (Buswell et al., 1982). The conversion of vanillic acid to protocatechuic acid has also been observed in other ascomycetes (Henderson, 1961) as well as some basidiomycetes, such as *S. commune* (Tsujiyama & Ueno, 2008). However, another study demonstrated the presence of MHQ as a product of vanillic acid in several ascomycetes, indicating that at least part of this phylum also possesses a vanillate decarboxylase (vanillate hydroxylase) (Guiraud et al., 1992). This was confirmed by a study with *P. variotii* (Rahouti et al., 1989), suggesting that the conversion to both MHQ and protocatechuic acid existed in the ancestral fungus before the ascomycetes and basidiomycetes split into two different phyla. After the split most fungi apparently maintained both pathways, whereas some species lost one or both of them (Haider & Trojanowski, 1975).

In some species, vanillin was described as an intermediate in the conversion of ferulic acid to vanillic acid, such as in basidiomycete *S. commune* (Ghosh & Nanda, 1994; Tsujiyama & Ueno, 2008), ascomycete *P. variotii* (Rahouti et al., 1989), and yeast *Debaromyces hansenii* (Mathew et al., 2007). In contrast, in other fungi the direct conversion of ferulic acid into vanillic acid and vanillin into vanillic acid have been described (Henderson & Farmer, 1955), as well as vanillic acid as an intermediate in the production of vanillin (Falconnier et al., 1994; Krings et al., 2001; Thibault et al., 1998; Tilay, Bule, & Annapure, 2010). Because this was usually based on the relative amounts of the two compounds and their conversion is reversible, it is difficult to say what the most common pathway is in fungi for vanillic acid and vanillin.

Veratric acid and related compounds became interesting when it was shown that veratryl alcohol was synthesized by the lignin-degrading white-rot fungus *P. chrysosporium* (Lundquist & Kirk, 1978), and it was suggested that veratryl alcohol may act as radical mediator in reactions catalyzed by LiP. Its significance in lignin degradation by other fungi producing LiP is not clear because they do not produce veratryl alcohol or the amounts are very low (Hatakka, 2001; Hatakka et al., 1991). However, veratric acid is both reduced to veratraldehyde and veratryl alcohol and demethoxylated to vanillic acid and further to vanillin and vanillyl alcohol by *P. cinnabarinus* (Hatakka, 1985). The whole genome of this fungus was recently sequenced which may elucidate the participating enzymes (Levasseur et al., 2014). The conversion of veratric acid in *A. japonicus* was suggested to go directly to protocatechuic acid and subsequent ring cleavage (Figure 17) (Milstein et al., 1983). However, in another study using *Penicillium* and two other ascomycetes, veratric acid was converted to vanillic acid (Figure 19) (Henderson, 1957), suggesting that in these fungi the vanillic acid pathway is also required for metabolizing veratric acid, similar to what was described for *P. cinnabarinus* (Hatakka, 1985). It is likely that veratric acid can also be converted to veratraldehyde and then to veratryl alcohol. In *P. chrysosporium* an alternative aromatic ring-cleavage pathway was reported for veratryl alcohol resulting in two isomeric products (Figure 20) (Leisola, Schmidt, Thanei-Wyss, & Fiechter, 1985).

7.2 Conversion of *p*-Coumaryl Alcohol and *p*-Coumaric Acid

p-Coumaryl alcohol is one of the components of lignin and can converted in two steps to form *p*-coumaric acid through *p*-coumaric aldehyde in *Aspergillus flavus* (Iyayi & Dart, 1982). *p*-Coumaric acid is subsequently in three

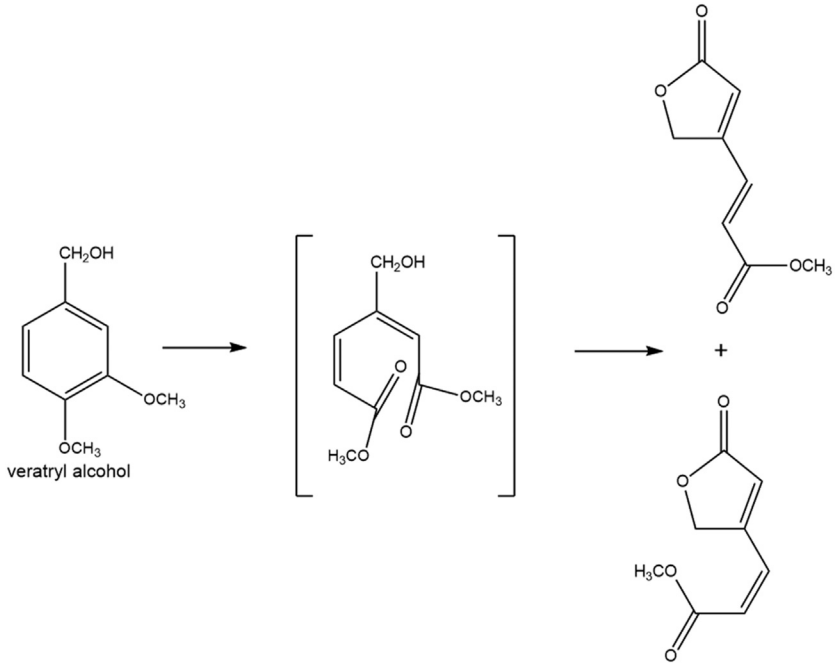


Figure 20 Alternative aromatic ring cleavage through veratryl alcohol. *Redrawn based on Leisola et al. (1985) with permission from Elsevier.*

steps converted to *p*-hydroxybenzoic acid with β-hydroxy-(*p*-hydroxyphenyl)-propionic acid and (*p*-hydroxybenzyl)acetic acid as intermediates (Figure 21).

An alternative pathway was reported for *P. variotii*, in which *p*-coumaric acid was converted in two steps into *p*-hydroxybenzaldehyde, which was subsequently converted into *p*-hydroxybenzoic acid and then to protocatechuic acid (Sachan, Ghosh, & Mitra, 2006). This pathway was also reported for the ascomycete *F. solani* in which the initial reaction product of *p*-coumaric acid was identified as *p*-vinyl-phenol (Nazareth & Mavinkurve, 1986).

In the basidiomycete *P. cinnabarinus* a more diverse set of metabolites were identified when *p*-coumaric acid was added to the growth medium, suggesting a different metabolism than in the ascomycetes described above (Estrada Alvarado et al., 2001). The oxidative degradation of the *p*-coumaric acid side-chain resulted in *p*-hydroxybenzoic acid. This compound was then either reduced to *p*-hydroxybenzaldehyde and *p*-hydroxybenzoic alcohol or alternatively hydroxylated and reduced to the protochatechuic acid derivatives. The formation of *p*-hydroxybenzoic acid from *p*-coumaric acid in *S.*

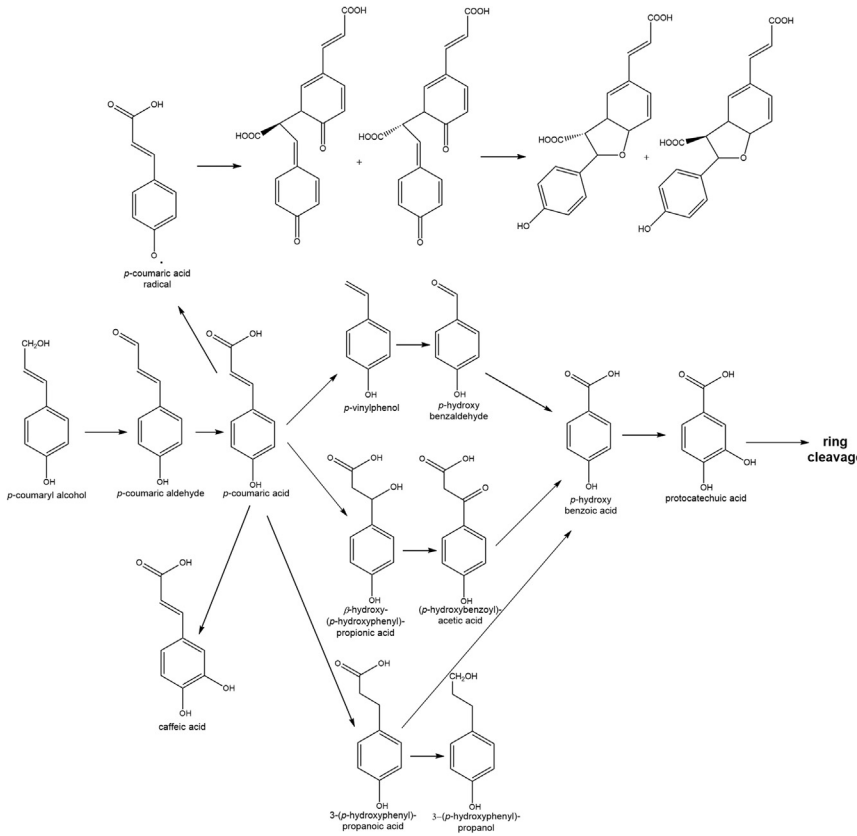


Figure 21 Fungal conversion of *p*-coumaric acid and related aromatic compounds. *Based on references of Section 7.2.*

commune (Sachan, Ghosh, & Mitra, 2010) and *Polyporus* (*Inonotus*) *hispidus* (French, Vance, & Towers, 1976) suggests at least a partly similar pathway for this basidiomycete as for *P. cinnabarinus*.

Two alternative pathways were reported for *p*-coumaric acid conversion in *P. cinnabarinus*. One is a second reductive pathway resulting in 3-(*p*-hydroxyphenyl)-propanol (Estrada Alvarado et al., 2001), whereas the other is a hydroxylation to form caffeic acid (Estrada-Alvarado, Navarro, Record, Asther, & Asther, 2003). The later pathway was also described for the ascomycete *Gliocladium deliquescens* (Torres & Rosazza, 2001).

The dimerization of *p*-coumaric acid was observed by the ascomycete fungus *Curvularia lunata* and a mechanism for the formation of this compound was suggested (Figure 21) (Torres & Rosazza, 2001). The presence

of laccase and peroxidase activities in this fungus led the authors to suggest a radical-based coupling process.

7.3 Conversion of Cinnamic Acid and its Methoxylated Derivatives

In *A. niger* cinnamic acid is converted to styrene (Clifford, Faulkner, Walker, & Woodcock, 1969; Plumridge, Stratford, Lowe, & Archer, 2008) (Figure 22). This conversion was also reported for *A. japonicus*, but two other pathways were also suggested in this fungus, one to benzoic acid and one to cinnamic aldehyde, which was then converted to cinnamyl alcohol (Milstein et al., 1983).

Different pathways have been described for the conversion of methoxylated cinnamic acids. In *Lentinula edodes* 3,4-dimethoxycinnamic acid is

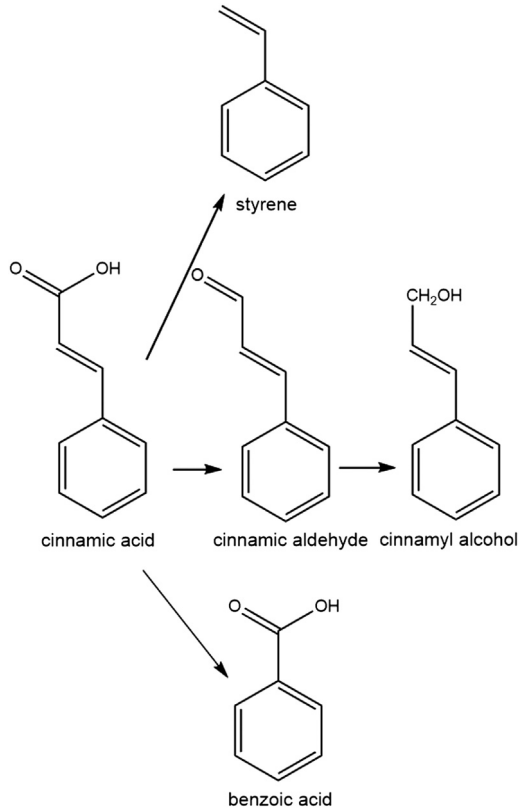


Figure 22 Fungal conversion of cinnamic acid. *Based on Clifford et al. (1969), Milstein et al. (1983), Plumridge et al. (2008).*

converted into ferulic acid or 3,4-dimethoxycinnamic aldehyde, whereas *p*-hydroxycinnamic acid is also converted into ferulic acid (Crestini & Sermani, 1994). The pathway through 3,4-dimethoxycinnamic aldehyde was also described for *P. radiata* (Cho et al., 2009) and *Coriolus versicolor* (Kamaya & Higuchi, 1984b), which then proceeds through 3,4-dimethoxycinnamyl alcohol, followed by the removal of the aliphatic chain and further reduction to 3,4-dimethoxy-benzyl alcohol, followed by ring cleavage (Figure 23). In addition, a second pathway for 3,4-dimethoxycinnamic acid conversion was described in this study, consisting of modification of the aliphatic chain, followed by the simultaneous demethoxylation and hydroxylation and removal of the aliphatic chain resulting in 2-methoxy phenol, after which ring cleavage takes place.

The hypothetical pathway for the degradation of substituted cinnamic acids in white-rot fungus *P. radiata* occurs via 3,4-dimethoxystyrene or 3-methoxy,4-hydroxystyrene to vanillate (Cho et al., 2009) which further goes through decarboxylation, aliphatic chain cleavage, demethylation in position 4, followed by the demethylation in the position 3, and final step of ring cleavage (Rogalski, 2003). *C. versicolor* growing in the cultures with low nitrogen and high oxygen, oxidized 3,4-dimethoxycinnamic alcohol to veratrylglycerol, which was converted by the $C_\alpha - C_\beta$ cleavage of the side chain to the veratrylaldehyde, and subsequently reduced to veratryl alcohol (Kamaya & Higuchi, 1984a).

7.4 Conversion of Benzoic Acid and Related Compounds

In most fungi benzoic acid is hydroxylated to *p*-hydroxybenzoic acid, which is then also hydroxylated to protochatechuic acid, resulting in ring cleavage and the β-ketoadipate pathway (Figure 24) (Wright, 1993). The first step of this pathway is catalyzed by benzoate-*para*-hydroxylase (BphA) in *A. niger* (van Gorcom et al., 1990), and cytochrome P450 monooxygenases (see Section 7.7.1) in *Cochliobolus lunatus* (Korosec et al., 2013), *P. chrysosporium* (Matsuzaki & Wariishi, 2005) and *Rhodotorula minuta* (Fukuda, Nakamura, Sukita, Ogawa, & Fujii, 1996). *P. chrysosporium* enzyme (PcCYP1f) also catalyzes the conversion of 3-hydroxybenzoic acid into protocatechuate. *A. niger* BphA differs from the other enzymes being tetrahydropteridine dependent. It acts together with a cytochrome P450 reductase (CprA) (van den Brink, van den Hondel, & van Gorcom, 1996) and both *bphA* and *cprA* are induced by benzoate (van den Brink, Punt, van Gorcom, & van den Hondel, 2000). The analysis of the expression of *bphA* in the presence of a range of aromatic compounds (de Vries et al., 2002) demonstrated that

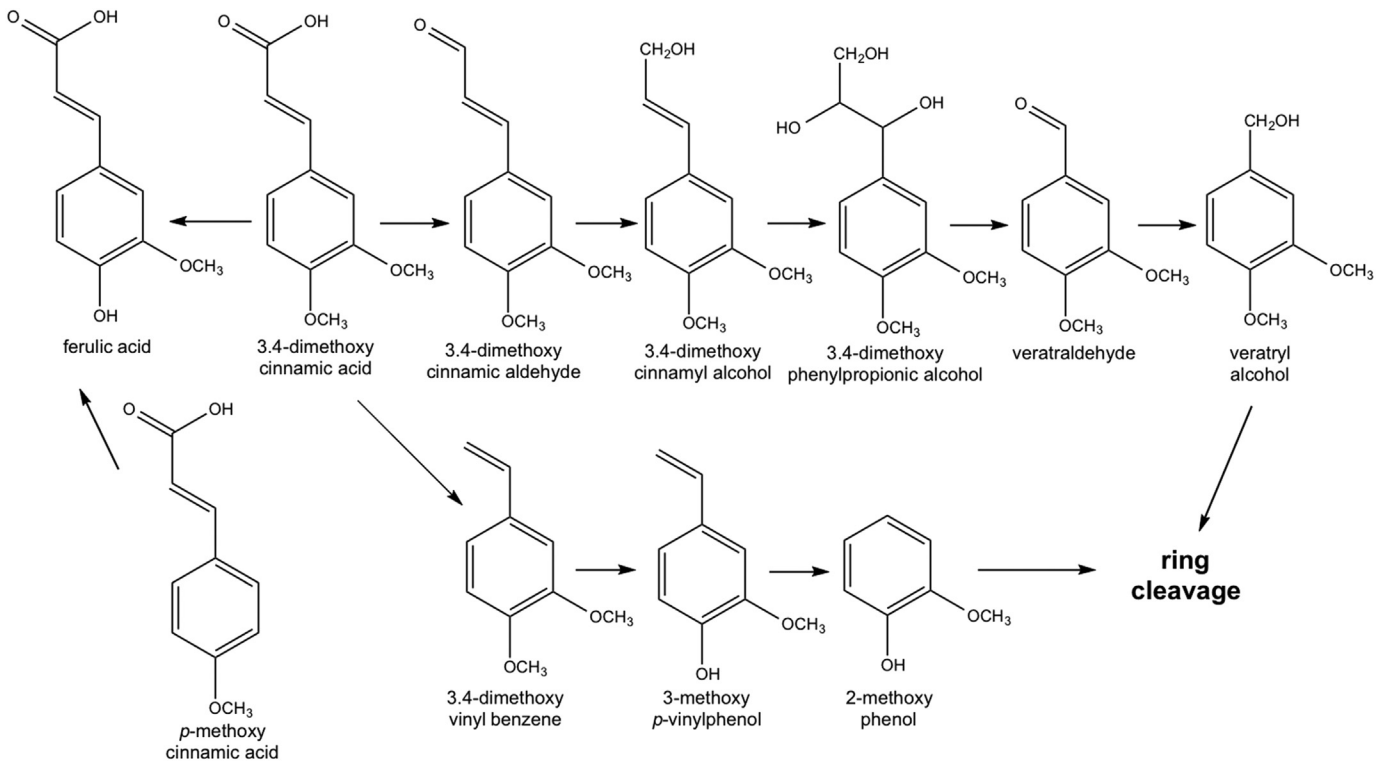


Figure 23 Fungal conversion of methoxylated cinnamic acids. *Based on Cho et al. (2009), Crestini and Sermani (1994), Kamaya and Higuchi (1984b).*

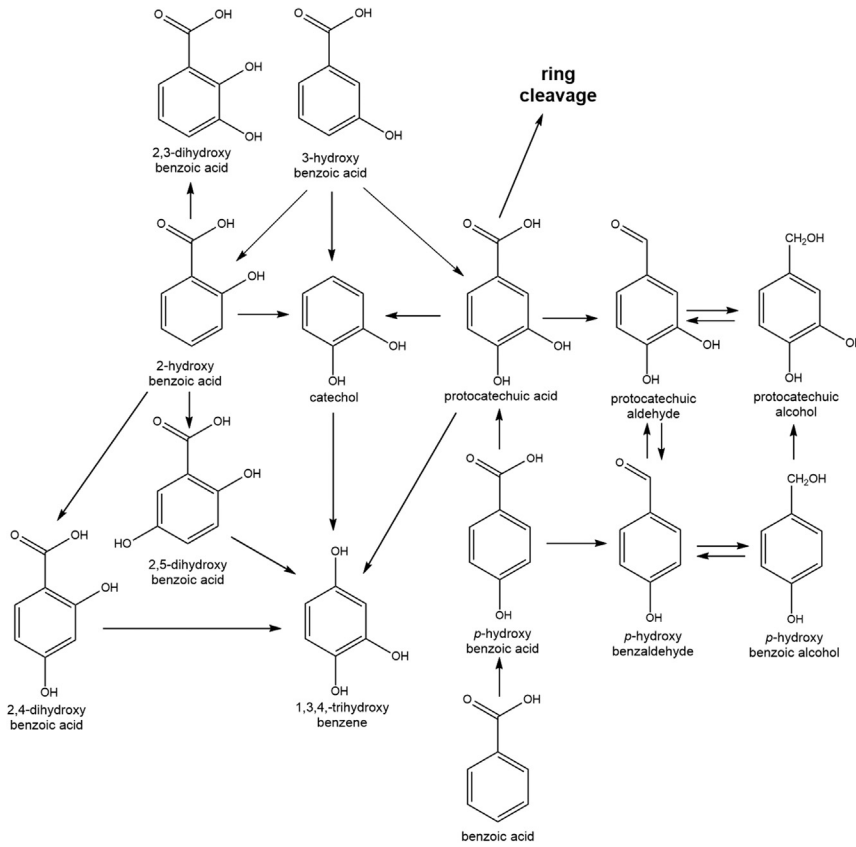


Figure 24 Fungal conversion of benzoic acid and related compounds. *Based on Cain et al. (1968), Halsall et al. (1969), Wright (1993).*

it was specifically expressed in the presence of compounds that were all suggested to be metabolized through *p*-hydroxy benzoic acid in *A. japonicus* (Milstein et al., 1983), a close relative of *A. niger*.

Additional pathways for the conversion of benzoic acid related compounds have also been described and were reviewed by Wright (Wright, 1993) (Figure 24). These additional conversions included enzymes of the catechol pathway (Cain, Bilton, & Darrah, 1968), but also other metabolic activities. The conversion of *p*-hydroxybenzoate to 3,4-dihydrooxybenzoate was described for *A. niger*, several Penicillia, and *Schizophyllum commune* (Halsall, Darrah, & Cain, 1969), and this pathway was also reported in several other fungi as were conversions of other hydroxylated benzoic acid compounds (Wright, 1993). Also unspecific peroxygenases (UPO;

EC 1.11.2.1) are able to hydroxylate benzene and toluene to respective hydroxylated compounds (Hofrichter & Ullrich, 2014).

7.5 Conversion of Sinapic Acid and Syringic Acid and Related Compounds

The conversion of syringic acid has been studied in detail in the white-rot basidiomycete *Sporotrichum pulverulentum* (an anamorph of *P. chrysosporium*) (Eriksson, Gupta, Nishida, & Rao, 1984). This pathway involves the reduction of the carboxyl group, simultaneous decarboxylation and hydroxylation, and demethylation and methylation (Figure 25). These conversions were also investigated in two (ascomycete) soft-rot fungi and two brown-rot fungi, demonstrating significant differences in metabolic ability (Eriksson et al., 1984). The two soft-rot fungi were able to methylate the *p*-hydroxyl group, but this was not observed for the two brown-rot fungi, who overall poorly metabolized syringic acid.

The conversion of sinapic acid to syringic acid was reported for *P. variotii* (Mukherjee, Sachan, Ghosh, & Mitra, 2006). In this pathway, sinapic acid was first converted to syringic aldehyde, which was oxidized to syringic acid (Figure 25). The accumulation of syringic acid suggests that this fungus is not capable of converting syringic acid further as was described above for *S. pulverulentum*. The conversion of syringic aldehyde to syringic acid appears to be a common pathway for many ascomycete soil fungi, as they commonly accumulate syringic acid in the presence of the aldehyde (Henderson & Farmer, 1955).

Recently, it was reported that *A. oryzae* can convert gallic acid to progallin A, pyrogallic acid, and methyl gallate (Guo et al., 2014). Progallin A and methyl gallate were also suggested to be converted to pyrogallic acid and all three metabolites could result in ring cleavage through the β-oxidation pathway.

7.6 Ring Cleavage of Aromatic Compounds

Most fungal aromatic pathways end either in protocatechuic acid or catechol. These compounds can undergo ring cleavage and conversion through the well-studied β-ketoadipate pathway (Figure 26) (Cain et al., 1968; Fuchs, Boll, & Heider, 2011; Gross, Gafford, & Tatum, 1956; Harwood & Parales, 1996; Henderson, 1963). Catechol is converted in four steps to β-ketoadipate through the sequential action of catechol-1,2-dioxgenase, *cis*,*cis*-muconate lactonizing enzyme, muconolactone isomerase, and enol-lactone hydrolase. The conversion of protocatechuate to β-ketoadipate occurs in

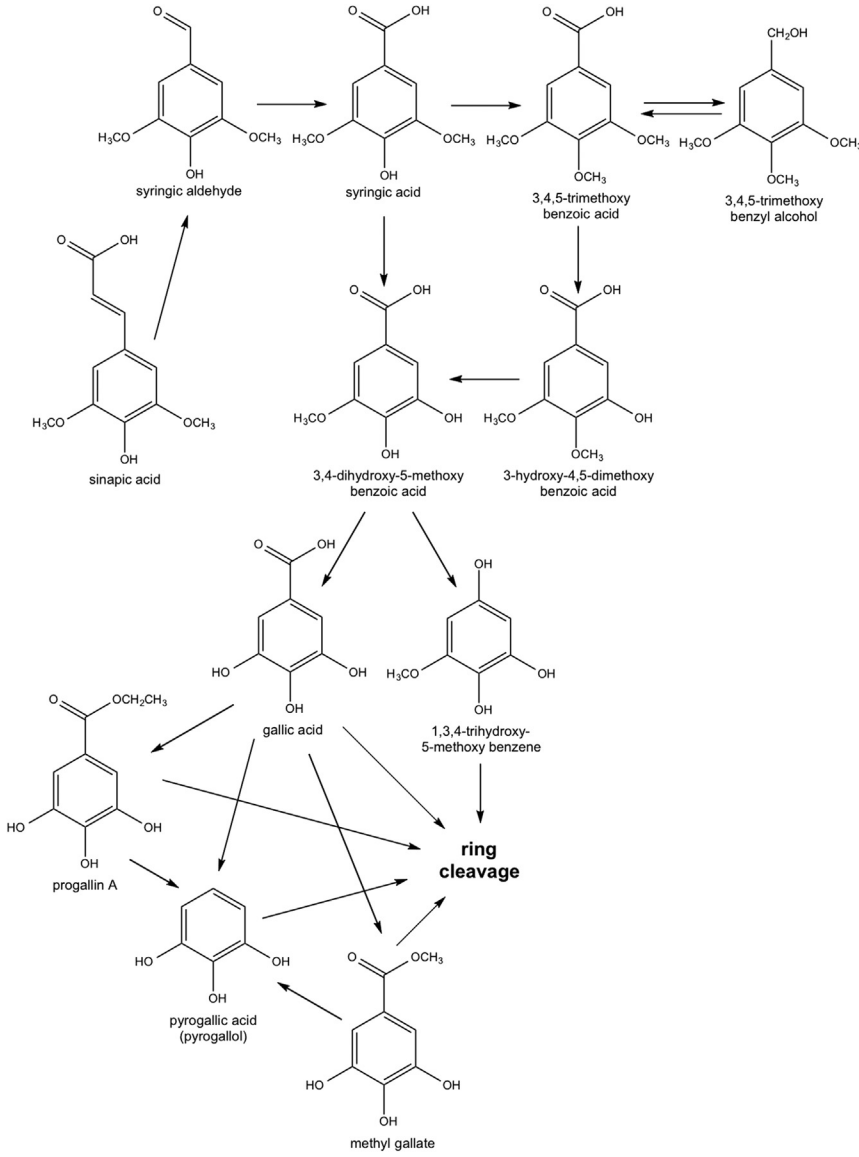


Figure 25 Fungal conversion of sinapic and syringic acid. *Based on Eriksson et al. (1984), Henderson and Farmer (1955), Mukherjee et al. (2006).*

three steps mediated by protocatechuate-3,4-dioxygenase (Wojtas-Wasilewska & Trojanowski, 1980), β-carboxy-*cis*,*cis*,-muconate lactonizing enzyme, and γ-carboxymucanolactone decarboxylase. β-Ketoadipate is then converted to β-ketoadipyl-CoA by β-ketoadipate:succinyl-CoA

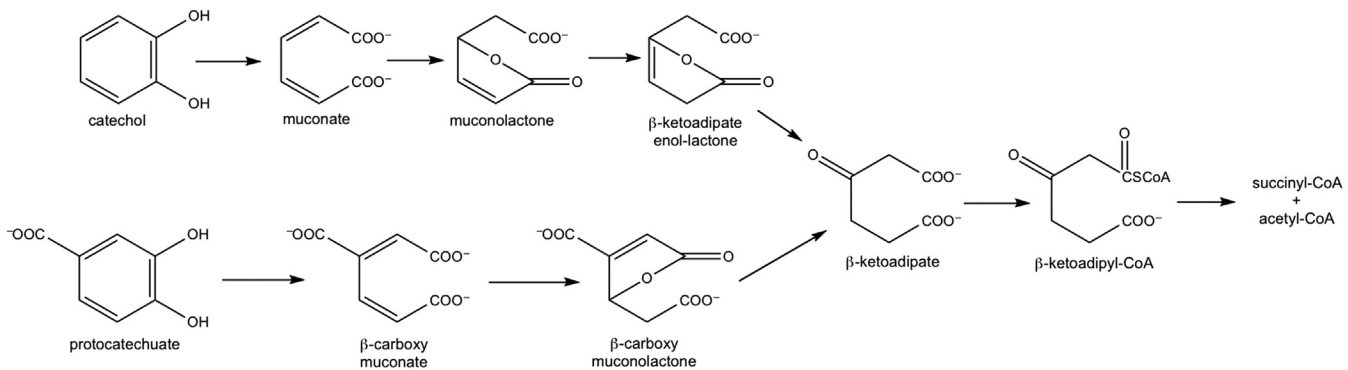


Figure 26 The fungal β-ketoadipate pathway. *Based on Cain et al. (1968), Fuchs et al. (2011), Gross et al. (1956), Harwood and Parales (1996), Henderson (1963).*

transferase, which is subsequently converted to succinyl-CoA and acetyl-CoA by β-ketoadipyl-CoA thiolase.

7.7 Candidate Enzymes Involved in Aromatic Metabolism

So far, very few enzymes and genes involved in aromatic metabolism in fungi have been characterized. Some have been mentioned above (e.g., vanillate hydroxylase, benzoate-*para*-hydroxylase) and here some additional enzymes that have been implicated in aromatic metabolism are mentioned.

7.7.1 Cytochrome P450 Enzymes

Cytochrome P450 monooxygenases (P450s) are intracellular enzymes that are involved in aromatic conversions (Matsuzaki & Wariishi, 2004). They belong to the superfamily of heme-thiolate proteins and are able to catalyze various enzymatic reactions including the metabolism of aliphatic, alicyclic, and aromatic molecules in reactions resulting in hydroxylation, epoxidation, dealkylation, sulfoxydation, deamination, desulphuration, dehalogenation, and *N*-oxide reduction (Sono, Roach, Coulter, & Dawson, 1996). P450s are ubiquitous enzymes found in all life forms. Although, the highest number of individual P450 enzymes so far has been observed in plants (Mao, Seebeck, Schrenker, & Yu, 2013), putative P450 enzyme-encoding genes are also abundantly present in the wood-decaying basidiomycetes (Chen et al., 2014; Doddapaneni, Chakraborty, & Yadav, 2005; Eastwood et al., 2011; Ide, Ichinose, & Wariishi, 2012; Martinez et al., 2004; Martinez et al., 2009). For instance, the 149 putative P450 monooxygenases-encoding genes comprise about 1% of the coding genome of the white-rot fungus *P. chrysosporium* (Doddapaneni et al., 2005; Martinez et al., 2004). It is assumed that the high number of P450 isoforms found in wood-decaying fungi could reflect the ability of these fungi to metabolize and mineralize aromatic compounds resulting from the extracellular oxidation of wood. The whole-genome-based observations imply the involvement of multiple P450 monooxygenases in lignin-degrading process, with the hypothesis that these intracellular or membrane-bound monooxygenases catalyze the subsequent oxidation of the peroxidase-depolymerized lignin derivatives leading to the complete mineralization of lignin to CO_2 (Subramanian & Yadav, 2008).

7.7.2 Glutathione S-Transferases

Similarly to cytochrome P450 monooxygenases, glutathione *S*-transferases (GSTs, EC 2.5.1.18) are intracellular enzymes suggested to be involved in different detoxification processes in fungal cells. GSTs are also involved in

stress responses and defense systems, and some bacterial GSTs are known to have β-etherase activity (i.e., catalyze the cleavage of β-O-4 linkages) (Morel, Ngadin, Droux, Jacquot, & Gelhaye, 2009). In fungi, multiple groups of GSTs have been identified and the group of etherase-like GSTs is named fungal specific GST class A (GSTFuA) (Mathieu et al., 2012). In proteomic studies of white-rot fungus *P. chrysosporium*, the upregulation of GSTFu-encoding gene has been detected in response to vanillin. As vanillin is one of the key intermediate products found during lignin degradation, GSTFus may have a role in fungal cleavage of β-O-4 linkages (Shimizu, Yuda, Nakamura, Tanaka, & Wariishi, 2005). Wood-degrading fungi harbor from 4 up to 20 GSTFu-encoding genes (Morel, Meux, Mathieu, Thuillier, Chibani, Harvengt et al., 2013). Interestingly, also ectomycorrhizal fungi possess GSTFu isoforms (from 3 to 10). However, the ability of GSTFus to cleave β-O-4 linkages has not been studied.

7.7.3 Alcohol Oxidases

Alcohol oxidases have been implicated in aromatic metabolism in fungi. Extracellular aryl-alcohol oxidases (AAO; EC 1.1.3.7) from CAZy family AA3_2 generate H_2O_2 in collaboration with intracellular aryl-alcohol dehydrogenases in redox cycling of aromatic fungal metabolites, including lignin-derived compounds *p*-anisaldehyde such as phenolic aromatic aldehydes and acids (Ferreira et al., 2005; Guillén, Martínez, Martínez, & Evans, 1994; Gutierrez, Caramelo, Prieto, Martinez, & Martinez, 1994; Hernandez-Ortega, Ferreira, & Martinez, 2012; Kirk & Farrell, 1987; Shimada & Higuchi, 1991).

AAO is a FAD dependent enzyme, which has been identified in several white-rot fungal species and ascomycetes, such as *Amauroderma boleticeum*, *Bjerkandera adusta*, *P. chrysosporium* and *Pleurotus* species, *Fusarium* spp. and *Geotrichum candidum* (Asada, Watanabe, Ohtsu, & Kuwahara, 1995; Farmer, Henderson, & Russell, 1960; Muheim, Waldner, Leisola, & Fiechter, 1990; Rosazza, Huang, Dostal, Volm, & Rousseau, 1995; Saparrat, Guillen, Arambarri, Martinez, & Martinez, 2002).

Intracellular vanillyl-alcohol oxidases (VAO; EC 1.1.3.38) catalyze the oxidation of *p*-hydroxybenzyl alcohols to the corresponding aldehydes, such as vanillyl alcohol into vanillin (Furukawa, Wieser, Morita, Sugio, & Nagasawa, 1999; Pannala, Razaq, Halliwell, Singh, & Rice-Evans, 1998). In addition, it also can perform the deamination of 4-hydroxybenzylamines and the oxidative demethylation of 4-(methoxymethyl) phenols. A gene encoding this enzyme was first described in *Penicillium simplicissimum* (Benen et al., 1998). Interestingly, a novel oxidase has been described from *B. adusta*

that combines the catalytic properties of VAO and AAO by converting both phenolic and nonphenolic benzyl alcohols (Romero et al., 2009).

7.7.4 O-Methyl Transferase

O-methyl transferase is an intracellular enzyme that converts methyl-*p*-coumarate to methyl-*p*-methoxycinnamate and that was first reported for the basidiomycete *Lentinus lepideus* (Shimazono, 1959). It is specific for methyl-esters of hydroxycinnamic acids and cannot convert free cinnamic acids, or free benzoic acids or methyl-esters. It only converts the OH group linked to the aromatic ring at the *para* position, and its activity reduces when additional OH groups are present at other positions of the aromatic ring (Wat & Towers, 1975). Wood-rotting fungi synthesize methylated aromatic compounds, e.g., veratryl alcohol *de novo* from glucose, but it was found that the white-rot fungi *P. chrysosporium*, *P. radiata*, and *Coriolus versicolor* use chloromethane (CH_3Cl) as methyl donor in these reactions (Harper et al., 1990). Two types of *O*-methyl transferases utilizing *S*-adenosyl methionine were purified from mycelial extracts of *P. chrysosporium,* an enzyme methylating phenolic compounds in *para* (Jeffers, McRoberts, & Harper, 1997) or and another enzyme in *meta* position (Coulter, Kennedy, McRoberts, & Harper, 1993).

8. APPLICATIONS OF PLANT-BASED AROMATIC COMPOUNDS

Despite the large annual worldwide production of plant biomass only small quantities of chemicals are derived from renewable resources (Lucia, Argyropoulos, Adamopoulos, & Gaspar, 2006). The commercial use of the polysaccharide fraction of plant biomass is well established, but the aromatic components (e.g., lignin) are less well used. However, lignin is a versatile raw material with many (potential) applications. It can be used (1) as carbon source for energy production or is converted in energy carriers such as syngas and syngas products (methanol, ethanol, mixed alcohols); (2) in high molecular mass applications such as carbon fibers, wood adhesives (binders), and pharmaceuticals; (3) for production of polymer building blocks and low molecular weight chemicals such as benzene, toluene, xylene, phenol, guaiacol, vanillic acid, and vanillin (Gandini, 2011; Sampaio, 1995). The latter two groups are considered to be value-added lignin applications. Lignin is especially interesting from the industrial point of view because it is, up till now the only

renewable resource available in sufficient quantities for the production of aromatics. This is an attractive but also a very challenging goal to achieve. The recalcitrance of lignin is one of the major obstacles in biofuel production process and therefore the microbial degradation of lignin is receiving a great deal of attention. Plant-based aromatic compounds are receiving increasing attention in several applications in industry, in particular, in the pharmaceutical industry. The best-studied role of plant-based phenolic compounds is as natural antioxidants (Cai et al., 2004a; Surveswaran et al., 2007; Wojdylo et al., 2007). Ferulic acid is one of the best studied aromatic compounds for this purpose, due to its abundance in nature (Graf, 1992; Kanski, Aksenova, Stoyanova, & Butterfield, 2002; Pannala et al., 1998; Schroeter, Williams, Matin, Iversen, & Rice-Evans, 2000). However, other beneficial effect of plant-based aromatic compounds have also been reported, such as a role as antimicrobial, anti-inflammatory, hepatoprotective, antidiabetic, anticholesterolemic, neuroprotective, anticarcinogenic, ultraviolet (UV) protective, and radioprotective agents (de Paiva et al., 2013). For instance, flavonoids have been shown to have antioxidant, anticancer, antiallergic, anti-inflammatory, anticarcinogenic, and gastroprotective properties (Yao et al., 2004), whereas ferulic acid inhibited the growth of colon cancer cells (Hudson, Dinh, Kokubun, Simmonds, & Gescher, 2000; Mori et al., 1999). The skin-protection effect of ferulic acid is also considered desirable from a cosmetic affect, as the UV-protective effect of ferulic acid is applied in sunscreens and whitening agents (Murray et al., 2008).

Modified versions of natural phenolics can have even better properties than their natural starting compound. A modified form of ferulic acid has better properties with respect to the suppression of inflammatory responses and skin tumor promotion (Murakami et al., 2002), whereas ferulic acid and gallic acid based polyphenols inhibit Epstein—Barr virus activation (Nomura et al., 2002). As an alternative to chemical synthesis, the heterologous production of phenolic-based pharmaceuticals has already been performed in yeast (Huang et al., 2008).

The role of ferulic acid as an antioxidant also has applications in the food industry, such as in the prevention of discoloration of Red Sea bream (Maoka et al., 2008), but in this sector it is best known as a precursor for flavor compounds, such as vanillin (Lesage-Meessen et al., 2002). However, ferulic acid and other monomeric plant-based aromatics can be starting compounds for the production of a range of valuable molecules such as styrenes, polymers, alkylbenzenes, and catechols (Rosazza et al., 1995).

9. CONCLUDING REMARKS

The high potential of plant-based aromatics for applications in the pharmaceutical, chemical, and food industry has resulted in an increasing interest in the microbial (in particular fungal) release of these compounds from plant biomass. We have a reasonable understanding of the release of aromatic compounds from common plant-based polymers, such as the role of various peroxidases in lignin degradation and feruloyl esterases in releasing aromatic compounds from plant cell wall polysaccharides, which is further enhanced by the availability of a rapidly increasing number of fungal genomes. In contrast, relatively little is known about the fungal enzymes and genes involved in the conversion of monomeric aromatics and many of the industrial conversions are still based on chemical processes.

The push to a biobased economy and society is stimulating the development of microbial conversions as alternatives for existing chemical processes. To also apply this to the field of aromatic conversion, significant effort should be devoted to the identification of the genes involved in fungal aromatic pathways, their regulation and the variety of compounds that can be made by them.

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REFERENCES

Adler, E. (1977). Lignin chemistry — past, present and future. *Wood Science and Technology, 11*, 169—218.

Agarwal, U. P., & Atalla, R. H. (1986). In-situ Raman microprobe studies of plant cell walls: macromolecular organization and compositional variability in the secondary wall of *Picea mariana* (Mill.) B.S.P. *Planta, 169*, 325—332.

Aguilar, C. N., Augur, C., Favela-Torres, E., & Viniegra-Gonzalez, G. (2001). Production of tannase by *Aspergillus niger* Aa-20 in submerged and solid-state fermentation: influence of glucose and tannic acid. *Journal of Industrial Microbiology and Biotechnology, 26*, 296—302.

Aguilar, C. N., Rodriguez, R., Gutierrez-Sanchez, G., Augur, C., Favela-Torres, E., Prado-Barragan, L. A., et al. (2007). Microbial tannases: advances and perspectives. *Applied Microbiology and Biotechnology, 76*, 47—59.

Akin, D. E. (2008). Plant cell wall aromatics: influence on degradation of biomass. *Biofuels, Bioproducts and Biorefining, 2*, 288—303.

Alexandre, G., & Zhulin, I. B. (2000). Laccases are widespread in bacteria. *Trends in Biotechnology, 18*, 41—42.

Amthor, J. S. (2003). Efficiency of lignin biosynthesis: a quantitative analysis. *Annali di Botanica, 91*, 673—695.

Ander, P., Hatakka, A., & Eriksson, K.-E. (1980). Vanillic acid metabolism by the white-rot fungus *Sporotrichum pulverulentum*. *Archives of Microbiology, 125*, 189–202.

Argyropoulos, D. S., & Menachem, S. B. (1997). Lignin. *Advances in Biochemical Engineering/ Biotechnology, 57*, 127–158.

Asada, Y., Watanabe, A., Ohtsu, Y., & Kuwahara, M. (1995). Purification and characterization of an aryl-alcohol oxidase from the lignin-degrading basidiomycete *Phanerochaete chrysosporium*. *Bioscience, Biotechnology, and Biochemistry, 59*, 1339–1341.

Asgher, M., Ahmad, Z., & Iqbal, H. M. N. (2013). Alkali and enzymatic delignification of sugarcane bagasse to expose cellulose polymers for saccharification and bio-ethanol production. *Industrial Crops and Products, 44*, 488–495.

Baciocchi, E., Bietti, M., Gerini, M. F., Lanzalunga, O., & Mancinelli, S. (2001). Oxidation of non-phenolic small β-*O*-aryl-lignin model dimers catalysed by lignin peroxidase. Comparison with the oxidation induced by potassium 12-tungstocobalt(III)ate. *Journal of the Chemical Society, Perkin Transactions, 2*, 1506–1511.

Baldrian, P. (2006). Fungal laccases—occurrence and properties. *FEMS Microbiology Reviews, 30*, 215–242.

Baldrian, P., & Šnajdr, J. (2006). Production of ligninolytic enzymes by litter-decomposing fungi and their ability to decolorize synthetic dyes. *Enzyme and Microbial Technology, 39*, 1023–1029.

Ballard, R. G., Walsh, M. A., & Cole, W. E. (1984). The penetration and growth of blue-stain fungi in the sapwood of lodgepole pine attacked by mountain pine beetle. *Canadian Journal of Botany, 62*, 1724–1729.

Bao, W., Fukushima, Y., Jensen, K. A., Jr., Moen, M. A., & Hammel, K. E. (1994). Oxidative degradation of non-phenolic lignin during lipid peroxidation by fungal manganese peroxidase. *FEBS Letters, 354*, 297–300.

Bartolome, B., Faulds, C. B., Kroon, P. A., Waldron, K., Gilbert, H. J., Hazlewood, G., et al. (1997). An *Aspergilllus niger* esterase (ferulic acid esterase III) and a recombinant *Pseudomonas fluorescens* subsp. *cellulosa* esterase (Xy1D) release a 5-5′ ferulic dehydrodimer (diferulic acid) from barley and wheat cell walls. *Applied and Environmental Microbiology, 63*, 208–212.

Benen, J. A. E., Sanchez-Torres, P., Wagemaker, M. J. M., Fraaijes, M. W., van Berkel, W. J. H., & Visser, J. (1998). Molecular cloning, sequencing, and heterologous expression of the *vaoA* gene from *Penicillium simplicissimum* CBS 170.90 encoding vanillyl-alcohol oxidase. *Journal of Biological Chemistry, 273*, 7865–7872.

Benoit, I., Danchin, E. G., Bleichrodt, R. J., & de Vries, R. P. (2008). Biotechnological applications and potential of fungal feruloyl esterases based on prevalence, classification and biochemical diversity. *Biotechnology Letters, 30*, 387–396.

Bentley, W. E., & Payne, G. F. (2013). Materials science. Nature's other self-assemblers. *Science, 341*, 136–137.

Billa, E., & Monties, B. (1995). Molecular variability of lignin fractions isolated from wheat straw. *Research on Chemical Intermediates, 21*, 303–311.

Blanchette, R. A. (2003). Deterioration in historic and archaeological woods from terrestrial sites. In R. J. Koestler, V. R. Koestler, A. E. Charola, & F. E. Nieto-Fernandez (Eds.), *Art, biology, and conservation: Biodeterioration of works of art* (pp. 328–347). New York: The Metropolitan Museum of Art.

Blanchette, R. A., Held, B. W., & Farrell, R. L. (2002). Defibration of wood in the expedition huts of Antarctica: an unusual deterioration process occurring in the polar environment. *Polar Record, 38*, 313–322.

Blanchette, R. A., Krueger, E. W., Haight, J. E., Akhtar, M., & Akin, D. E. (1997). Cell wall alterations in loblolly pine wood decayed by the white-rot fungus, *Ceriporiopsis subvermispora*. *Journal of Biotechnology, 53*, 203–213.

Bödeker, I. T., Clemmensen, K. E., de Boer, W., Martin, F., Olson, A., & Lindahl, B. D. (2014). Ectomycorrhizal *Cortinarius* species participate in enzymatic oxidation of humus in northern forest ecosystems. *New Phytologist, 203*, 245−256.

Bödeker, I. T., Nygren, C. M. A., Taylor, F., Olson, A., & Lindahl, B. D. (2009). Class II peroxidase-encoding genes are present in a phylogenetically wide range of ectomycorrhizal fungi. *ISME Journal, 3*, 1387−1395.

Boerjan, W., Ralph, J., & Baucher, M. (2003). Lignin biosynthesis. *Annual Review of Plant Biology, 54*, 519−546.

Bradoo, S., Gupta, R., & Saxena, R. K. (1997). Parametric optimization and biochemical regulation of extracellular tannase from *Aspergillus japonicus*. *Process Biochemistry, 32*, 135−139.

Briens, C., Piskorz, J., & Berruti, F. (2008). Biomass valorization for fuel and chemicals production − a review. *International Journal of Chemical Reactor Engineering, 6*, R2.

Brunow, G. (2006). Lignin line and lignin-based product family trees. In B. Kamm, P. R. Gruber, & M. Kamm (Eds.), *Biorefineries − Industrial processes and products*. Weinheim: Wiley-VCH Verlag GmbH & Co.

Brunow, G., & Lundquist, K. (2010). Functional groups and bonding patterns in lignin (including the lignin-carbohydrate-complexes). In C. Heitner, D. Dimmel, & J. Schmidt (Eds.), *Lignin and Lignans: Advances in chemistry* (pp. 267−299). Boca Rotan, FL: CRC Press.

Buranov, A. U., & Mazza, G. (2008). Lignin in straw of herbaceous crops. *Industrial Crops and Products, 28*, 237−259.

Buswell, J. A., Ander, P., Pettersson, B., & Eriksson, K.-E. (1979). Oxidative decarboxylation of vanillic acid by *Sporotrichum pulverulentum*. *FEBS Letters, 103*, 98−101.

Buswell, J. A., & Eriksson, K.-E. (1988). Vanillate hydroxylase from *Sporotrichum pulverulentum*. *Methods in Enzymology, 161*, 274−281.

Buswell, J. A., & Eriksson, K.-E. (1994). Effect of lignin-related phenols and their methylated derivatives on the growth of eight white-rot fungi. *World Journal of Microbiology and Biotechnology, 10*, 169−174.

Buswell, J. A., Eriksson, K.-E., Gupta, J. K., Hamp, S. G., & Nordh, I. (1982). Vanillic acid metabolism by selected soft-rot, brown-rot, and white-rot fungi. *Archives of Microbiology, 131*, 366−374.

Cai, Y. J., Buswell, J. A., & Chang, S. T. (1993). Effect of lignin-derived phenolic monomers on the growth of the edible mushrooms *Lentinus edodes, Pleurotus sajor-caju* and *Volvariella volvacea*. *World Journal of Microbiology and Biotechnology, 9*, 503−507.

Cai, Y., Luo, Q., Sun, M., & Corke, H. (2004). Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sciences, 74*, 2157−2184.

Cain, R. B., Bilton, R. F., & Darrah, J. A. (1968). The metabolism of aromatic acids by micro-organisms. Metabolic pathways in the fungi. *Biochemical Journal, 108*, 797−828.

Cai, Y., Sun, M., Xing, J., & Corke, H. (2004). Antioxidant phenolic constituents in roots of *Rheum officinale* and *Rubia cordifolia*: structure-radical scavenging activity relationships. *Journal of Agricultural and Food Chemistry, 52*, 7884−7890.

Call, H. P., & Mücke, I. (1997). History, overview and applications of mediated ligninolytic systems, especially laccase-mediator-systems (Lignozym®-process). *Journal of Biotechnology, 53*, 163−202.

Camarero, S., Ibarra, D., Martinez, M. J., & Martinez, A. T. (2005). Lignin-derived compounds as efficient laccase mediators for decolorization of different types of recalcitrant dyes. *Applied and Environmental Microbiology, 71*, 1775−1784.

Campbell, M. M., & Sederoff, R. R. (1996). Variation in lignin content and composition (mechanisms of control and implications for the genetic improvement of plants). *Plant Physiology, 110*, 3−13.

Cartwright, N. J., & Buswell, J. A. (1967). The separation of vanillate *O*-demethylase from protocatechuate 3,4-oxygenase by ultracentrifugation. *Biochemical Journal, 105*, 767–770.

Chakar, F. S., & Ragauskas, A. J. (2004). Review of current and future softwood kraft lignin process chemistry. *Industrial Crops and Products, 20*, 131–141.

Chen, C. L., & Chang, H. M. (1985). Chemistry of lignin biodegradation. In T. Higuchi (Ed.), *Biosynthesis and biodegradation of wood components* (pp. 535–556). Academic Press.

Chen, W., Lee, M. K., Jefcoate, C., Kim, S. C., Chen, F., & Yu, J. H. (2014). Fungal cytochrome p450 monooxygenases: their distribution, structure, functions, family expansion, and evolutionary origin. *Genome Biology and Evolution, 6*, 1620–1634.

Chen, F., Tobimatsu, Y., Havkin-Frenkel, D., Dixon, R. A., & Ralph, J. (2012). A polymer of caffeyl alcohol in plant seeds. *Proceedings of the National Academy of Sciences of the United States of America, 109*, 1772–1777.

Cherney, J. H., Anliker, K. S., Albrecht, K. A., & Wood, K. V. (1989). Soluble phenolic monomers in forage crops. *Journal of Agricultural and Food Chemistry, 37*, 345–350.

Cho, N.-S., Rogalski, J., Deptula, T., Staszczak, M., Janusz, G., Cho, H.-Y., et al. (2009). Biodegradation of cinnamates by white-rot fungus, *Phlebia radiata*. *Journal of the Faculty of Agriculture, Kyushu University, 54*, 285–291.

Claus, H. (2004). Laccases: structure, reactions, distribution. Oxford, England, 1993 *Micron, 35*, 93–96.

Clifford, D. R., Faulkner, J. K., Walker, J. R. L., & Woodcock, D. (1969). Metabolism of cinnamic acid by *Aspergillus niger*. *Phytochemistry, 8*, 549–552.

Collins, C., Keane, T. M., Turner, D. J., O'Keeffe, G., Fitzpatrick, D. A., & Doyle, S. (2013). Genomic and proteomic dissection of the ubiquitous plant pathogen, *Armillaria mellea*: toward a new infection model system. *Journal of Proteome Research, 12*, 2552–2570.

Corradini, E., Foglia, P., Giansanti, P., Gubbiotti, R., Samperi, R., & Lagana, A. (2011). Flavonoids: chemical properties and analytical methodologies of identification and quantitation in foods and plants. *Natural Product Research, 25*, 469–495.

Coulter, C., Kennedy, J. T., McRoberts, W. C., & Harper, D. B. (1993). Purification and properties of an *S*-adenosylmethionine: 2,4-disubstituted phenol *O*-methyltransferase from *Phanerochaete chrysosporium*. *Applied and Environmental Microbiology, 59*, 706–711.

Crawford, R. L., & Perkins Olson, P. (1978). Microbial catabolism of vanillate: decarboxylation to guaiacol. *Applied and Environmental Microbiology, 36*, 539–543.

Crepin, V. F., Faulds, C. B., & Connerton, I. F. (2004a). Functional classification of the microbial feruloyl esterases. *Applied Microbiology and Biotechnology, 63*, 647–652.

Crepin, V. F., Faulds, C. B., & Connerton, I. F. (2004b). Identification of a type-D feruloyl esterase from *Neurospora crassa*. *Applied Microbiology and Biotechnology, 63*, 567–570.

Crestini, C., & Argyropoulos, D. S. (1998). The early oxidative biodegradation steps of residual kraft lignin models with laccase. *Bioorganic and Medicinal Chemistry, 6*, 2161–2169.

Crestini, C., Melone, F., Sette, M., & Saladino, R. (2011). Milled wood lignin: a linear oligomer. *Biomacromolecules, 12*, 3928–3935.

Crestini, C., & Sermani, G. G. (1994). Oxidation and aromatic ring cleavage of 4-methoxy and 3,4-dimethoxycinnamic acid by *Lentinus edodes*. *Biotechnology Letters, 16*, 995–1000.

D'Acunzo, F., & Lanzalunga, O. (2004). First synthesis of a polysaccharide-supported lignin model compound and study of its oxidation promoted by lignin peroxidase. *Biochemical and Biophysical Research Communications, 313*, 17–21.

De Jesus, M., Nicola, A. M., Rodrigues, M. L., Janbon, G., & Casadevall, A. (2009). Capsular localization of the *Cryptococcus neoformans* polysaccharide component galactoxylomannan. *Eukaryot Cell, 8*, 96–103.

de Paiva, L. B., Goldbeck, R., dos Santos, W. D., & Squina, F. M. (2013). Ferulic acid and derivatives: moleculaes with potential application in the pharmaceutical field. *Brazilian Journal of Pharmaceutical Sciences, 49*, 395–411.

de Vries, R. P., Kester, H. C. M., vanKuyk, P. A., & Visser, J. (2002). The *Aspergillus niger faeB* gene encodes a second feruloyl esterase involved in pectin and xylan degradation, and is specifically induced on aromatic compounds. *Biochemical Journal, 363*, 377–386.

de Vries, R. P., & Visser, J. (1999). Regulation of the feruloyl esterase (*faeA*) gene from *Aspergillus niger*. *Applied and Environmental Microbiology, 65*, 5500–5503.

de Vries, R. P., & Visser, J. (2001). *Aspergillus* enzymes involved in degradation of plant cell wall polysaccharides. *Microbiology and Molecular Biology Reviews, 65*, 497–522.

Desentis-Mendoza, R. M., Hernandez-Sanchez, H., Moreno, A., Rojas del c, E., Chel-Guerrero, L., Tamariz, J., et al. (2006). Enzymatic polymerization of phenolic compounds using laccase and tyrosinase from *Ustilago maydis*. *Biomacromolecules, 7*, 1845–1854.

Dey, S., Maiti, T. K., & Bhattacharyya, B. C. (1994). Production of some extracellular enzymes by a lignin peroxidase-producing brown rot fungus, *Polyporus ostreiformis*, and its comparative abilities for lignin degradation and dye decolorization. *Applied and Environmental Microbiology, 60*, 4216–4218.

DiGuistini, S., Wang, Y., Liao, N. Y., Taylor, G., Tanguay, P., Feau, N., et al. (2011). Genome and transcriptome analyses of the mountain pine beetle-fungal symbiont *Grosmannia clavigera*, a lodgepole pine pathogen. *Proceedings of the National Academy of Sciences of the United States of America, 108*, 2504–2509.

Dix, N. J., & Webster, J. (1995). *Fungal ecology*. Springer, The Netherlands.

Doddapaneni, H., Chakraborty, R., & Yadav, J. S. (2005). Genome-wide structural and evolutionary analysis of the P450 monooxygenase genes (P450ome) in the white rot fungus Phanerochaete chrysosporium: evidence for gene duplications and extensive gene clustering. *BMC Genomics, 6*, 92.

Doyle, W. A., Blodig, W., Veitch, N. C., Piontek, K., & Smith, A. T. (1998). Two substrate interaction sites in lignin peroxidase revealed by site-directed mutagenesis. *Biochemistry, 37*, 15097–15105.

Dykes, L., & Rooney, L. W. (2007). Phenolic compounds in cereal grains and their health benefits. *Cereal Foods World, 52*, 105–111.

Eastwood, D. C., Floudas, D., Binder, M., Majcherczyk, A., Schneider, P., Aerts, A., et al. (2011). The plant cell wall-decomposing machinery underlies the functional diversity of forest fungi. *Science, 333*, 762–765.

Enoki, A., & Gold, M. H. (1982). Degradation of the diarylpropane lignin model compound 1-(3′,4′-diethoxyphenyl)-1,3-dihydroxy-2-(4′-methoxyphenyl)-propane and derivatives by the basidiomycete *Phanerochaete chrysosporium*. *Archives of Microbiology, 132*, 123–130.

Enoki, A., Goldsby, G. P., & Gold, M. H. (1981). β-Ether cleavage of the lignin model compound 4-ethoxy-3-methoxyphenylglycerol-β-guaiacyl ether and derivatives by *Phanerochaete chrysosporium*. *Archives of Microbiology, 129*, 141–145.

Eraso, F., & Hartley, R. D. (1990). Monomeric and dimeric phenolic constituents of plant cell walls—possible factors influencing wall biodegradability. *Journal of the Science of Food and Agriculture, 51*, 163–170.

Eriksson, K.-E., Blanchette, R. A., & Ander, P. (1990). *Microbial and enzymatic degradation of Wood and Wood components*. Berlin, Germany: Springer-Verlag.

Eriksson, K.-E., Gupta, J. K., Nishida, A., & Rao, M. (1984). Syringic acid metabolism by some white-rot, soft-rot and brown-rot fungi. *Journal of General Microbiology, 130*, 2457–2464.

Estrada Alvarado, I., Lomascolo, A., Navarro, D., Delattre, M., Asther, M., & Lesage-Meessen, L. (2001). Evidence of a new biotransformation pathway of *p*-coumaric acid into *p*-hydroxybenzaldehyde in *Pycnoporus cinnabarinus*. *Applied Microbiology and Biotechnology, 57*, 725–730.

Estrada-Alvarado, I., Navarro, D., Record, E., Asther, M., & Asther, M. (2003). Fungal biotransformation of *p*-coumaric acid into caffeic acid by *Pycnoporus cinnabarinus*: an alternative for producing a strong natural antioxidant. *World Journal of Microbiology & Biotechnology, 19*, 157–160.

Fagerstedt, K. V., Kukkola, E. M., Koistinen, V. V., Takahashi, J., & Marjamaa, K. (2010). Cell wall lignin is polymerised by class III secretable plant peroxidases in Norway spruce. *Journal of Integrative Plant Biology, 52*, 186–194.

Falconnier, B., Lapierre, C., Lesage-Meessen, L., Yonnet, G., Brunerie, P., Colonna-Ceccaldi, B., et al. (1994). Vanillin as a product of ferulic acid biotransformation by the white-rot fungus *Pycnoporus cinnabarinus* I-937: Identification of metabolic pathways. *Journal of Biotechnology, 37*, 123–132.

Farmer, V. C., Henderson, M. E., & Russell, J. D. (1960). Aromatic-alcohol-oxidase activity in the growth medium of *Polystictus versicolor*. *Biochemical Journal, 74*, 257–262.

Faulds, C. B., Mandalari, G., LoCurto, R., Bisignano, G., & Waldron, K. W. (2004). Arabinoxylan and mono- and dimeric ferulic acid release from brewer's grain and wheat bran by feruloyl esterases and glycosyl hydrolases from *Humicola insolens*. *Applied Microbiology and Biotechnology, 64*, 644–650.

Faulds, C. B., Sancho, A. I., & Bartolome, B. (2002). Mono- and dimeric ferulic acid release from brewer's spent grain by fungal feruloyl esterases. *Applied Microbiology and Biotechnology, 60*, 489–494.

Faulds, C. B., Zanichelli, D., Crepin, V. F., Connerton, I. F., Juge, N., Bhat, M. K., et al. (2003). Specificity of feruloyl esterases for water-extractable and water-unextractable feruloylated polysaccharides: influence of xylanase. *Journal of Cereal Science, 38*, 281–288.

Ferreira, P., Medina, M., Guillen, F., Martinez, M. J., Van Berkel, W. J., & Martinez, A. T. (2005). Spectral and catalytic properties of aryl-alcohol oxidase, a fungal flavoenzyme acting on polyunsaturated alcohols. *Biochemical Journal, 389*, 731–738.

Floudas, D., Binder, M., Riley, R., Barry, K., Blanchette, R. A., Henrissat, B., et al. (2012). The Paleozoic origin of enzymatic lignin decomposition reconstructed from 31 fungal genomes. *Science, 336*, 1715–1719.

French, C. J., Vance, C. P., & Towers, G. H. N. (1976). Conversion of *p*-coumaric acid to *p*-hydroxybenzoic acid by cell free extracts of potato tubers and *Polyporus hispidus*. *Phytochemistry, 15*, 564–566.

Freudenberg, K., & Neish, A. C. (1968). *Constitution and biosynthesis of lignin*. New York: Springer-Verlag.

Fry, S. C. (1982). Phenolic components of the primary cell wall. Feruloylated disaccharides of D-galactose and L-arabinose from spinach polysaccharides. *Biochemistry Journal, 203*, 493–504.

Fry, S. C. (1983). Feruloylated pectin substances from the primary cell wall: their structure and possible functions. *Planta, 157*, 111–123.

Fry, S. C. (1986). Cross-linking of matrix polymers in the growing cell walls of angiosperms. *Annual Review of Plant Physiology, 37*, 165–186.

Fuchs, G., Boll, M., & Heider, J. (2011). Microbial degradation of aromatic compounds—from one strategy to four. *Nature Reviews Microbiology, 9*, 803–816.

Fukuda, H., Nakamura, K., Sukita, E., Ogawa, T., & Fujii, T. (1996). Cytochrome P450rm from *Rhodotorula* minuta catalyzes 4-hydroxylation of benzoate. *Journal of Biochemistry, 119*, 314–318.

Furukawa, H., Wieser, M., Morita, H., Sugio, T., & Nagasawa, T. (1999). Purification and characterization of vanillyl-alcohol oxidase from *Byssochlamys fulva* V107. *Journal of Bioscience and Bioengineering, 87*, 285–290.

Gandini, A. (2011). The irruption of polymers from renewable resources on the scene of macromolecular science and technology. *Green Chemistry, 13*, 1061–1083.

Garcia-Conesa, M. T., Ostergaard, P., Kauppinen, S., & Williamson, G. (2001). Hydrolysis of diethyl diferulates by a tannase from *Aspergillus oryzae*. *Carbohydrate Polymers, 44*, 319–324.

Ghosh, M., & Nanda, G. (1994). Purification and some properties of a xylanase from *Aspergillus sydowii* MG49. *Applied and Environmental Microbiology, 60*, 4620–4623.

Ghosh, S., Sachan, A., & Mitra, A. (2005). Degradation of ferulic acid by a white rot fungus *Schizophyllum commune*. *World Journal of Microbiology and Biotechnology, 21*, 385–388.

Graf, E. (1992). Antioxidant potential of ferulic acid. *Free Radical Biology & Medicine, 13*, 435–448.

Gross, G. G. (2008). From lignins to tannins: forty years of enzyme studies on the biosynthesis of phenolic compounds. *Phytochemistry, 69*, 3018–3031.

Gross, S. R., Gafford, R. D., & Tatum, E. L. (1956). The metabolism of protocatechuic acid by *Neurospora*. *Journal of Biological Chemistry, 219*, 781–796.

Guillén, F., Martínez, A. T., Martínez, M. J., & Evans, C. S. (1994). Hydrogen peroxide-producing system of *Pleurotus erygii* involving the extracellular enzyme aryl-alcohol oxidase. *Applied Microbiology and Biotechnology, 41*, 465–470.

Guiraud, P., Steiman, R., Seigle-Murandi, F., & Benoit-Guyod, J.-L. (1992). Metabolism of vanillic acid by micromycetes. *World Journal of Microbiology and Biotechnology, 8*, 270–275.

Guiraud, P., Steiman, R., Seigle-Murandi, F., & Benoit-Guyod, J. L. (1995). Comparison of the toxicity of various lignin-related aromatic compounds towards selected fungi perfecti and fungi imperfecti. *Ecotoxicology and Environmental Safety, 32*, 29–33.

Guo, D., Zhang, Z., Liu, D., Zheng, H., Chen, H., & Chen, K. (2014). A comparative study on the degradation of gallic acid by *Aspergillus oryzae* and *Phanerochaete chrysosporium*. *Water Science and Technology, 70*, 175–181.

Gupta, J. K., Hamp, S. G., Buswell, J. A., & Eriksson, K.-E. (1981). Metabolism of *trans*-ferulic acid by the white-rot fungus *Sporotrichum pulverulentum*. *Archives of Microbiology, 128*, 349–354.

Gutiérrez, A., Babot, E. D., Ullrich, R., Hofrichter, M., Martínez, A. T., & del Río, J. C. (2011). Regioselective oxygenation of fatty acids, fatty alcohols and other aliphatic compounds by a basidiomycete heme-thiolate peroxidase. *Archives of Biochemistry and Biophysics, 514*, 33–43.

Gutierrez, A., Caramelo, L., Prieto, A., Martinez, M. J., & Martinez, A. T. (1994). Anisaldehyde production and aryl-alcohol oxidase and dehydrogenase activities in ligninolytic fungi of the genus *Pleurotus*. *Applied and Environmental Microbiology, 60*, 1783–1788.

Hagerman, A. E., Riedl, K. M., Jones, G. A., Sovik, K. N., Ritchard, N. T., Hartzfeld, P. W., et al. (1998). High molecular weight plant polyphenolics (tannins) as biological antioxidants. *Journal of Agricultural and Food Chemistry, 46*, 1887–1892.

Hage, A., Schoemaker, H. E., Wever, R., Zennaro, E., & Heipieper, H. J. (2001). Determination of the toxicity of several aromatic carbonylic compounds and their reduced derivatives on *Phanerochaete chrysosporium* using a *Pseudomonas putida* test system. *Biotechnology and Bioengineering, 73*, 69–73.

Haider, K., & Trojanowski, J. (1975). Decomposition of specifically ^{14}C-labelled phenols and dehydrophenols of coniferyl alcohols as models for lignin degradation by soft and white rot fungi. *Archives of Microbiology, 105*, 33–41.

Halsall, B. E., Darrah, J. A., & Cain, R. B. (1969). The regulation of enzymes of aromatic-ring fission in fungi: organisms using both catechol and protocatechuate pathways. *Biochemical Journal, 114*, 75P–76P.

Hammel, K. E., & Cullen, D. (2008). Role of fungal peroxidases in biological ligninolysis. *Current Opinion in Plant Biology, 11*, 349–355.

Hammel, K. E., Jensen, K. A. J., Mozuch, M. D., Landucci, L. L., Tien, M., & Pease, E. A. (1993). Ligninolysis by a purified lignin peroxidase. *Journal of Biological Chemistry, 268*, 12274–12281.

Hammel, K. E., Tien, M., Kalyanaraman, B., & Kirk, T. K. (1985). Mechanism of oxidative C_{α}-C_{β} cleavage of a lignin model dimer by *Phanerochaete chrysosporium* ligninase. Stoichiometry and involvement of free radicals. *Journal of Biological Chemistry, 260*, 8348–8353.

Harper, D. B., Buswell, J. A., Kennedy, J. T., & Hamilton, J. T. (1990). Chloromethane, methyl donor in veratryl alcohol biosynthesis in *Phanerochaete chrysosporium* and other lignin-degrading fungi. *Applied and Environmental Microbiology, 56*, 3450–3457.

Harvey, P. J., Gilardi, G.-F., Goble, M. L., & Palmer, J. M. (1993). Charge transfer reactions and feedback control of lignin peroxidase by phenolic compounds: significance in lignin degradation. *Journal of Biotechnology, 30*, 57–69.

Harwood, C. S., & Parales, R. E. (1996). The beta-ketoadipate pathway and the biology of self-identity. *Annual Review of Microbiology, 50*, 553–590.

Haslam, E. (2007). Vegetable tannins—lessons of a phytochemical lifetime. *Phytochemistry, 68*, 2713–2721.

Hatakka, A. (1985). Degradation of veratric acid and other lignin-related aromatic compounds by the white-rot fungus *Pycnoporus cinnabarinus*. *Archives of Microbiology, 141*, 22–28.

Hatakka, A. (2001). Biodegradation of lignin. In M. Hofrichter, & A. Steinbüchel (Eds.), *Lignin, humic substances and Coal: Vol. 1. Biopolymers*. Weinheim: Wiley-Blackwell.

Hatakka, A., & Hammel, K. E. (2010). Fungal biodegradation of lignocelluloses. In K. Esser, & M. Hofrichter (Eds.), *The Mycota: A comprehensive Treatise on fungi as experimental systems for basic and applied research. Industrial applications* (2nd ed.). (pp. 319–340). Berlin, Heidelberg: Springer.

Hatakka, A., Lundell, T. K., Tervilä-Wilo, A. L. M., & Brunow, G. (1991). Metabolism of non-phenolic β-*O*-4 lignin model compounds by the white-rot fungus *Phlebia radiata*. *Applied Microbiology and Biotechnology, 36*, 270–277.

Henderson, M. E. (1957). Metabolism of methoxylated aromatic compounds by soil fungi. *Journal of General Microbiology, 16*, 686–695.

Henderson, M. E. (1961). The metabolism of aromatic compounds related to lignin by some hyphomycetes and yeast-like fungi of soil. *Journal of General Microbiology, 26*, 155–165.

Henderson, M. E. K. (1963). Fungal metabolism of certain aromatic compounds related to lignin. *Pure and Applied Chemistry, 7*, 589–602.

Henderson, M. E., & Farmer, V. C. (1955). Utilization by soil fungi of *p*-hydroxybenzaldehyde, ferulic acid, syringaldehyde and vanillin. *Journal of General Microbiology, 12*, 37–46.

Henriksson, G. (2009). Wood chemistry and wood biotechnology. In M. Ek, G. Gellerstedt, & G. Henriksson (Eds.), *Pulp and paper chemistry and technology* (pp. 121–146). Berlin: Walter de Gruyter.

Hernandez-Ortega, A., Ferreira, P., & Martinez, A. T. (2012). Fungal aryl-alcohol oxidase: a peroxide-producing flavoenzyme involved in lignin degradation. *Applied Microbiology and Biotechnology, 93*, 1395–1410.

Hibbett, D. S., Gilbert, L. B., & Donoghue, M. J. (2000). Evolutionary instability of ectomycorrhizal symbioses in basidiomycetes. *Nature, 407*, 506–508.

Higuchi, T. (2006). Look back over the studies of lignin biochemistry. *Journal of Wood Science, 52*, 2–8.

Hilden, L., Johansson, G., Pettersson, G., Li, J., Ljungquist, P., & Henriksson, G. (2000). Do the extracellular enzymes cellobiose dehydrogenase and manganese peroxidase form a pathway in lignin biodegradation? *FEBS Letters, 477*, 79–83.

Hofrichter, M. (2002). Lignin conversion by manganese peroxidase (MnP). *Enzyme and Microbial Technology, 30*, 454–466.

Hofrichter, M., & Fritsche, W. (1997). Depolymerization of low-rank coal by extracellular fungal enzyme systems. III. In vitro depolymerization of coal humic acids by a crude

preparation of manganese peroxidase of the white-rot fungus *Nematoloma frowardii* b19. *Applied Microbiology and Biotechnology, 47*, 566–571.
Hofrichter, M., Scheibner, K., Schneegaß, I., & Fritsche, W. (1998). Enzymatic combustion of aromatic and aliphatic compounds by manganese peroxidase of *Nematoloma frowardii*. *Applied and Environmental Microbiology, 64*, 399–404.
Hofrichter, M., & Ullrich, R. (2014). Oxidations catalyzed by fungal peroxygenases. *Current Opinion in Chemical Biology, 19*, 116–125.
Hofrichter, M., Ullrich, R., Pecyna, M. J., Liers, C., & Lundell, T. (2010). New and classic families of secreted fungal heme peroxidases. *Applied Microbiology and Biotechnology, 87*, 871–897.
Huang, Z., Dostal, L., & Rosazza, J. P. (1993). Mechanisms of ferulic acid conversions to vanillic acid and guaiacol by *Rhodotorula rubra*. *Journal of Biological Chemistry, 268*, 23954–23958.
Huang, B., Guo, J., Yi, B., Yu, X., Sun, L., & Chen, W. (2008). Heterologous production of secondary metabolites as pharmaceuticals in *Saccharomyces cerevisiae*. *Biotechnology Letters, 30*, 1121–1137.
Hudson, E. A., Dinh, P. A., Kokubun, T., Simmonds, M. S., & Gescher, A. (2000). Characterization of potentially chemopreventive phenols in extracts of brown rice that inhibit the growth of human breast and colon cancer cells. *Cancer Epidemiology, Biomarkers and Prevention, 9*, 1163–1170.
Ide, M., Ichinose, H., & Wariishi, H. (2012). Molecular identification and functional characterization of cytochrome P450 monooxygenases from the brown-rot basidiomycete *Postia placenta*. *Archives of Microbiology, 194*, 243–253.
Ikeda, R., Sugita, T., Jacobson, E. S., & Shinoda, T. (2003). Effects of melanin upon susceptibility of *Cryptococcus* to antifungals. *Microbiology and immunology, 47*, 271–277.
Ishii, T. (1991). Isolation and characterization of a diferuloyl arabinoxylan hexasaccharide from bamboo shoot cell-walls. *Carbohydrate Research, 219*, 15–22.
Ishii, T. (1994). Feruloylated oligosaccharides from cell walls of suspension-cultured spinach (*Spinacia oleracea*) cells and sugar-beet (*Beta vulgaris*) pulp. *Plant and Cell Physiology, 35*, 701–704.
Ishii, T. (1997). Structure and functions of feruloylated polysaccharides. *Plant Science, 127*, 111–127.
Ishii, T., & Hiroi, T. (1990a). Isolation and characterization of feruloylated arabinoxylan oligosaccharides from bamboo shoot cell-walls. *Carbohydrate Research, 196*, 175–183.
Ishii, T., & Hiroi, T. (1990b). Linkage of phenolic acids to cell-wall polysaccharides of bamboo shoot. *Carbohydrate Research, 206*, 297–310.
Ishii, T., Hiroi, T., & Thomas, J. R. (1990). Feruloylated xyloglucan and *p*-coumaroyl arabinoxylan oligosaccharides from bamboo shoot cell-walls. *Phytochemistry, 29*, 1999–2003.
Ishii, T., & Tabita, T. (1993). Structural characterisation of feruloyl oligosaccharides from spinach-leaf cell walls. *Carbohydrate Research, 248*, 179–190.
Ishikawa, H., Schubert, W. J., & Nord, F. F. (1963). Investigations on lignins and lignification. 28. The degradation by *Polyporus versicolor* and *Fomes fomentarius* of aromatic compounds structurally related to softwood lignin. *Archives of Biochemistry and Biophysics, 100*, 140–149.
Isroi Millati, R., Syamsiah, S., Niklasson, C., Cahyanto, M., Ludquist, K., & Taherzadeh, M. (2011). Biological pretreatment of lignocelluloses with white-rot fungi and its applications: a review. *BioResources, 6*, 5224–5259.
Iyayi, C. B., & Dart, R. K. (1982). The degradation of *p*-coumaroyl alcohol by *Aspergillus flavus*. *Journal of General Microbiology, 128*, 1473–1482.
Jeffers, M. R., McRoberts, W. C., & Harper, D. B. (1997). Identification of a phenolic 3-*O*-methyltransferase in the lignin-degrading fungus *Phanerochaete chrysosporium*. *Microbiology, 143*, 1975–1981.

Jensen, K. A., Bao, W., Kawai, S., Srebotnik, E., & Hammel, K. E. (1996). Manganese-dependent cleavage of nonphenolic lignin structures by *Ceriporiopsis subvermispora* in the absence of lignin peroxidase. *Applied and Environmental Microbiology, 62*, 3679–3686.

Kaisoon, O., Siriamornpun, S., Weerapreeyakul, N., & Meeso, N. (2011). Phenolic compounds and anti-oxidant activities of edible flowers from Thailand. *Journal of Functional Foods, 3*, 88–99.

Kamaya, J., & Higuchi, T. (1984a). Degradation of lignin substructure models with biphenyl linkage by *Phanerochaete chrysosporium*. *Wood Research, 70*, 25–28.

Kamaya, Y., & Higuchi, T. (1984b). Metabolism of 3,4-dimethoxycinnamyl alcohol and derivatives by *Coriolus versicolor*. *FEMS Microbiol Letters, 24*, 225–229.

Kamaya, Y., Nakatsubo, F., Higuchi, T., & Iwahara, S. (1981). Degradation of d,l-syringaresinol, β-β′ linked lignin model compound, by *Fusarium solani* M-13-1. *Archives of Microbiology, 129*, 305–309.

Kanski, J., Aksenova, M., Stoyanova, A., & Butterfield, D. A. (2002). Ferulic acid antioxidant protection against hydroxyl and peroxyl radical oxidation in synaptosomal and neuronal cell culture systems in vitro: structure-activity studies. *Journal of Nutritional Biochemistry, 13*, 273–281.

Kapich, A., Hofrichter, M., Vares, T., & Hatakka, A. (1999). Coupling of manganese peroxidase-mediated lipid peroxidation with destruction of nonphenolic lignin model compounds and ^{14}C-labeled lignins. *Biochemical and Biophysical Research Communications, 259*, 212–219.

Kapich, A. N., Steffen, K. T., Hofrichter, M., & Hatakka, A. (2005). Involvement of lipid peroxidation in the degradation of a non-phenolic lignin model compound by manganese peroxidase of the litter-decomposing fungus *Stropharia coronilla*. *Biochemical and Biophysical Research Communications, 330*, 371–377.

Katayama, Y., Nishida, T., Morohoshi, N., & Kuroda, K. (1989). The metabolism of biphenyl structures in lignin by the wood-rotting fungus *Coriolus versicolor*. *FEMS Microbiology Letters, 61*, 307–314.

Kato, Y., & Nevin, D. J. (1985). Isolation and identification of *O*-(5-*O*-feruloyl-arabinofuranosyl)-(1-3)-*O*-D-xylopyranosyl-(1-3)-xylopyranose as a component of *Zea* shoot cell walls. *Carbohydrate Research, 137*, 139–150.

Kawai, S., Iwatsuki, M., Nakagawa, M., Inagaki, M., Hamabe, A., & Ohashi, H. (2004). An alternative β-ether cleavage pathway for a non-pehnolic β-*O*-4 lignin model dimer catalyzed by a laccase-mediator system. *Enzyme and Microbial Technology, 35*, 154–160.

Kawai, S., Nakagawa, M., & Ohashi, H. (2002). Degradation mechanisms of a nonphenolic β-*O*-4 lignin model dimer by *Trametes versicolor* laccase in the present of 1-hydroxybenzotriazole. *Enzyme and Microbial Technology, 30*, 482–489.

Kawai, S., Umezawa, T., Shimada, M., & Higuchi, T. (1988). Aromatic ring cleavage of 4,6-di(tert-butyl)guaiacol, a phenolic lignin model compound, by laccase of *Coriolus versicolor*. *FEBS Letters, 236*, 309–311.

Kerem, Z., & Hammel, K. E. (1999). Biodegradative mechanism of the brown rot basidiomycete *Gloeophyllum trabeum*: evidence for an extracellular hydroquinone-driven fenton reaction. *FEBS Letters, 446*, 49–54.

Kim, S. J., & Shoda, M. (1999). Purification and characterization of a novel peroxidase from *Geotrichum candidum* Dec 1 involved in decolorization of dyes. *Applied and Environmental Microbiology, 65*, 1029–1035.

Kinne, M., Poraj-Kobielska, M., Ullrich, R., Nousiainen, P., Sipilä, J., Scheibner, K., et al. (2011). Oxidative cleavage of non-phenolic β-*O*-4 lignin model dimers by an extracellular aromatic peroxygenase. *Holzforschung, 65*, 673–679.

Kirk, T. K., & Farrell, R. L. (1987). Enzymatic "combustion": the microbial degradation of lignin. *Annual Review of Microbiology, 41*, 465–505.

Koide, K., Osono, T., & Takeda, H. (2005). Colonization and lignin decomposition of *Camellia japonica* leaf litter by endophytic fungi. *Mycoscience, 46*, 280–286.

Korosec, B., Sova, M., Turk, S., Krasevec, N., Novak, M., Lah, L., et al. (2013). Antifungal activity of cinnamic acid derivatives involves inhibition of benzoate 4-hydroxylase (CYP53). *Journal of Applied Microbiology, 116*, 955–966.

Kosuge, T., & Conn, E. E. (1959). The metabolism of aromatic compounds in higher plants. I. Coumarin and *o*-coumaric acid. *Journal of Biological Chemistry, 234*, 2133–2137.

Kosuge, T., & Conn, E. E. (1961). The metabolism of aromatic compounds in higher plants. III. The beta-glucosides of *o*-coumaric, coumarinic, and melilotic acids. *Journal of Biological Chemistry, 236*, 1617–1621.

Krings, U., Pilawa, S., Theobald, C., & Berger, R. G. (2001). Phenyl propenoic side chain degradation of ferulic acid by *Pycnoporus cinnabarinus*—elucidation of metabolic pathways using [5-2H]-ferulic acid. *Journal of Biotechnology, 85*, 305–314.

Kroon, P. A., Faulds, C. B., Brezillon, C., & Williamson, G. (1997). Methyl phenylalkanoates as substrates to probe the active sites of esterases. *European Journal of Biochemistry, 248*, 245–251.

Kroon, P. A., Garcia-Conesa, M. T., Fillingham, I. J., Hazlewood, G. P., & Williamson, G. (1999). Release of ferulic acid dehydrodimers from plant cell walls by feruloyl esterases. *Journal of the Science of Food and Agriculture, 79*, 428–434.

Kuhad, R. C., Singh, A., & Eriksson, K.-E. L. (1997). Microorganisms and enzymes involved in the degradation of plant fiber cell walls. *Advances in Biochemical Engineering/Biotechnology, 57*, 47–125.

Kumar, S., & Pandey, A. K. (2013). Chemistry and biological activities of flavonoids: an overview. *Scientific World Journal, 2013*, 162750.

Lahtinen, M., Kruus, K., Heinonen, P., & Sipilä, J. (2009). On the reactions of two fungal laccases differing in their redox potential with lignin model compounds: products and their rate of formation. *Journal of Agricultural and Food Chemistry, 57*, 8357–8365.

Leisola, M., Schmidt, B., Thanei-Wyss, U., & Fiechter, A. (1985). Aromatic ring-cleavage of veratryl alcohol by *Phanerochaete chrysosporium*. *FEBS Letters, 189*, 267–270.

Lesage-Meessen, L., Delattre, M., Haon, M., Thibault, J. F., Ceccaldi, B. C., Brunerie, P., et al. (1996). A two-step bioconversion process for vanillin production from ferulic acid combining *Aspergillus niger* and *Pycnoporus cinnabarinus*. *Journal of Biotechnology, 50*, 107–113.

Lesage-Meessen, L., Lomascolo, A., Bonnin, E., Thibault, J. F., Buleon, A., Roller, M., et al. (2002). A biotechnological process involving filamentous fungi to produce natural crystalline vanillin from maize bran. *Applied Biochemistry and Biotechnology, 102–103*, 141–153.

Levasseur, A., Drula, E., Lombard, V., Coutinho, P. M., & Henrissat, B. (2013). Expansion of the enzymatic repertoire of the CAZy database to integrate auxiliary redox enzymes. *Biotechnology for Biofuels, 6*, 41.

Levasseur, A., Lomascolo, A., Chabrol, O., Ruiz-Duenas, F. J., Boukhris-Uzan, E., Piumi, F., et al. (2014). The genome of the white-rot fungus *Pycnoporus cinnabarinus*: a basidiomycete model with a versatile arsenal for lignocellulosic biomass breakdown. *BMC Genomics, 15*, 486.

Liers, C., Bobeth, C., Pecyna, M., Ullrich, R., & Hofrichter, M. (2010). DyP-like peroxidases of the jelly fungus *Auricularia auricula-judae* oxidize nonphenolic lignin model compounds and high-redox potential dyes. *Applied Microbiology and Biotechnology, 85*, 1869–1879.

Li, K., & Helm, R. F. (1995). Synthesis and rearrangement reactions of ester-linked lignin-carbohydrate model compounds. *Journal of Agricultural and Food Chemistry, 43*, 2098–2103.

Li, K., Xu, F., & Eriksson, K.-E. (1999). Comparison of fungal laccases and redox mediators in oxidation of a nonphenolic lignin model compound. *Applied and Environmental Microbiology, 65*, 2654–2660.

Lomascolo, A., Lesage-Meessen, L., Labat, M., Navarro, D., Delattre, M., & Asther, M. (1999). Enhanced benzaldehyde formation by a monokaryotic strain of *Pycnoporus cinnabarinus* using a selective solid adsorbent in the culture medium. *Canadian Journal of Microbiology, 45*, 653–657.

Lucia, L. A., Argyropoulos, D. S., Adamopoulos, L., & Gaspar, A. R. (2006). Chemicals and energy from biomass. *Canadian Journal of Chemistry, 84*, 960–970.

Lundell, T. K., Mäkelä, M. R., de Vries, R. P., & Hildén, K. S. (2014). Genomics, life-styles and future prospects of wood-decaying and litter-decomposing Basidiomycota. In F. Martin (Ed.), *Genomics of plant-related fungi* (pp. 329–370). London: Elsevier Academic Press.

Lundell, T., Mäkelä, M. R., & Hildén, K. (2010). Lignin-modifying enzymes in filamentous basidiomycetes: ecological, functional and phylogenetic review. *Journal of Basic Microbiology, 50*, 5–20.

Lundell, T., Wever, R., Floris, R., Harvey, P., Hatakka, A., Brunow, G., et al. (1993). Lignin peroxidase L3 from *Phlebia radiata*. Pre-steady-state and steady-state studies with veratryl alcohol and a non-phenolic lignin model compound 1-(3,4-dimethoxyphenyl)-2-(2-methoxyphenoxy)propane-1,3-diol. *European Journal of Biochemistry, 211*, 391–402.

Lundquist, K., & Kirk, T. K. (1978). *De novo* synthesis and decomposition of veratryl alcohol by a lignin-degrading basidiomycete. *Phytochemistry, 17*, 1676.

Mäkelä, M. R., Hildén, K. S., & de Vries, R. P. (2014). Degradation and modification of plant biomass by fungi. In M. Nowrousian (Ed.), *Mycota Vol XIII fungal Genomics* (2nd ed.). (pp. 175–208). Berlin: Springer-Verlag.

Maoka, T., Tanimoto, F., Sano, M., Tsurukawa, K., Tsuno, T., Tsujiwaki, S., et al. (2008). Effects of dietary supplementation of ferulic acid and gamma-oryzanol on integument color and suppression of oxidative stress in cultured red sea bream, *Pagrus major*. *Journal of Oleo Science, 57*, 133–137.

Mao, G., Seebeck, T., Schrenker, D., & Yu, O. (2013). CYP709B3, a cytochrome P450 monooxygenase gene involved in salt tolerance in *Arabidopsis thaliana*. *BMC Plant Biology, 13*, 169.

Martin, F., Aerts, A., Ahren, D., Brun, A., Danchin, E. G., Duchaussoy, F., et al. (2008). The genome of *Laccaria bicolor* provides insights into mycorrhizal symbiosis. *Nature, 452*, 88–92.

Martínez, A. T. (2002). Molecular biology and structure-function of lignin-degrading heme peroxidases. *Enzyme and Microbial Technology, 30*, 425–444.

Martinez, D., Challacombe, J., Morgenstern, I., Hibbett, D., Schmoll, M., Kubicek, C. P., et al. (2009). Genome, transcriptome, and secretome analysis of wood decay fungus *Postia placenta* supports unique mechanisms of lignocellulose conversion. *Proceedings of the National Academy of Sciences of the United States of America, 106*, 1954–1959.

Martinez, D., Larrondo, L. F., Putnam, N., Gelpke, M. D., Huang, K., Chapman, J., et al. (2004). Genome sequence of the lignocellulose degrading fungus *Phanerochaete chrysosporium* strain RP78. *Nature Biotechnology, 22*, 695–700.

Martinez, M. J., Ruiz-Dueñas, F. J., Guillen, F., & Martinez, A. T. (1996). Purification and catalytic properties of two manganese peroxidase isoenzymes from *Pleurotus eryngii*. *European Journal of Biochemistry, 237*, 424–432.

Martins, L. O., Soares, C. M., Pereira, M. M., Teixeira, M., Costa, T., Jones, G. H., et al. (2002). Molecular and biochemical characterization of a highly stable bacterial laccase that occurs as a structural component of the *Bacillus subtilis* endospore coat. *Journal of Biological Chemistry, 277*, 18849–18859.

Mathew, S., Abraham, T. E., & Sudheesh, S. (2007). Rapid conversion of ferulic acid to 4-vinyl guaiacol and vanillin metabolites by *Debaromyces hansenii*. *Journal of Molecular Catalysis B: Enzymatic, 44*, 48–52.

Mathieu, Y., Prosper, P., Buee, M., Dumarcay, S., Favier, F., Gelhaye, E., et al. (2012). Characterization of a *Phanerochaete chrysosporium* glutathione transferase reveals a novel

structural and functional class with ligandin properties. *Journal of Biological Chemistry, 287*, 39001–39011.

Matsuzaki, F., & Wariishi, H. (2004). Functional diversity of cytochrome P450s of the white-rot fungus *Phanerochaete chrysosporium*. *Biochemical and Biophysical Research Communications, 324*, 387–393.

Matsuzaki, F., & Wariishi, H. (2005). Molecular characterization of cytochrome P450 catalyzing hydroxylation of benzoates from the white-rot fungus *Phanerochaete chrysosporium*. *Biochemical and Biophysical Research Communications, 334*, 1184–1190.

Mayer, A. M., & Staples, R. C. (2002). Laccase: new functions for an old enzyme. *Phytochemistry, 60*, 551–565.

McCann, M. C., Bush, M., Milioni, D., Sado, P., Stacey, N. J., Catchpole, G., et al. (2001). Approaches to understanding the functional architecture of the plant cell wall. *Phytochemistry, 57*, 811–821.

McLeod, M. N. (1974). Plant tannins—their role in forage quality. *Nutrition Abstracts and Reviews, 44*, 803–815.

Mendonça Neves, F., Kawano, C. Y., & Said, S. (2005). Effect of benzene compounds from plants on the growth and hyphal morphology in *Neurospora crassa*. *Brazilian Journal of Microbiology, 36*, 190–195.

Mester, T., Ambert-Balay, K., Ciofi-Baffoni, S., Banci, L., Jones, A. D., & Tien, M. (2001). Oxidation of a tetrameric nonphenolic lignin model compound by lignin peroxidase. *Journal of Biological Chemistry, 276*, 22985–22990.

Micard, V., Grabber, J. H., Ralph, J., Renard, C. M. G. C., & Thibault, J.-F. (1997). Dehydrodiferulic acids from sugar beet pulp. *Phytochemistry, 44*, 1365–1368.

Miki, K., Renganathan, V., & Gold, M. H. (1986). Mechanism of β-aryl ether fimeric lignin model compound oxidation by lignin peroxidase of *Phanerochaete chrysosporium*. *Biochemistry, 25*, 4790–4796.

Miki, K., Renganathan, V., Mayfield, M. B., & Gold, M. H. (1987). Aromatic ring cleavage of a β-biphenyl ether dimer catalyzed by lignin peroxidase of *Phanerochaete chrysosporium*. *FEBS Letters, 210*, 199–203.

Milstein, O., Vered, Y., Shragina, L., Gressel, J., Flowers, H. M., & Huttermann, A. (1983). Metabolism of lignin related aromatic compounds by *Aspergillus japonicus*. *Archives of Microbiology, 135*, 147–154.

Morel, M., Ngadin, A. A., Droux, M., Jacquot, J.-P., & Gelhaye, E. (2009). The fungal glutathione *S*-transferase system. Evidence of new classes in the wood-degrading basidiomycete *Phanerochaete chrysosporium*. *Cellular and Molecular Life Sciences, 66*, 3711–3725.

Morel, M., Meux, E., Mathieu, Y., Thuillier, A., Chibani, K., Harvengt, L., et al. (2013). Xenomic networks variability and adaptation traits in wood decaying fungi. *Microbial Biotechnology, 6*, 248–263.

Mori, H., Kawabata, K., Yoshimi, N., Tanaka, T., Murakami, T., Okada, T., et al. (1999). Chemopreventive effects of ferulic acid on oral and rice germ on large bowel carcinogenesis. *Anticancer Research, 19*, 3775–3778.

Moura, J. C., Bonine, C. A., de Oliveira Fernandes Viana, J., Dornelas, M. C., & Mazzafera, P. (2010). Abiotic and biotic stresses and changes in the lignin content and composition in plants. *Journal of Integrative Plant Biology, 52*, 360–376.

Muheim, A., Waldner, R., Leisola, M. S. A., & Fiechter, A. (1990). An extracellular aryl-alcohol oxidase from the white-rot fungus *Bjerkandera adusta*. *Enzyme and Microbial Technology, 12*, 204–209.

Mukherjee, G., Sachan, A., Ghosh, S., & Mitra, A. (2006). Conversion of sinapic acid to syringic acid by a filamentous fungus *Paecilomyces variotii*. *Journal of General and Applied Microbiology, 52*, 131–135.

Murakami, A., Nakamura, Y., Koshimizu, K., Takahashi, D., Matsumoto, K., Hagihara, K., et al. (2002). FA15, a hydrophobic derivative of ferulic acid, suppresses inflammatory

responses and skin tumor promotion: comparison with ferulic acid. *Cancer Letters, 180*, 121–129.

Murray, J. C., Burch, J. A., Streilein, R. D., Iannacchione, M. A., Hall, R. P., & Pinnell, S. R. (2008). A topical antioxidant solution containing vitamins C and E stabilized by ferulic acid provides protection for human skin against damage caused by ultraviolet irradiation. *Journal of the American Academy of Dermatology, 59*, 418–425.

Nagendran, S., Hallen-Adams, H. E., Paper, J. M., Aslam, N., & Walton, J. D. (2009). Reduced genomic potential for secreted plant cell-wall-degrading enzymes in the ectomycorrhizal fungus *Amanita bisporigera*, based on the secretome of *Trichoderma reesei*. *Fungal Genetics and Biology, 46*, 427–435.

Naik, S. N., Goud, V. V., Rout, P. K., & Dalai, A. K. (2010). Production of first and second generation biofuels: a comprehensive review. *Renewable and Sustainable Energy Reviews, 14*, 578–597.

Nakatsubo, F., Kirk, T. K., Shimada, M., & Higuchi, T. (1981). Metabolism of a phenylcoumaran substructure lignin model compound in ligninolytic cultures of *Phanerochaete chrysosporium*. *Archives of Microbiology, 128*, 416–420.

Nazareth, S., & Mavinkurve, S. (1986). Degradation of ferulic acid via 4-vinylguaiacol by *Fusarium solani* (Mart.) Sacc. *Canadian Journal of Microbiology, 32*, 494–497.

Newby, V. K., Sablon, R., Synge, R. L. M., Casteele, K. V., & van Sumere, C. F. (1980). Free and bound phenolic acids of Lucerne (*Medicago sativa* cv. Europe). *Phytochemistry, 19*, 651–657.

Niemenmaa, O., Uusi-Rauva, A., & Hatakka, A. (2006). Wood stimulates the demethoxylation of [$O^{14}CH_3$]-labeled lignin model compounds by the white-rot fungi *Phanerochaete chrysosporium* and *Phlebia radiata*. *Archives of Microbiology, 185*, 307–315.

Niemenmaa, O., Uusi-Rauva, A., & Hatakka, A. (2008). Demethoxylation of [$O^{14}CH_3$]-labelled lignin model compounds by the brown-rot fungi *Gloeophyllum trabeum* and *Poria (Postia) placenta*. *Biodegradation, 19*, 555–565.

Nishida, A., & Fukuzumi, T. (1978). Formation of coniferyl alcohol from ferulic acid by the white rot fungus *Trametes*. *Phytochemistry, 17*, 417–419.

Nomura, E., Hosoda, A., Morishita, H., Murakami, A., Koshimizu, K., Ohigashi, H., et al. (2002). Synthesis of novel polyphenols consisted of ferulic and gallic acids, and their inhibitory effects on phorbol ester-induced Epstein-Barr virus activation and superoxide generation. *Bioorganic and Medicinal Chemistry, 10*, 1069–1075.

Obst, J. R. (1982). Frequency and alkali resistance of lignin-carbohydrate bonds in wood. *Tappi Journal, 65*, 109–112.

Olson, A., Aerts, A., Asiegbu, F., Belbahri, L., Bouzid, O., Broberg, A., et al. (2012). Insight into trade-off between wood decay and parasitism from the genome of a fungal forest pathogen. *New Phytologist, 194*, 1001–1013.

Önnerud, H., Zhang, L., Gellerstedt, G., & Henriksson, G. (2002). Polymerization of monolignols by redox shuttle-mediated enzymatic oxidation: a new model in lignin biosynthesis I. *Plant Cell, 14*, 1953–1962.

Osono, T., & Takeda, H. (2001). Effects of organic chemical quality and mineral nitrogen addition on lignin and holocellulose decomposition of beech leaf litter by *Xylaria* sp. *European Journal of Soil Biology, 37*, 17–23.

Pannala, A. S., Razaq, R., Halliwell, B., Singh, S., & Rice-Evans, C. A. (1998). Inhibition of peroxynitrite dependent tyrosine nitration by hydroxycinnamates: nitration or electron donation? *Free Radical Biology and Medicine, 24*, 594–606.

Passardi, F., Cosio, C., Penel, C., & Dunand, C. (2005). Peroxidases have more functions than a Swiss army knife. *Plant Cell Reports, 24*, 255–265.

Pawlus, A. D., Sahli, R., Bisson, J., Riviere, C., Delaunay, J. C., Richard, T., et al. (2013). Stilbenoid profiles of canes from *Vitis* and *Muscadinia* species. *Journal of Agricultural and Food Chemistry, 61*, 501–511.

Perez-Boada, M., Ruiz-Dueñas, F. J., Pogni, R., Basosi, R., Choinowski, T., Martinez, M. J., et al. (2005). Versatile peroxidase oxidation of high redox potential aromatic compounds: site-directed mutagenesis, spectroscopic and crystallographic investigation of three long-range electron transfer pathways. *Journal of Molecular Biology, 354*, 385–402.

Plumridge, A., Stratford, M., Lowe, K. C., & Archer, D. B. (2008). The weak-acid preservative sorbic acid is decarboxylated and detoxified by a phenylacrylic acid decarboxylase, PadA1, in the spoilage mold *Aspergillus niger*. *Applied and Environmental Microbiology, 74*, 550–552.

Prior, R. L., & Gu, L. (2005). Occurrence and biological significance of proanthocyanidins in the American diet. *Phytochemistry, 66*, 2264–2280.

Rahouti, M., Seigle-Murandi, F., Steiman, R., & Eriksson, K.-E. (1989). Metabolism of ferulic acid by *Paecilomyces variotii* and *Pestalotia palmarum*. *Applied and Environmental Microbiology, 55*, 2391–2398.

Ralet, M.-C., Faulds, C. B., Williamson, G., & Thibault, J.-F. (1994). Degradation of feruloylated oligosaccharides from sugar beet pulp and wheat bran by ferulic acid esterases from *Aspergillus niger*. *Carbohydrate Research, 263*, 257–269.

Ralph, J., Grabber, J. H., & Hatfield, R. D. (1995). Lignin-ferulate crosslinks in grasses: active incorporation of ferulate polysaccharide esters into ryegrass lignins. *Carbohydrate Research, 275*, 167–178.

Ralph, J., Lundquist, K., Brunow, G., Lu, F., Kim, H., & Schatz, P. F. (2004). Lignins: natural polymers from oxidativecoupling of 4-hydroxyphenylpropanoids. *Phytochemistry Reviews, 3*, 29–60.

Renganathan, V., Miki, K., & Gold, M. H. (1986). Role of molecular oxygen in lignin peroxidase reactions. *Archives of Biochemistry and Biophysics, 246*, 155–161.

Riley, R., Salamov, A. A., Brown, D. W., Nagy, L. G., Floudas, D., Held, B. W., et al. (2014). Extensive sampling of basidiomycete genomes demonstrates inadequacy of the white-rot/brown-rot paradigm for wood decay fungi. *Proceedings of the National Academy of Sciences of the United States of America, 111*, 9923–9928.

Rinaldi, A. C., Comandini, O., & Kuyper, T. W. (2008). Ectomycorrhizal fungal diversity: separating the wheat from the chaff. *Fungal Diversity, 33*, 1–45.

Rineau, F., Roth, D., Shah, F., Smits, M., Johansson, T., Canbäck, B., et al. (2012). The ectomycorrhizal fungus *Paxillus involutus* converts organic matter in plant litter using a trimmed brown-rot mechanism involving Fenton chemistry. *Environmental Microbiology, 14*, 1477–1487.

Rogalski, J. (2003). Degradation of vanillic acid by white rot fungus *Phlebia radiata*. In *4th internat. sym. for. prod. bioSci.* (pp. 271–277). Cheongju, Korea: Chungbuk Nat. Univ.

Rogalski, J., Niemenmaa, O., Uusi-Rauva, A., & Hatakka, A. (1996). De novo synthesis of aromatic compounds and metabolism of [^{14}C]-glucose in the presence and absence of phenolic compounds by *Phlebia radiata*. In E. Srebotnik, & K. Messner (Eds.), *Biotechnology in the pulp and paper industry: Advances in applied and fundamental research (Proceedings of the 6th international conference on biotechnology in the pulp and paper industry)* (pp. 451–454). Vienna, Austria: Facultas-Universitätsverlag.

Rombouts, F. M., & Thibault, J.-F. (1986). Feruloylated pectic substances from sugar-beet pulp. *Carbohydrate Research, 154*, 177–187.

Romero, E., Ferreira, P., Martinez, A. T., & Martinez, M. J. (2009). New oxidase from *Bjerkandera* arthroconidial anamorph that oxidizes both phenolic and nonphenolic benzyl alcohols. *Biochimica et Biophysica Acta - Proteins and Proteomics, 1794*, 689–697.

Rosazza, J. P., Huang, Z., Dostal, L., Volm, T., & Rousseau, B. (1995). Review: biocatalytic transformations of ferulic acid: an abundant aromatic natural product. *Journal of Industrial Microbiology, 15*, 457–471.

Rubin, E. M. (2008). Genomics of cellulosic biofuels. *Nature, 454*, 841–845.

Ruiz-Dueñas, F. J., Morales, M., García, E., Miki, Y., Martínez, M. J., & Martínez, A. T. (2009). Substrate oxidation sites in versatile peroxidase and other basidiomycete peroxidases. *Journal of Experimental Botany, 60*, 441–452.

Rytioja, J., Hildén, K. S., Yuzon, J., Hatakka, A., de Vries, R. P., & Mäkelä, M. R. (2014). Plant polysaccharide degrading enzymes from basidiomycetes. *Microbiology and Molecular Biology Reviews, 78*, 614–649.

Sachan, A., Ghosh, S., & Mitra, A. (2006). Biotransformation of *p*-coumaric acid by *Paecilomyces variotii*. *Letters in Applied Microbiology, 42*, 35–41.

Sachan, A., Ghosh, S., & Mitra, A. (2010). Transforming *p*-coumaric acid into *p*-hydroxybenzoic acid by the mycelial culture of a white rot fungus *Schizophyllum commune*. *African Journal of Microbiology Research, 4*, 267–273.

Sampaio, J. P. (1995). Utilization of low molecular weight lignin-related aromatic compounds for the selective isolation of yeasts: *Rhodotorula vanillica*, a new basidiomycetous yeast species. *Systematic and Applied Microbiology, 17*, 613–619.

Saparrat, M. C., Guillen, F., Arambarri, A. M., Martinez, A. T., & Martinez, M. J. (2002). Induction, isolation, and characterization of two laccases from the white rot basidiomycete *Coriolopsis rigida*. *Applied and Environmental Microbiology, 68*, 1534–1540.

Saulnier, L., Vigouroux, J., & Thibault, J.-F. (1995). Isolation and partial characterization of feruloylated oligosaccharides from maize bran. *Carbohydrate Research, 272*, 241–253.

Schoemaker, H. E., Harvey, P. J., Bowen, R. M., & Palmer, J. M. (1985). On the mechanism of enzymatic lignin breakdown. *FEBS Letters, 183*, 7–12.

Schooneveld-Bergmans, M. E. F., Hopman, A. M. C. P., Beldman, G., & Voragen, A. G. J. (1998). Extraction and partial characterization of feruloylated glucuronoarabinoxylans from wheat bran. *Carbohydrate Polymers, 35*, 39–47.

Schroeter, H., Williams, R. J., Matin, R., Iversen, L., & Rice-Evans, C. A. (2000). Phenolic antioxidants attenuate neuronal cell death following uptake of oxidized low-density lipoprotein. *Free Radicals in Biology and Medicine, 29*, 1222–1233.

Sethuraman, A., Akin, D. E., Eisele, J. G., & Eriksson, K.-E. (1998). Effect of aromatic compounds on growth and ligninolytic enzyme production of two white rot fungi. *Ceriporiopsis subvermispora* and *Cyathus stercoreus*. Canadian Journal of Microbiology, *44*, 872–885.

Shary, S., Kapich, A. N., Panisko, E. A., Magnuson, J. K., Cullen, D., & Hammel, K. E. (2008). Differential expression in *Phanerochaete chrysosporium* of membrane-associated proteins relevant to lignin degradation. *Applied and Environmental Microbiology, 74*, 7252–7257.

Shibuya, N., & Nakane, R. (1984). Pectic polysaccharides of rice endosperm cell walls. *Phytochemistry, 23*, 1425–1429.

Shimada, M., & Higuchi, T. (1991). Microbial, enzymatic and biomimetic degradation of lignin. In D. N. S. Hon, & N. Shiraishi (Eds.), *Wood and cellulosic chemistry* (pp. 557–619). New York: Marcel Dekker.

Shimazono, H. (1959). Investigations on lignins and lignification. XXI. Identification of phenolic esters in the culture medium of *Lentinus lepideus* and the *O*-methylation of methyl *p*-coumarate to methyl *p*-methoxycinnamate *in vivo*. *Archives of Biochemistry and Biophysics, 83*, 206–215.

Shimizu, M., Yuda, N., Nakamura, T., Tanaka, H., & Wariishi, H. (2005). Metabolic regulation at the tricarboxylic acid and glyoxylate cycles of the lignin-degrading basidiomycete *Phanerochaete chrysosporium* against exogenous addition of vanillin. *Proteomics, 5*, 3919–3931.

Sjöström, E. (1993). *Wood chemistry, fundamentals and applications* (2nd ed.). San Diego: Academic press.

Smith, B. G., & Harris, P. J. (2001). Ferulic acid is esterified to glucuronoarabinoxylans in pineapple cell walls. *Phytochemistry, 56*, 513–519.

Sono, M., Roach, M. P., Coulter, E. D., & Dawson, J. H. (1996). Heme-containing oxygenases. *ChemicalReviews, 96*, 2841–2888.

Srebotnik, E., & Hammel, K. E. (2000). Degradation of nonphenolic lignin by the laccase/1-hydroxybenzotriazole system. *Journal of Biotechnology, 81*, 179–188.

Srebotnik, E., Jensen, K. A., Jr., & Hammel, K. E. (1994). Fungal degradation of recalcitrant nonphenolic lignin structures without lignin peroxidase. *Proceedings of the National Academy of Sciences of the United States of America, 91*, 12794–12797.

Steffen, K. T., Hofrichter, M., & Hatakka, A. (2000). Mineralisation of ^{14}C-labelled synthetic lignin and ligninolytic enzyme activities of litter-decomposing basidiomycetous fungi. *Applied Microbiology and Biotechnology, 54*, 819–825.

Subramanian, V., & Yadav, J. S. (2008). Regulation and heterologous epression of P450 enzyme system components of the white rot fungus *Phanerochaete chrysosporium*. *Enzyme and Microbial Technology, 43*, 205–213.

Sugano, Y. (2009). DyP-type peroxidases comprise a novel heme peroxidase family. *Cellular and Molecular Life Sciences, 66*, 1387–1403.

Surveswaran, S., Cai, Y.-Z., Corke, H., & Sun, M. (2007). Systematic evaluationof natural phenolic antioxidants from 133 Indian medicinal plants. *Food Chemistry, 102*, 938–953.

Suseela, R. G., & Nandy, S. C. (1983). Isolation, purification and some properties of *Penicillium chrysogenum* tannase. *Applied and Environmental Microbiology, 46*, 525–527.

Tedersoo, L., May, T., & Smith, M. (2010). Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. *Mycorrhiza, 20*, 217–263.

Thibault, J. F., Asther, M., Ceccaldi, B. C., Couteau, D., Delattre, M., Duarte, J. C., et al. (1998). Fungal bioconversion of agricultural by-products to vanillin. *Lebensmittel-Wissenschaft und Technologie, 31*, 530–536.

Thurston, C. F. (1994). The structure and function of fungal laccases. *Microbiology, 140*, 19–26.

Tien, M., & Kirk, T. K. (1983). Lignin-degrading enzyme from the hymenomycete *Phanerochaete chrysosporium* Burds. *Science, 221*, 661–663.

Tilay, A., Bule, M., & Annapure, U. (2010). Production of biovanillin by one-step biotransformation using fungus *Pycnoporous cinnabarinus*. *Journal of Agricultural and Food Chemistry, 58*, 4401–4405.

Tillett, R., & Walker, J. R. L. (1990). Metabolism of ferulic acid by a *Penicillium* sp. *Archives of Microbiology, 154*, 206–208.

Topakas, E., Christakopoulos, P., & Faulds, C. B. (2005). Comparison of mesophilic and thermophilic feruloyl esterases: characterization of their substrate specificity for methyl phenylalkanoates. *Journal of Biotechnology, 115*, 355–366.

Topakas, E., Kalogeris, E., Kekos, D., Macris, B. J., & Christakopoulos, P. (2003). Bioconversion of ferulic acid into vanillic acid by the thermophilic fungus *Sporotrichum thermophile*. *Lebensmittel-Wissenschaft und Technologie, 36*, 561–565.

Torres, Y. T. J. L., & Rosazza, J. P. (2001). Microbial transformations of *p*-coumaric acid by *Bacillus megaterium* and *Curvularia lunata*. *Journal of Natural Products, 64*, 1408–1414.

Tsujiyama, S., & Ueno, M. (2008). Formation of 4-vinyl guaiacol as an intermediate in bioconversion of ferulic acid by *Schizophyllum commune*. *Bioscience, Biotechnology, and Biochemistry, 72*, 212–215.

Tuor, U., Wariishi, H., Schoemaker, H. E., & Gold, M. H. (1992). Oxidation of phenolic arylglycerol beta-aryl ether lignin model compounds by manganese peroxidase from *Phanerochaete chrysosporium*: oxidative cleavage of an alpha-carbonyl model compound. *Biochemistry, 31*, 4986–4995.

Ullrich, R., & Hofrichter, M. (2005). The haloperoxidase of the agaric fungus *Agrocybe aegerita* hydroxylates toluene and naphthalene. *FEBS Letters, 579*, 6247–6250.

Umezawa, T., & Higuchi, T. (1985). Aromatic ring cleavage in degradation of β-*O*-4 lignin substructure by *Phanerochaete chrysosporium*. *FEBS Letters, 182*, 257–259.

Umezawa, T., & Higuchi, T. (1987). Mechanism of aromatic ring cleavage of β-*O*-4 lignin substructure models by lignin peroxidase. *FEBS Letters, 218*, 255–260.

Umezawa, T., & Higuchi, T. (1989). Cleavages of aromatic ring and β-*O*-4 bond of synthetic lignin (DHP) by lignin peroxidase. *FEBS Letters, 242*, 325–329.

Umezawa, T., Nakatsubo, F., & Higuchi, T. (1982). Lignin degradation by *Phanerochaete chrysosporium*: metabolism of a phenolic phenylcoumaran substructure model compound. *Archives of Microbiology, 131*, 124–128.

Umezawa, T., Shimada, M., Higuchi, T., & Kusai, K. (1986). Aromatic ring cleavage of β-*O*-4-lignin substructure model dimers by lignin peroxidase of *Phanerochaete chrysosporium*. *FEMS Microbiology Letters, 205*, 287–292.

Uthandi, S., Saad, B., Humbard, M. A., & Maupin-Furlow, J. A. (2010). LccA, an archaeal laccase secreted as a highly stable glycoprotein into the extracellular medium by *Haloferax volcanii*. *Applied and Environmental Microbiology, 76*, 733–743.

Vallverdu-Queralt, A., Regueiro, J., Martinez-Huelamo, M., Rinaldi Alvarenga, J. F., Leal, L. N., & Lamuela-Raventos, R. M. (2014). A comprehensive study on the phenolic profile of widely used culinary herbs and spices: rosemary, thyme, oregano, cinnamon, cumin and bay. *Food Chemistry, 154*, 299–307.

van Bloois, E., Torres Pazmino, D. E., Winter, R. T., & Fraaije, M. W. (2009). A robust and extracellular heme-containing peroxidase from *Thermobifida fusca* as prototype of a bacterial peroxidase superfamily. *Applied Microbiology and Biotechnology, 86*, 1419–1430.

van den Brink, J. M., Punt, P. J., van Gorcom, R. F., & van den Hondel, C. A. (2000). Regulation of expression of the *Aspergillus niger* benzoate para-hydroxylase cytochrome P450 system. *Molecular and General Genetics, 263*, 601–609.

van den Brink, J. M., van den Hondel, C. A., & van Gorcom, R. F. (1996). Optimization of the benzoate-inducible benzoate *p*-hydroxylase cytochrome P450 enzyme system in *Aspergillus niger*. *Applied Microbiology and Biotechnology, 46*, 360–364.

van Diepeningen, A. D., Debets, A. J., Varga, J., van der Gaag, M., Swart, K., & Hoekstra, R. F. (2004). Efficient degradation of tannic acid by black *Aspergillus* species. *Mycological Research, 108*, 919–925.

van Gorcom, R. F. M., Boschloo, J. G., Kuijvenhoven, A., Lange, J., van Vark, A. J., Bos, C. J., et al. (1990). Isolation and molecular characterisation of the benzoate-para-hydroxylase gene (*bph*A) of *Aspergillus niger*: a member of a new gene family of the cytochrome P450 superfamily. *Molecular and General Genetics, 233*, 192–197.

Vanholme, R., Demedts, B., Morreel, K., Ralph, J., & Boerjan, W. (2010). Lignin biosynthesis and structure. *Plant Physiology, 153*, 895–905.

Veneault-Fourrey, C., Plett, J. M., & Martin, F. (2013). Who is controlling whom within the ectomycorrhizal symbiosis: Insights from genomic and functional analyses. In F. J. de Bruijn (Ed.), *Molecular microbial ecology of the rhizosphere* (pp. 501–512). Hoboken, NJ: John Wiley & Sons, Inc.

Ververidis, F., Trantas, E., Douglas, C., Vollmer, G., Kretzschmar, G., & Panopoulos, N. (2007). Biotechnology of flavonoids and other phenylpropanoid-derived natural products. Part I: chemical diversity, impacts on plant biology and human health. *Journal of Biotechnology, 2*, 1214–1234.

Wat, C.-K., & Towers, G. H. N. (1975). Phenolic *O*-methyltransferase from *Lentinus lepideus* (basidiomycete). *Phytochemistry, 14*, 663–666.

Westermark, U., & Eriksson, K.-E. (1974). Cellobiose:quinone oxidoreductase, a new wood degrading enzyme from white-rot fungi. *Acta Chemica Scandinavica, 28*, 209–214.

Williamson, P. R. (1994). Biochemical and molecular characterization of the diphenol oxidase of *Cryptococcus neoformans*: identification as a laccase. *Journal of Bacteriology, 176*, 656–664.

Wojdylo, A., Oszmianski, J., & Czemerys, R. (2007). Antioxidant activity and phenolic compounds in 32 selected herbs. *Food Chemistry, 105*, 940–949.

Wojtas-Wasilewska, M., & Trojanowski, J. (1980). Purification and properties of protocatechuate 3,4-dioxygenase from *Chaetomium piluliferum* induced with *p*-hydroxybenzoic acid. *Acta Biochimica Polonica, 27*, 21–34.

Wong, D. W. (2006). Feruloyl esterase: a key enzyme in biomass degradation. *Applied Biochemistry and Biotechnology, 133*, 87–112.

Wood, P. M. (1994). Pathways for production of Fenton's reagent by wood-rotting fungi. *FEMS Microbiology Reviews, 13*, 313–320.

Wright, J. D. (1993). Fungal degradation of benzoic acid and related compounds. *World Journal of Microbiology and Biotechnology, 9*, 9–16.

Yajima, Y., Enoki, A., Mayfield, M. B., & Gold, M. H. (1979). Vanillate hydroxylase from the white rot basidiomycete *Phanerochaete chrysosporium*. *Archives of Microbiology, 123*, 319–321.

Yao, L. H., Jiang, Y. M., Shi, J., Tomas-Barberan, F. A., Datta, N., Singanusong, R., et al. (2004). Flavonoids in food and their health benefits. *Plant Foods for Human Nutrition, 59*, 113–122.

Youn, H.-D., Hah, Y. C., & Kang, S.-O. (1995). Role of laccase in lignin degradation by white-rot fungi. *FEMS Microbiology Letters, 132*, 183–188.

Zelena, K., Hardebusch, B., Hülsdau, B., Berger, R. G., & Zorn, H. (2009). Generation of norisoprenoid flavors from carotenoids by fungal peroxidases. *Journal of Agricultural and Food Chemistry, 57*, 9951–9955.

CHAPTER THREE

Candida Survival Strategies

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Contents

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Abstract

Only few *Candida* species, e.g., *Candida albicans*, *Candida glabrata*, *Candida dubliniensis*, and *Candida parapsilosis*, are successful colonizers of a human host. Under certain circumstances these species can cause infections ranging from superficial to life-threatening disseminated candidiasis. The success of *C. albicans*, the most prevalent and best studied *Candida* species, as both commensal and human pathogen depends on its genetic, biochemical, and morphological flexibility which facilitates adaptation to a wide range of host niches. In addition, formation of biofilms provides additional protection from adverse environmental conditions. Furthermore, in many host niches *Candida* cells coexist with members of the human microbiome. The resulting fungal—bacterial interactions have a major influence on the success of *C. albicans* as commensal and also influence disease development and outcome. In this chapter, we review the current knowledge of important survival strategies of *Candida* spp., focusing on fundamental fitness and virulence traits of *C. albicans*.

1. INTRODUCTION

Candida albicans is a polymorphic fungus that is a member of the endogenous human microbiota colonizing the oropharynx, genital, and

gastrointestinal (GI) mucosa of 30–70% of healthy individuals (Kauffman, 2006; Odds, 1987; Pappas, 2006; Pfaller & Diekema, 2007). On the other hand, *Candida* spp. are also common causes of fungal infections. *Candida* infections range from superficial mucosal manifestations, e.g., vulvovaginal candidiasis and oropharyngeal candidiasis (OPC), to serious and life-threatening systemic infections such as disseminated candidiasis and fungemia (Calderone, 2002; Perlroth, Choi, & Spellberg, 2007; Yapar, 2014). *Candida* species are the fourth-most common hospital-acquired bloodstream infections in the US and systemic candidiasis is still associated with high mortality rates of up to 50% (Perlroth et al., 2007). Common risk factors for the development of candidemia include extended treatment in intensive care units, central venous catheters, GI surgery, polytrauma, extremes of age, severe immunosuppression, neutropenia, solid tumors, and hematological malignancies (Brown, Denning, et al., 2012; Koh, Kohler, Coggshall, Van Rooijen, & Pier, 2008; Spellberg et al., 2012; Yapar, 2014). Challenges in early diagnosis of candidemia, the present lack of antifungal vaccines and the limited number of antimycotic substances available contribute to the difficulties in treating systemic candidiasis (Brown, Denning, et al., 2012; Spellberg et al., 2012; Yapar, 2014). Longitudinal molecular typing studies revealed that most *Candida* infections arise from one patients' own commensal strains rather than by vertical or longitudinal transfer (Nucci & Anaissie, 2001; Odds et al., 2006). Therefore, the understanding of survival and virulence mechanisms of this human pathogenic yeast is of major importance to develop better treatment prospects.

Of the approximately 200 *Candida* species that have been described so far only 15 have been isolated from infections in humans (Moran, Coleman, & Sullivan, 2012; Yapar, 2014). Furthermore, the vast majority of *Candida* infections are caused by only five species, *C. albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis*, and *Candida krusei* (Bassetti et al., 2013; Diekema, Arbefeville, Boyken, Kroeger, & Pfaller, 2012; Lewis, 2009; Maubon, Garnaud, Calandra, Sanglard, & Cornet, 2014; Pappas, 2006; Pfaller & Diekema, 2007), which suggests that these species in particular are well adjusted for life within the human host. In the last decades, the incidence of *C. albicans* infections, the leading pathogenic *Candida* species so far, has declined while non-albicans *Candida* have increased (Maubon et al., 2014; Perlroth et al., 2007; Yapar, 2014). In Northern countries, especially *C. glabrata* is more frequently found which might be attributed to the increased use of fluconazole in treating *Candida* infections, for which *C. glabrata* is intrinsically resistant (Guinea, 2014; Maubon et al., 2014;

Ostrosky-Zeichner et al., 2003; Pfaller et al., 2004; Tortorano et al., 2006; Yapar, 2014). Some *Candida* spp. have been associated with certain risk groups suggesting that differences in their colonization and survival strategies promote infections only under distinct preliminary circumstances. For example, *C. glabrata* and *C. tropicalis* are more commonly seen in patients with hematological or solid organ malignancies and neutropenia; and *C. krusei* infections occur especially in patients that have undergone hematopoietic stem cell transplantation (Yapar, 2014). *Candida parapsilosis* is associated with infections in neonates, rather than adults, and is a common pathogen of catheter-related infections (Yapar, 2014), probably due to its role as member of the skin microbiota (van Asbeck, Clemons, & Stevens, 2009; Trofa, Gacser, & Nosanchuk, 2008). However, regardless of the *Candida* species and the colonized or infected host niche, *Candida* spp. have to cope with mechanical (e.g., epithelial) barriers, biochemical, chemical, and physical antagonists (e.g., bile, mucus, pH, and antimicrobial peptides (AMPs)), microbial competition (normal human microbiota), and the innate and adaptive immune system of the human host (see Figure 1). Especially for those *Candida* species that are only found in association with warm-blooded hosts, such as *C. albicans* and *C. glabrata*, maintenance in its chosen host niche, ideally without causing damage to the host, is essential for survival. Therefore, different *Candida* species have evolved different strategies (recently reviewed in Brunke & Hube, 2013). As *C. albicans* is the most common cause of candidiasis and best investigated model organism of a human pathogenic yeast, the following chapter will focus on *C. albicans*. The most intriguing skill of *C. albicans* is its versatility: During its long coevolution with the human host *C. albicans* gained the ability to strive in host niches that differ dramatically in their environmental conditions, e.g., in regard

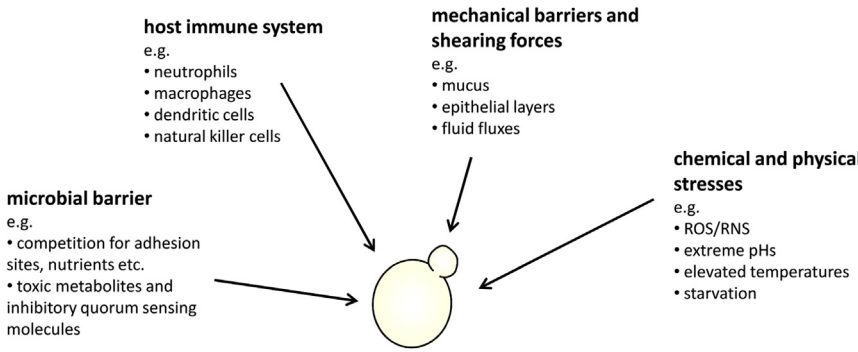

Figure 1 In vivo challenges for *Candida albicans* in the human host.

to pH, nutrient availability, O_2 and CO_2 levels, and the presence of immune cells (Calderone, 2002). In the following sections, we will outline the importance of different *C. albicans* virulence and fitness traits for survival and describe how different phenotypes and the morphological switch between budding yeast and the filamentous hyphal growth form contribute to survival. We will provide examples that show that *C. albicans* on the one hand is very flexible, and has the ability to rapidly respond and adapt to changing environmental conditions, but on the other hand can specialize to certain microniches to use the full capacity of the available resources (Hube, 2009). A summary of important survival mechanisms can be found in Figure 2. Furthermore, because of the emergence of medical device associated and polymicrobial infections in recent years (Bonhomme & d'Enfert, 2013; Bouza et al., 2013; Harriott & Noverr, 2011; Klotz, Chasin, Powell, Gaur, & Lipke, 2007; Morales & Hogan, 2010; Pulimood, Ganesan, Alangaden, & Chandrasekar, 2002; Ramage, Mowat, Jones, Williams, & Lopez-Ribot, 2009; Shirtliff, Peters, & Jabra-Rizk, 2009), part of this chapter will focus on biofilms as multicellular communities and their role as " safe haven" for the yeast. We will review the importance of intercellular and interspecies signaling mechanisms for *C. albicans* in order to build and maintain these multicellular structures. In this context, we will moreover address the interaction of *C. albicans* with members of the human microbiome and bacterial human opportunistic pathogens. These interactions can be beneficial or detrimental for the fungus, and thereby have a tremendous impact on *C. albicans* survival within the host.

2. THE ROLE OF YEAST-TO-HYPHAE TRANSITION AND OTHER PHENOTYPIC CHANGES FOR *C. ALBICANS* SURVIVAL

2.1 Morphogenesis and the Road to Infection

Candida albicans is a polymorphic fungus which can grow in diverse morphological forms, such as round budding yeast cells, pseudohyphae which are ellipsoid cells that are constricted at their septa, or parallel-walled cells with no visible constrictions, so-called true hyphae (Berman & Sudbery, 2002; Odds, 1988; Sudbery, Gow, & Berman, 2004). It was long believed that the formation of filamentous growth forms, morphogenesis, was essential for *C. albicans* virulence, since *C. albicans* mutant strains that are locked in either morphological form were attenuated in their virulence in murine infection models (Braun, Head, Wang, & Johnson, 2000;

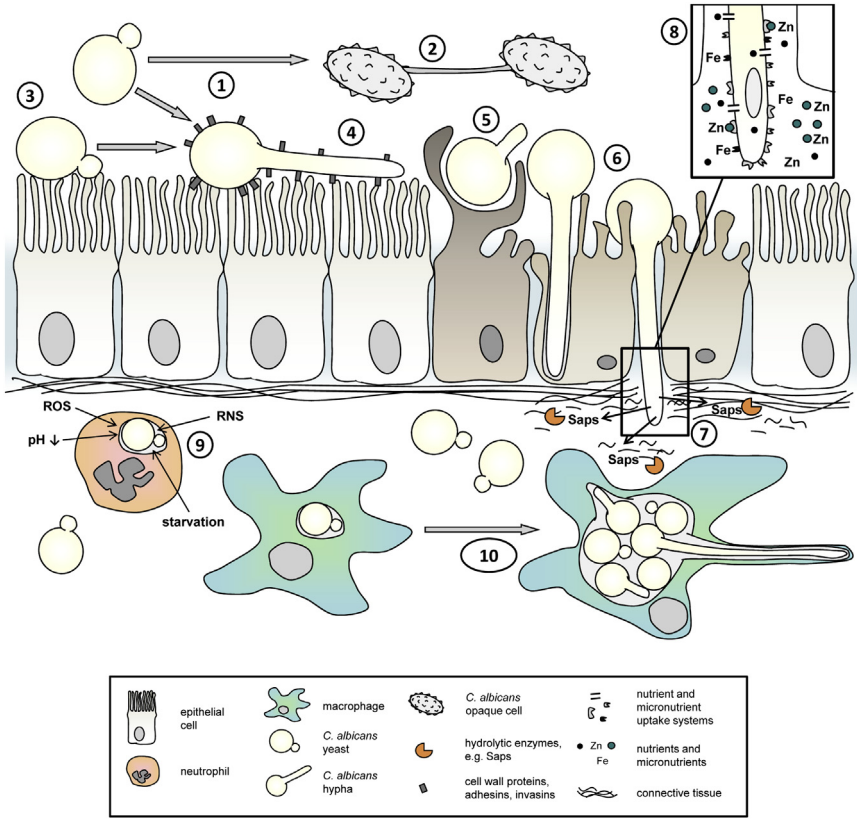

Figure 2 *Candida albicans* survival strategies. (1) morphological flexibility; (2) white-to-opaque switching and mating; (3) contact-induced filamentation; (4) hypha-associated expression of adhesins; invasion into host cells by induced endocytosis (5) or active penetration (6); (7) release of hydrolytic enzymes (e.g., secreted aspartic proteases (Saps)) that support penetration and the breakdown of tissue material; (8) acquisition of nutrients and micronutrients from host cells, e.g., zinc and iron uptake systems; (9) stress response pathways facilitating resistance to adverse environmental conditions, e.g., reactive oxygen species (ROS), reactive nitrogen species (RNS), low pH, and starvation; (10) active modification of the phagosome to promote hyphal growth, facilitating macrophage damage and escape.

Braun, Kadosh, & Johnson, 2001; Lo et al., 1997; Murad et al., 2001). However, recent research suggests that this hypothesis is oversimplified as some strains that are defective for morphogenesis are still virulent in a systemic mouse model (Banerjee et al., 2008; Noble, French, Kohn, Chen, & Johnson, 2010; Saville, Lazzell, Chaturvedi, Monteagudo, & Lopez-Ribot, 2008; Spiering et al., 2010); furthermore, hypha formation is coregulated

with several virulence-associated factors, rendering it difficult to determine to which extend the formation of hyphae per se contributes to virulence (Kumamoto & Vinces, 2005b). Therefore, the current model integrates both, yeast and hyphae, as important players during *C. albicans* infection with distinct roles of the different morphologies during different steps of infection (Gow, Brown, & Odds, 2002; Kumamoto & Vinces, 2005b; Saville, Lazzell, Monteagudo, & Lopez-Ribot, 2003). Both growth forms can be found during systemic infections; yeast forms have been proposed to be important for dissemination via the bloodstream while hyphae formation appears to be associated with invasion of tissue (Jacobsen et al., 2012; Martin, Wachtler, Schaller, Wilson, & Hube, 2011; Zhu & Filler, 2010). The morphology might furthermore reflect niche-specific fungal responses (Jacobsen et al., 2012).

While the role of filamentation for virulence has been studied in detail, comparatively little is known about morphology during commensal growth. In contrast to infection, where invasive growth and host damage are intrinsically linked, commensalism is a balanced state that allows fungal growth without inflicting host damage (Gow & Hube, 2012). Although yeast cells are thought to be the dominant morphology during GI tract colonization, genetic analysis revealed that colonization is associated with high levels of hypha-associated gene (HAG) expression, such as upregulation of *EFH1*, *ECE1*, *RBT4*, and *RBT1* in yeast cells (Doedt et al., 2004; d'Enfert, 2009; Rosenbach, Dignard, Pierce, Whiteway, & Kumamoto, 2010; White et al., 2007). This might promote the maintenance of the yeast cells in the GI tract and is independent of morphogenesis. Efg1, a major regulator of filamentation, was also shown to be important for the regulation of GI tract colonization (d'Enfert, 2009; Kumamoto & Vinces, 2005b; Pierce, Dignard, Whiteway, & Kumamoto, 2013; Pierce & Kumamoto, 2012; Stoldt, Sonneborn, Leuker, & Ernst, 1997). *EFG1* expression in the GI tract can either be high, promoting immune evasion, or rather low, supporting commensal growth. Based on these observations, Pierce et al. hypothesized that variations in Efg1 levels in the GI tract lead to subpopulations of cells with different characteristics, enabling host-dependent shaping and diversity of the colonizing population (Pierce et al., 2013).

2.1.1 Regulation of Morphogenesis

Many virulence-associated traits of pathogenic *Candida* species have possibly evolved to facilitate survival as a commensal, e.g., in the fluctuating environment of the gut and in competition with commensal bacteria. Mechanisms

acquired by the fungus to deal with adverse conditions as a commensal can also promote virulence, since they provide the fungus with the necessary weaponry to overcome host barriers (Hube, 2009; Pierce et al., 2013). Indeed, hypha formation is triggered by environmental signals that resemble unfavorable growth conditions or indicate a putatively hostile environment. Such factors include the presence of serum, elevated temperature, neutral pH, presence of certain nutrients, starvation signals, matrix embedding, CO_2 and O_2 levels, cell density, and contact to physical surfaces (Inglis & Sherlock, 2013; Sudbery, 2011). It is intriguing how many of these factors resemble growth conditions the fungus might encounter in the human host (Cottier & Muhlschlegel, 2009; Sudbery, 2011). In each niche or microniche, *C. albicans* will be affected by a unique combination of biological and chemical factors, which either promote or inhibit morphogenesis (Cottier & Muhlschlegel, 2009). It has recently been proposed that a distinct combination of these environmental conditions might be necessary for the shift from a commensal to pathogenic lifestyle in *C. albicans* (Kadosh & Lopez-Ribot, 2013; Lu, Su, Solis, Filler, & Liu, 2013).

Morphogenesis generally requires two steps, hyphal initiation and hyphal maintenance (Lu, Su, Wang, & Liu, 2011; Martin, Moran, et al., 2011; Sudbery, 2011). Hyphal initiation in response to elevated temperature requires the removal of the filamentation repressor Nrg1 from the promoter regions of HAGs (Lu et al., 2011). In the second step, the absence of Nrg1 allows binding of Brg1, a GATA-transcription factor, to the HAG promoter that recruits the histone deacetylase Hda1. Hda1 in turn leads to chromatin remodeling and the establishment of a filamentous chromatin state promoting hyphal maintenance and the expression of HAGs (Lu et al., 2011; Lu, Su, & Liu, 2012; Su, Lu, & Liu, 2013). However, combinatorial environmental signals have been shown to bypass the requirement for Brg1 and Hda1 by a newly identified O_2 sensor and an uncharacterized CO_2 sensor (Lu et al., 2013).

The yeast-to-hyphae induction is furthermore influenced by a range of small molecules, e.g., cell cycle inhibitors, quorum sensing molecules (QSMs; e.g., farnesol, tyrosol, homoserine lactone (HSL)), fatty acids (e.g., butyric, capric, palmitoleic, linoleic, and arachidonic acid), eicosanoids, peptides and proteins, rapamycin, geldanamycin, and histone deacetylase inhibitors (Shareck & Belhumeur, 2011). Some of these molecules are produced by the fungus itself in order to autoregulate hyphal formation in the presence of environmental stimuli (e.g., QSM, eicosanoids), others may be produced by the host or the host microbiome to manipulate *C. albicans* morphogenesis

(e.g., QSM, fatty acids, peptides, proteins) (Albuquerque & Casadevall, 2012; Hogan, 2006; Nickerson, Atkin, & Hornby, 2006; Shareck & Belhumeur, 2011; Sudbery, 2011). Only recently it was described that a glucanase, secreted by *C. albicans*, has the ability to induce filamentation, which may be an adaptive response to cell wall damaging enzymes (Xu, Nobile, & Dongari-Bagtzoglou, 2013). A range of additional signaling pathways, activated by various environmental signals, stimulate morphogenesis and expression of HAGs. These pathways include the inhibition of heat shock protein 90 (Hsp90) by elevated temperatures and subsequent activation of Ras1, the cAMP/PKA-signaling pathway via direct or indirect activation of the adenylyl cyclase Cyr1, mitogen activated protein kinase (MAPK) signaling via Ras1/Hst7 and Cek1, activation of the Rim101 pathway by neutral to alkaline pH, Czf1 activation under embedded conditions via Rac1, hypoxia-induced Efg1/Efh1 activation, and reactive oxygen species (ROS) signaling induced by genotoxic stress (Gow, van de Veerdonk, Brown, & Netea, 2012; Huang, 2012; Inglis & Sherlock, 2013; Shapiro & Cowen, 2012; Shapiro, Robbins, & Cowen, 2011; Sudbery, 2011). Nrg1, Tup1, and Rfg1, as well as the stress-activated Hog1 pathway are important negative regulators of morphogenesis (Inglis & Sherlock, 2013; Shapiro et al., 2011; Sudbery, 2011). Especially Ras1 and Cyr1 play a crucial role since they integrate a wide range of environmental signals, and in case of Ras1 may even activate a variety of cell signaling cascades (Hogan & Muhlschlegel, 2011; Inglis & Sherlock, 2013). This leads to simultaneous integration of diverse signals to regulate morphogenesis, and allows the coregulation of morphogenesis with a wide variety of other fitness/virulence attributes. Consequently, most pathways do not regulate filamentation discretely but are part of a complex regulatory network that is yet not fully understood. More detailed information on the involved pathways can be found in several recent reviews (Gow et al., 2012; Hogan & Muhlschlegel, 2011; Huang, 2012; Inglis & Sherlock, 2013; Shapiro et al., 2011; Sudbery, 2011). Finally, the different signaling pathways lead to activation or inhibition of key transcriptional regulators, e.g., Efg1, Czf1, Cph1, Tec1, Flo8, and Nrg1, that control expression of genes necessary for hypha formation and hypha-associated genes (Brown, Giusani, Chen, & Kumamoto, 1999; Cao, Lane, Raniga, Lu, Zhou, Ramon, et al., 2006; Giusani, Vinces, & Kumamoto, 2002; Huang, 2012; Kumamoto & Vinces, 2005b; Leberer, Harcus, Broadbent, Clark, Dignard, Ziegelbauer, et al., 1996; Liu, Kohler, & Fink, 1994; Murad et al., 2001; Schweizer, Rupp, Taylor, Rollinghoff, & Schroppel, 2000; Shapiro et al., 2011; Stoldt et al., 1997; Vinces, Haas, & Kumamoto, 2006).

UME6, *HGC1*, and *EED1* are key players in hypha formation (Kumamoto & Vinces, 2005b). Ume6 is an important transcription factor that controls the expression of many HAGs and biofilm formation (Banerjee et al., 2008, 2013; Carlisle et al., 2009; Carlisle & Kadosh, 2010; Zeidler et al., 2009). It has been shown that the ectopic expression of *UME6* can induce hypha formation independent of Efg1 and Cph1, while native *UME6* expression depends on some hyphal regulators (Banerjee et al., 2008; Carlisle et al., 2009; Carlisle & Kadosh, 2010; Zeidler et al., 2009). Only recently it was shown that *UME6* expression is not only regulated on transcriptional, but also on posttranscriptional level via an exceptional long 5′ UTR region that controls the stability of the *UME6* transcript, which underlines the importance of this gene during morphogenesis (Childers, Mundodi, Banerjee, & Kadosh, 2014). Hgc1 is the cyclin partner of Cdk1, a kinase with multiple functions during polarized growth and the inhibition of cell separation (Zheng, Wang, & Wang, 2004). The maintenance of *HGC1* expression depends on Ume6 and, in turn, *UME6* expression depends on *EED1* (Epithelial Escape and Dissemination 1) (Carlisle & Kadosh, 2010; Martin, Moran, et al., 2011). The cellular function of Eed1 is still unknown, but it was shown that this protein is essential for the maintenance of hyphal growth, since mutants lacking *EED1* are still able to initiate filamentous growth, but are unable to maintain polarized growth and switch back to yeast growth (Martin, Moran, et al., 2011; Zakikhany et al., 2007).

Hypha formation is a form of hyperpolarized growth, and the cell biology of hyphal growth, i.e., cell cycle and cell division, is substantially different to yeast cell growth. Mechanisms of hyphal growth have been reviewed in detail elsewhere (Arkowitz & Bassilana, 2011; Steinberg, 2007; Sudbery, 2011; Virag & Harris, 2006) and describing the mechanism of this hyperpolarized growth is beyond the aim of this chapter.

2.1.2 Tropisms—To Know Where to Go

Filamentation can be seen as an escape mechanism or an alternative to movement. As such, the growth has to be directed, for example, toward a signal (tropism) or through barriers toward a new environment. For *C. albicans*, tropism includes the fungal ability to reorientate the growth direction of the hyphal tip in response to environmental cues (Brand & Gow, 2009). Thigmotropism, the orientation of hyphal growth depending on the surface configuration, is the best studied form of tropism in *C. albicans*. This tropism is facilitated by mechanosensors in the hyphal membrane e.g., Fig1/Mid1, which activate the influx of extracellular calcium via Ca^{2+}-channels (e.g.,

Cch1) (Brand & Gow, 2009; Brand, Lee, Veses, & Gow, 2009; Kumamoto, 2008a; Yang et al., 2011; Yu et al., 2012). The resulting localized Ca^{2+} gradients then direct the reorientation of growth (Brand & Gow, 2009). Galvanotropism (orientation within an electric field), aerotropism (orientation via oxygen levels) and chemotropism (orientation by chemicals, e.g., pheromones, chemokines) have also been described for *C. albicans* (Aoki, Ito-Kuwa, Nakamura, Vidotto, & Takeo, 1998; Brand et al., 2007; Crombie, Gow, & Gooday, 1990; Daniels, Srikantha, Lockhart, Pujol, & Soll, 2006; Gooday & Adams, 1993). Tropisms of *C. albicans* may play an important role for epithelial and tissue penetration and damage, as mutants that fail to reorient hyphal growth were shown to be attenuated in their ability to penetrate and damage epithelial cells (Brand & Gow, 2009; Brand, Vacharaksa, et al., 2008; Davies, Stacey, & Gilligan, 1999). Chemotropism along a pheromone gradient plays a key role for mating (see below).

2.1.3 *Contribution of Hypha Formation and Hypha-Associated Genes to* C. albicans *Survival and Pathogenesis*

The complex regulatory network of morphogenesis described above facilitates the concomitant expression of factors that do not directly affect morphogenesis, termed HAGs. Some HAGs are important virulence factors that contribute to the role of filamentation in pathogenesis. As the complex contribution of HAGs to survival and pathogenesis of *C. albicans* has been reviewed in detail elsewhere (Kumamoto & Vinces, 2005b), we will only provide a summary of the role of selected HAGs for virulence. HAGs can encode intracellular, cell wall, or secreted components, with a range of functions. Some of these genes are involved in initiation and maintenance of hypha formation itself. Upon induction of environmental signaling the corresponding proteins are activated and promote the formation of hyperpolarized growth, e.g., Efg1, Cph1, Ras1, Tec1, Hgc1, Ume6, and Eed1 (Banerjee et al., 2008; Feng, Summers, Guo, & Fink, 1999; Leberer et al., 1996; Liu et al., 1994; Martin, Moran, Jacobsen, Heyken, Domey, Sullivan, et al., 2011; Schweizer et al., 2000; Stoldt et al., 1997; Zakikhany et al., 2007; Zeidler et al., 2009; Zheng et al., 2004). Hyphae-associated cell wall proteins and secreted proteins include Als3, Hwp1, Rbt1, and Rbt4, that promote tight adhesion as the first step for invasive growth (see section *From Attachment to Disease: Adhesion, Invasion, and Damage*) (Braun & Johnson, 1997; Kumamoto & Vinces, 2005b; Staab, Bradway, Fidel, & Sundstrom, 1999; Staab, Datta, & Rhee, 2013; Sundstrom, Balish, & Allen, 2002; Zhao et al., 2004). Als3 is furthermore required for iron acquisition from host cells (Almeida et al., 2008; Almeida,

Wilson, & Hube, 2009). Hypha-associated hydrolytic enzymes (encoded by *SAP4*, *SAP5*, or *SAP6*) promote penetration and destruction of surrounding tissue, thereby liberating nutrients from host cells (Felk et al., 2002; Kumamoto & Vinces, 2005b; Naglik, Challacombe, & Hube, 2003). Many of the survival mechanisms described in the following sections, e.g., adhesion, invasion, biofilm formation, are tightly interlinked with morphogenesis and either depend upon the formation of filamentous growth forms itself, or indirectly require the expression of HAGs.

In summary, morphogenesis is indeed beneficial for *C. albicans* survival. Important HAGs help to deal with unfavorable or toxic growth conditions and stress. Furthermore, the formation of hyphae per se will give the fungus the opportunity to gain access to different host niches, to more easily gather nutrients and/or avoid competition for adhesion sites and nutrition by other microbes (Hube, 2004; Kumamoto, 2008b; Romani, Bistoni, & Puccetti, 2003). While yeast cells are able to proliferate in the tissue, hyphae are considered to be the motility form of *C. albicans* growth (Brand & Gow, 2009; Hube, 2009). They promote tissue dissemination, drive invasion by mechanical forces and HAG expression, and enable the fungus to escape from host cells (Gow, 2009; Kumamoto & Vinces, 2005a,b; Lorenz, Bender, & Fink, 2004; Whiteway & Oberholzer, 2004). In addition, hyphae are differentially recognized by major players of the human immune system leading to a rather anti-inflammatory response after recognition (Gow, 2013). However, the dissemination of *C. albicans* may lead to bloodstream infection associated with high mortality rates, which could ultimately lead to the elimination of the host and thereby also the fungus, for which no terrestrial life cycle exists (Gow, 2013; Romani et al., 2003). Since, from the evolutionary point of view, it cannot be the aim of the fungus to destroy its host, systemic infection might rather be an accident of *C. albicans* foraging or the search for shelter (Bliska & Casadevall, 2009; Casadevall, 2008; Hube, 2009; Romani et al., 2003). Indeed it is thought that *C. albicans* might in first line be a commensal of humans, the pathogenic and invasive form more the exception that occurs under certain incidents (d'Enfert, 2009). The high numbers of asymptomatic healthy carriers of *C. albicans*, as well as the severe risk factors that have to be met to develop disseminated candidiasis strongly support this hypothesis.

2.2 Phenotypic Switching—Sex, Commensalism and the Adaptation to the Host

In addition to the prominent transition between yeast and filamentous growth, *C. albicans* can undergo a range of other phenotypic transitions

(Soll, 2014). The term "phenotypic switching" has been introduced in 1985 as the ability of *C. albicans* to undergo spontaneous, reversible transition between a number of colony morphologies (Soll, 1992, 2014). The best studied phenotypic transition is the white-to-opaque switch, which is necessary for mating in *C. albicans* (Huang, 2012; Lohse & Johnson, 2009; Miller & Johnson, 2002; Morschhauser, 2010; Slutsky et al., 1987; Soll, 2014).

2.2.1 The White-to-Opaque Switch and Mating in C. albicans

Mating and sexual reproduction in natural *C. albicans* strains was first described in 2002 (Miller & Johnson, 2002). The default state of *C. albicans* is white cell growth, characterized by round to oval cells growing as smooth colonies on an agar surface. These diploid *C. albicans* cells are usually heterozygous at the mating type locus (MTL) in an a/α state (Lockhart et al., 2002; Soll, 2014). In order to become mating competent *C. albicans* cells have to undergo two major changes: First they have to become MTL homozygous (a/a or α/α) (Lockhart et al., 2002; Miller & Johnson, 2002). This is achieved by genetic changes on the chromosomal level, i.e., gene conversion, crossing-over events or loss of one copy of chromosome 5, that harbors the MTL, and subsequent duplication of the remaining copy (Soll, 2014; Wu, Lockhart, Pujol, Srikantha, & Soll, 2007; Wu, Pujol, Lockhart, & Soll, 2005). In the second step, the MTL homozygous white cells have to switch to the opaque cell phenotype to become mating competent (Lockhart, Zhao, Daniels, & Soll, 2003; Lockhart, Daniels, Zhao, Wessels, & Soll, 2003). This process is regulated by a complex network at different levels of regulation which have been reviewed in detail elsewhere (Bennett, 2009; Huang, 2012; Morschhauser, 2010; Soll, 2014). Important environmental cues that promote switching and thereby mating are elevated CO_2 levels, certain sugar sources, temperature, genotoxic and oxidative stress, and white blood cell metabolites (Soll, 2014). It is striking that many of these signals are considered unfavorable for *C. albicans* growth, supporting the idea that increased genetic heterogeneity by mating promotes the emergence of *C. albicans* subpopulations that might be better adapted to unfavorable conditions, thereby supporting *C. albicans* survival in the host. The master regulator of switching is Wor1, a transcription factor that binds to WOPR-boxes in its target genes and induces switching to mating competent opaque cells (Huang et al., 2006; Lohse et al., 2013; Srikantha et al., 2006; Tuch et al., 2010; Zordan, Galgoczy, & Johnson, 2006). Wor1 itself is regulated via Efg1, Czf1, and Wor2, as well as Ahr1 and Wor3 (Hernday et al., 2013; Huang, 2012; Zordan, Miller, Galgoczy, Tuch, & Johnson, 2007).

Furthermore the Ras1/cAMP/PKA pathway has been shown to be involved in white-opaque-switch regulation, probably by regulating transcription factors that in turn regulate *WOR1* expression (Inglis & Sherlock, 2013). During *C. albicans* mating, mating competent cells secrete pheromones (a-pheromones from a/a cells and α-pheromones from α/α cells), which stimulate cells of the opposite mating type, respectively, to form elongated evaginations along a pheromone gradient which ultimately fuse to form so-called conjugation tubes (schematically shown in Figure 2) (Bennett, Uhl, Miller, & Johnson, 2003; Lockhart, Zhao, et al., 2003; Panwar, Legrand, Dignard, Whiteway, & Magee, 2003; Soll, 2014). The nuclei of both mating cells subsequently migrate into the tube and fuse to form a tetraploid daughter cell at the fusion site (Bennett, Miller, Chua, Maxon, & Johnson, 2005; Lockhart, Zhao, et al., 2003). The tetraploid cell returns to a diploid state by random loss of chromosomes and recombination events (Bennett & Johnson, 2003; Forche et al., 2008).

White and opaque cells differ in their cellular morphology, mating competence, and gene expression, especially in genes for metabolism (Lan et al., 2002; Soll, 2014). Both white and opaque cells are able to filament, but use different regulatory pathways (Guan et al., 2013; Si, Hernday, Hirakawa, Johnson, & Bennett, 2013). Interestingly, opaque cells have lost their ability to secrete important chemoattractants for polymorphonuclear neutrophils (PMNs) and are therefore invisible to these immune cells under certain conditions (Geiger, Wessels, Lockhart, & Soll, 2004; Sasse, Hasenberg, Weyler, Gunzer, & Morschhauser, 2013). Furthermore, white cells are more virulent during systemic murine infection, whereas opaque cells are better colonizers in a murine skin model (Geiger et al., 2004; Kvaal et al., 1999; Kvaal, Srikantha, & Soll, 1997; Sasse et al., 2013). Thus, white—opaque switching can affect *C. albicans* survival in the host via mating, thereby promoting cell diversity, and by influencing immune recognition and virulence.

2.2.2 Gastrointestinally Induced Transition Cells, Gray Cells, and Other Phenotypic Variants—Diversity in Controlling Commensalism and Host Adaptation

Only recently new phenotypic variants of *C. albicans* have been discovered that might have a major impact on *C. albicans* survival within the host. In 2013, Pande et al. described a new cell type that emerged from a murine model of stable gastrointestinal (GI) colonization (Pande, Chen, & Noble, 2013). Propagation of the MTL-heterozygous strain within the mammalian GI tract induced overexpression of *WOR1* in a subpopulation of cells. This

was surprising since *WOR1* expression in vitro is restricted to MTL homozygous opaque cells. Although these GUT cells (for Gastrointestinally Induced Transition) shared some phenotypic characteristics with opaque cells, the two cell types were distinct. The major characteristic of the GUT cells was the enhanced fitness within the GI tract over wild type *C. albicans* cells, which was also reflected by a reorientation of their metabolism toward the nutrients available within the distal mammalian GI tract. Therefore, cues from the GI tract trigger the expression of *WOR1* in a subset of initially colonizing cells that become GUT cells, which subsequently promote colonization via metabolic adaptation.

In 2014, Tao et al. discovered a "gray" phenotype that is distinct from white and opaque cells in several cell biological aspects (Tao et al., 2014): Gray cells were induced by growth on yeast-peptone-dextrose (YPD) medium and showed an elongated, small cellular phenotype, high secreted aspartic protease (Sap) activity in bovine serum albumine (BSA)-containing media and substantial changes in global gene expression. Gray cell expression did neither require Efg1 nor Wor1, but deletion of both important regulators of white—opaque switching lead to constant gray cell phenotype. Gray cells, similar to opaque cells, had a decreased ability to form filaments and were less virulent in a systemic murine candidiasis model. In contrast, virulence in ex vivo tongue infection and in vivo skin infection models was increased, possibly due to increased Sap-activity.

A major difference between the recently described phenotypes, GUT and gray cells, is their stability under in vitro conditions. GUT cells were only formed during in vivo GI colonization and were not stable in vitro, whereas gray cells were discovered in vitro and were stable under a variety of culture conditions (Pande et al., 2013; Tao et al., 2014). The discovery of these cell types suggests that phenotypic switching may be a general feature of natural *C. albicans* strains. It is possible, that more yet unidentified phenotypic variants of *C. albicans* exist in certain host niches, representing specialized growth forms under environmental pressures. The high frequency and diversity of phenotypic and morphologic switching therefore likely promote survival of *C. albicans* within the human host (Soll, 2002).

3. HOW TO DEAL WITH STRESS—LESSONS FROM *C. ALBICANS*

In order to survive in the host, *C. albicans* has to adapt to changing conditions and host-derived stresses and the aptitude to cope with a wide

range of stresses is a prerequisite for life in the human host. Stress-responsive pathways and their downstream targets were shown to be necessary for colonization but also for virulence of *C. albicans* (Alonso-Monge et al., 1999; Csank et al., 1998; Diez-Orejas et al., 1997; Prieto, Roman, Correia, & Pla, 2014). Important adaptation mechanisms and recent insights in survival strategies under various stress conditions have recently been reviewed (Brown, Budge, et al.,2014; Brown, Haynes, Gow, & Quinn, 2012; Brown, Haynes, & Quinn, 2009). This section summarizes common stresses the fungus has to deal with and highlights the most important survival mechanisms.

3.1 The Heat Shock Response and Fungal Morphogenesis

Although *C. albicans* colonizes a thermally comparatively stable niche, functional heat shock responses have been retained in the fungus (Nicholls, Leach, Priest, & Brown, 2009). These responses include conserved reactions induced by diverse stressful conditions, which can cause protein-misfolding and nonspecific protein aggregation ultimately leading to cell death. Heat shock proteins (Hsps) thereby act as molecular chaperones that prevent misfolding and aggregation by binding and stabilizing their target proteins. In *C. albicans*, many cues that induce morphogenesis in vitro, e.g., high CO_2 levels and serum, also require a concomitant temperature shift to 37 °C to induce true hypha formation. A central regulator of this response is the chaperone Hsp90 (Shapiro & Cowen, 2012; Shapiro et al., 2009). Hsp90 is integrated in a complex regulatory network, interacting with a considerable proportion of the *C. albicans* proteome (Diezmann, Michaut, Shapiro, Bader, & Cowen, 2012). Below 37 °C, Hsp90 inhibits filamentation; elevating the temperature leads to an increase in protein-misfolding and Hsp90-mediated release of repression of morphogenesis (Diezmann et al., 2012; Shapiro & Cowen, 2012). Hsp90 furthermore impacts other signaling pathways and affects phenotypic switching, drug resistance, and biofilm formation in response to temperature shifts (Diezmann et al., 2012; Leach, Budge, et al., 2012; Leach, Klipp, Cowen, &, Brown, 2012; Leach, Tyc, Brown, & Klipp, 2012; Shapiro & Cowen, 2012). Further major Hsps with diverse biological roles in *C. albicans* include Hsp104, Hsp78, Ssa1, Ssa2, and Hsp60 (Mayer, Wilson, & Hube, 2013a). The expression of these Hsps is mainly controlled by the transcription factor Hsf1 (Sorger & Pelham, 1987, 1988). In addition, six small heat shock proteins (sHsps), that have to form multimeres to bind their client proteins, have been predicted in *C. albicans* (Mayer et al., 2013a). These include Hsp12 and Hsp21, which are both upregulated under a variety of stress conditions (Fu, De Sordi, & Muhlschlegel, 2012; Mayer, Wilson, & Hube,

2013b; Mayer, Wilson, Jacobsen, Miramon, Slesiona, et al., 2012). In addition, thermal and oxidative stress lead to the intracellular accumulation of trehalose, which can function as a "chemical chaperone" by stabilizing protein folding (Brown, Budge, et al., 2014). Hsp21 is crucial for regulating intracellular trehalose levels and enhances antifungal drug resistance (Mayer, Wilson, Jacobsen, Miramon, Slesiona, et al., 2012; Mayer et al., 2013b).

3.2 Hog1 and the Adaptation to Osmotic and Cationic Stresses

Responses to osmotic, oxidative, nitrosative, and cell wall stresses are regulated by a range of different MAPK pathways (Brown, Budge, et al., 2014; Brown, Haynes, et al., 2012; Monge, Roman, Nombela, & Pla, 2006). Environmental signals induce cascades of kinases that ultimately lead to the activation of downstream transcription factors that regulate the adaptive stress response (Monge et al., 2006). Hog1 (High-osmolarity glycerol 1), a kinase which is activated in response to osmotic, oxidative, thermal, heavy metal, and cell wall stresses, has key functions in the core stress response of *C. albicans* (Smith, Morgan, & Quinn, 2010; Smith, Nicholls, Morgan, Brown, & Quinn, 2004). Activation of Hog1 can be mediated via a two-component signaling pathway involving Sln1, and via signal input from the morphogenetic Cek1-MAPK pathway by Ste11 (Brown, Budge, et al., 2014; Monge et al., 2006). Hog1 also negatively regulates Cek1, thereby affecting morphogenesis, and consequently *hog1*Δ mutants are hyperfilamentous (Alonso-Monge et al., 1999; Eisman et al., 2006). Concurrent lack of Hog1 and Cek1 activation leads to a synthetic lethal phenotype upon osmotic stress, underlining the importance of both signaling pathways under this stress condition (Herrero-de-Dios, Alonso-Monge, & Pla, 2014). The adaptation to osmotic stress is specifically important in certain host niches. For example, NaCl concentrations can be extremely high in the kidneys and urine, and *C. albicans* has to deal with K^+-fluxes during phagocytosis (Brown, Budge, et al., 2014). Not surprisingly, mutants lacking Hog1 are strongly attenuated in a systemic mouse model of infection (Alonso-Monge et al., 1999).

3.3 A Toxic Weaponry—How to Deal with Oxidative and Nitrosative Stress

Oxidative stress mediated by ROS is a common byproduct of respiration. Furthermore ROS are produced by host immune cells as defense mechanisms, causing damage to DNA, proteins, and lipids (Bogdan, Rollinghoff, & Diefenbach, 2000; Brown et al., 2009). In order to overcome the toxic

effects of intracellular or extracellular ROS, *C. albicans* has developed a wide range of defense and detoxifying mechanisms (reviewed in Alonso-Monge, Roman, Arana, Pla, & Nombela, 2009; Bogdan et al., 2000; Brown, Budge, et al., 2014; Brown et al., 2009; Miramon, Kasper, & Hube, 2013): *C. albicans* encodes several superoxide dismutases (Sods) localized intracellulary (e.g., Sod1), mitochondrially (Sod2), or on the cell surface (Sod4 on yeast cells, Sod5 on hyphae) (Fradin et al., 2005; Frohner, Bourgeois, Yatsyk, Majer, & Kuchler, 2009; Gleason, Galaleldeen, et al., 2014; Gleason, Li, Odeh, & Culotta, 2014; Heilmann et al., 2011; Hwang, Baek, Yim, & Kang, 2003; Hwang et al., 1999, 2002; Lamarre, LeMay, Deslauriers, & Bourbonnais, 2001; Martchenko, Alarco, Harcus, & Whiteway, 2004; Rhie et al., 1999). Sods are responsible for the breakdown of superoxide radicals and are particularly important for dealing with phagocyte-derived oxidative stress (Chaves, Bates, Maccallum, & Odds, 2007; Fradin et al., 2005; Frohner et al., 2009; Hwang et al., 2002; Miramon et al., 2012). Superoxide detoxification ultimately leads to the production of hydrogen peroxide, that is detoxified via catalase (Cat1), or glutathione peroxidases (Gpx's) (Fradin et al., 2005; Lorenz et al., 2004; Nakagawa, Kanbe, & Mizuguchi, 2003; Wysong, Christin, Sugar, Robbins, & Diamond, 1998). The oxidized glutathione (GSH) is then recycled by at least two glutathione reductases, Grx2 and Grl1 (Miramon et al., 2013). Thioredoxins also play a role in peroxide detoxification in *C. albicans*, e.g., while facing neutrophils, but their role is not clear yet (Enjalbert, MacCallum, Odds, & Brown, 2007; Miramon et al., 2012; da Silva Dantas et al., 2010; Urban et al., 2005). Another important antioxidant mechanism involves the intracellular accumulation of trehalose (Martinez-Esparza et al., 2007; Mayer, Wilson, Jacobsen, Miramon, Slesiona, et al., 2012). This broad repertoire of ROS detoxifying mechanisms make *C. albicans* relatively resistant to oxidative stress, tolerating up to 20-mmol/L H_2O_2 (Brown, Budge, et al., 2014). The oxidative stress response in *C. albicans* is mainly regulated by the transcription factor Cap1 (Alarco & Raymond, 1999). Hog1, as core stress regulator, also contributes to the oxidative stress response although it only plays a minor role in the transcriptional response to ROS in *C. albicans* (Enjalbert et al., 2006; Smith et al., 2004, 2010). Immune cells also produce reactive nitrogen species (RNS) to combat pathogens. A nitrosative stress response is especially important for the survival of *C. albicans* in the interaction with neutrophils and is primarily regulated by the transcription factor Cta4 (Chiranand et al., 2008). The key detoxifying mechanisms for RNS rely on a flavohemoglobin Yhb1, a nitric oxide dioxygenase which converts

nitric oxide to ammonia (Hromatka, Noble, & Johnson, 2005; Miramon et al., 2013; Ullmann et al., 2004).

Global responses to ROS and RNS are upregulated in *C. albicans* after phagocytosis by neutrophils or macrophages, and following exposure to human blood or mucosal tissue, suggesting that *C. albicans* is exposed to oxidative and nitrosative stress during infection (Enjalbert et al., 2007; Fradin et al., 2005; Lorenz et al., 2004; Zakikhany et al., 2007). However, during tissue infection, e.g., in the kidneys, these responses were not as vital as expected, as shown by diagnostic green fluorescent protein (GFP)-fusions in a mouse model of disseminated infection (Enjalbert et al., 2007; Thewes et al., 2007; Walker et al., 2009; Wilson et al., 2009). Therefore, Brown et al. hypothesized that adaptation to oxidative stress might be crucial for the early stages of infection, but less important after establishment of systemic infection (Brown, Budge, et al., 2014; Brown et al., 2009).

3.4 Adaptation to Cell Wall Stresses

A variety of environmental conditions, including osmotic, oxidative, thermal, and cationic stresses, as well as exposure to certain antifungals ultimately affect stability, structure, and integrity of the cell wall. Attachment to a surface and induction of hyperpolarized growth require constant rearrangement of the cell wall structure, thereby presenting another form of cell wall stress (Klis, de Koster, & Brul, 2014). Not surprisingly, several signaling pathways are thus involved in the response to cell wall stress, including the three MAPK pathways with their key factors Hog1, Cek1, and Mkc1 (Brown, Budge, et al., 2014; Brown et al., 2009; Monge et al., 2006). Hog1 is a core stress regulator (see above). The Cek1 cascade, involving Cst20, Ste11, and Hst7, is primarily involved in morphogenesis but also cell wall integrity (Monge et al., 2006). The Cek1 pathway is regulated by growth signals and quorum sensing (Roman, Nombela, & Pla, 2005; Sato, Watanabe, Mikami, & Matsumoto, 2004), and the cascade involving Cek1 and Cek2 has been shown to be important for mating (Chen, Chen, Lane, & Liu, 2002; Chen, Wang, & Chen, 2000; Magee, Legrand, Alarco, Raymond, & Magee, 2002). The other important cell integrity pathway involves Mkc1 (Brown, Budge, et al., 2014; Monge et al., 2006). Mkc1 signaling is important under a broad range of stresses for maintaining the cell wall integrity and biogenesis, for invasive growth under embedded conditions and biofilm formation (Brown, Budge, et al., 2014; Monge et al., 2006). As cell wall stress is usually coupled with other forms of stressors, it is difficult to assess the relative contribution of resistance to cell wall stress to survival of *C. albicans* in the host; however, it

appears plausible that maintenance of a stable, intact cell wall is essential for replication of the fungus in vivo.

3.5 pH Adaptation in *C. albicans*

The pH in the human host differs dramatically according to the anatomical site. While the pH in the blood and tissues is rather neutral (pH 7.4 ± 0.1), the human vaginal cavity is acidic (pH ~4) and the pH along the digestive tract differs significantly, ranging from pH 2 to 8 (Davis, 2009). In contrast to bacteria, fungi prefer a slightly acidic pH for their growth. Why is the pH important for *C. albicans*? Nutrient uptake is driven to a large extend by proton gradients, which will not work at alkaline pH (Davis, 2009). Furthermore some essential micronutrients, e.g., iron are only soluble and available for the fungus at acidic pH (Davis, 2009). Furthermore, protein stability and enzyme function are affected by the pH. Alkaline pH might negatively affect the function of important fungal proteins and thus represents stress for *C. albicans* (Davis, 2009; Sosinska et al., 2011). In order to appropriately respond to environmental pH, signaling pathways that sense changes in the environmental pH and drive appropriate downstream responses have evolved. In *C. albicans*, the Rim101 signal transduction pathway is of major importance for the response to alkaline pH (Davis, 2009). Changes in pH are sensed by the plasma membrane receptors Dfg16 and Rim21, which in turn activate a cascade leading to the activation of the transcription factor Rim101 (Barwell, Boysen, Xu, & Mitchell, 2005; Castrejon, Gomez, Sanz, Duran, & Roncero, 2006; Rothfels et al., 2005; Thewes et al., 2007). Rim101 has been shown to be important for immune evasion, iron acquisition and full virulence in vivo (Bensen, Martin, Li, Berman, & Davis, 2004; Davis, Edwards, Mitchell, & Ibrahim, 2000; Davis, Wilson, & Mitchell, 2000; Nobile, Solis, et al., 2008). pH adaptation furthermore requires two functionally redundant cell wall β-glycosidases, *PHR1*, expressed at neutral/alkaline pH, and *PHR2*, expressed at acidic pH (Fonzi, 1999; Muhlschlegel & Fonzi, 1997). Phr1 supports filamentous growth of *C. albicans* and systemic infection, Phr2 and the Rim101 pathway yeast growth and vaginal infection (De Bernardis, Muhlschlegel, Cassone, & Fonzi, 1998; Fonzi, 1999). Other pH sensing pathways in *C. albicans* involve Mds3 and calcineurin, that are both required for full virulence (Davis, Bruno, Loza, Filler, & Mitchell, 2002; Kullas, Martin, & Davis, 2007; Zacchi, Gomez-Raja, & Davis, 2010). *Candida albicans* has furthermore developed mechanisms to actively change the pH of its environment (see *Nutrient and Micronutrient Acquisition and the Role of Metabolism for Candida Survival*).

3.6 How to Breathe—Adaptation to Hypoxia and Hypercapnia

Oxygen plays a central role in metabolism, but levels vary significantly in different host niches (Grahl, Shepardson, Chung, & Cramer, 2012). Especially regions of the human GI tract are considered to be hypoxic to anaerobic, and hypoxic conditions are also found in infected tissue (He et al., 1999; Karhausen et al., 2004). Therefore, *C. albicans* adaptation to hypoxia is important for both, successful colonization of the GI tract and infection. Low oxygen levels are often directly coupled with increased levels of carbon dioxide (hypercapnia), and both conditions trigger filamentation in *C. albicans* (Dubin & Estenssoro, 2008; Ernst & Tielker, 2009; Inglis & Sherlock, 2013; Klengel et al., 2005; Lu et al., 2013). *Candida albicans* can grow at very low oxygen conditions by fermenting glucose to ethanol (Rozpedowska et al., 2011). Hypoxia furthermore induces alterations of metabolism toward fatty acid metabolism and glycolysis, major changes in iron metabolism, ergosterol synthesis, and alterations of cell wall and membrane structure (Grahl et al., 2012). Molecules that require oxygen for their biosynthesis are indirect sensors for oxygen levels and stimulate changes in the overall fungal metabolism; ROS and RNS may play important roles as signaling molecules during hypoxic conditions (Cap, Vachova, & Palkova, 2012; Grahl et al., 2012). A major regulator of the hypoxic response is Efg1, however, Efh1, Tye7, and Ace2 are also involved (Bonhomme et al., 2011; Doedt et al., 2004; Mulhern, Logue, & Butler, 2006; Sellam et al., 2014; Setiadi, Doedt, Cottier, Noffz, & Ernst, 2006). Under hypoxic conditions Efg1 negatively regulates hypha formation, but induces genes associated with biofilm formation (Setiadi et al., 2006; Stichternoth & Ernst, 2009). Since Efg1 is an important regulator of GI tract colonization, a mostly hypoxic environment, Grahl et al. speculate that adaptation of hypoxia under these growth conditions may be important for colonization (Grahl et al., 2012).

3.7 Sequential and Combinatory Stresses, and the Situation In vivo

In a certain host niche, *C. albicans* most likely will not just be exposed to one but to multiple stresses, imposed sequentially or simultaneously. The adaptation to such sequential or "combinatorial" stresses has recently been reviewed comprehensively in Brown, Budge, et al. (2014). Exposure of *C. albicans* to nonlethal stresses can protect yeast cells against subsequent

exposure to the same (hormesis) or different stresses (stress cross-protection) (Brown, Budge, et al., 2014). Examples include thermotolerance, osmotolerance, and acquired tolerance to oxidative stress, as well as the cross-protection of thermal stress to subsequent oxidative stress (Arguelles, 1997; Enjalbert et al., 2007; Jamieson, Stephen, & Terriere, 1996; Leach, Klipp, et al., 2012; Leach, Tyc, et al., 2012; You et al., 2012). These responses rely on the initiation of molecular responses to the initial signal/stress, which leads to the activation and accumulation of key proteins and regulators that mediate not only adaptation to this specific signal/stress, but also activate a core transcriptional program, that subsequently mediates protection against other stresses (Brown, Budge, et al., 2014; Leach, Tyc, et al., 2012). From an evolutionary point of view, this may represent the adaptation of *C. albicans* to relatively predictable niches, where one stress is often followed by a second type of stress, called "adaptive prediction" (Brown, Budge, et al., 2014; Brunke, & Hube, 2014; Mitchell et al., 2009). One intriguing example is the activation of oxidative stress genes following the exposure to glucose, which could represent the situation in vivo, when *C. albicans* enters the bloodstream (which is relatively high in glucose levels compared to the surrounding tissues) where the fungus will likely face ROS produced by phagocytic cells (Brown, Budge, et al., 2014; Rodaki et al., 2009). Combinatorial stresses presumably more accurately describe the in vivo situation, where *C. albicans* has to cope with several simultaneous stresses, e.g., ROS, RNS, cationic fluxes, pH changes, and nutrient starvation in the phagolysosome (schematically illustrated in Figure 2) (Brown, Budge, et al., 2014; Miramon et al., 2013). The combination of several stressors enhances the likelihood of the host to overwhelm the adaptiveness of *C. albicans* and clear the fungus (Brown, Budge, et al., 2014; Kaloriti et al., 2014).

4. GENETIC FLEXIBILITY—ADAPTATION ON GENOMIC, TRANSCRIPTIONAL, AND TRANSLATIONAL LEVEL

4.1 Genomic Adaptation and Chromosome Instability in *C. albicans*

The preceding sections about morphological and phenotypic adaptations, and the ability to cope with a wide range of stress conditions already imply the tremendous ability of *C. albicans* to change its morphology and global transcriptional profile in response to unfavorable growth conditions. This phenotypic diversity is enhanced by the high variability of *C. albicans* on the genomic level. The genome of the naturally diploid yeast is notoriously

unstable (Barton & Scherer, 1994; Odds, Brown, & Gow, 2004; Rustchenko, 2007). Some laboratory strains were found to be aneuploids, e.g., WO-2 (Magee & Magee, 1997; Rustchenko-Bulgac, 1991), and the formation of random chromosome alterations also occurs in vivo (Chen, Magee, Dawson, Magee, & Kumamoto, 2004; Rustad, Stevens, Pfaller, & White, 2002). Aneuploidies and gene duplications can have immediate effects on adaptation by provoking gene dosage effects (Fischer, Hube, & Brunke, 2014). *Candida albicans* forms tetraploid cells during mating, and subsequent extensive chromosome loss will return the cell to a diploid state (Bennett & Johnson, 2005; Hull, Raisner, & Johnson, 2000; Johnson, 2003; Magee & Magee, 2000, 2004; Soll, 2004). This occurs in some cells more efficiently than in others, leading to a range of intermediate states between a diploid and tetraploid state within a population (Rustchenko, 2007). In addition, it has recently been shown that *C. albicans* can furthermore form viable haploid cells in vitro and in vivo (Hickman et al., 2013). These cells can undergo mating and phenotype changes similar to their diploid counterparts (Hickman et al., 2013). Altered karyotypes often come with a fitness cost, since they might interfere with important cellular functions (Rustchenko, 2007). However, it was shown by Rustchenko et al. that chromosome instability in *C. albicans* also changes the expression of catabolic pathways, which could favor the adaptation to changing nutrient availability (Rustchenko, Howard, & Sherman, 1997). In environments that are potentially lethal, *C. albicans* survives due to a rapid adaptation largely based on a reversible change in copy number of specific chromosomes (Harrison et al., 2014; Perepnikhatka et al., 1999; Rustchenko, 2007; Selmecki, Dulmage, Cowen, Anderson, & Berman, 2009; Selmecki, Forche, & Berman, 2006). A quite common phenomenon is monosomy of chromosome 5 which alters the compositions of the *C. albicans* cell wall and confers increased resistance to several stresses, e.g., L-sorbose, fluconazole, and amphotericin B (Janbon, Sherman, & Rustchenko, 1998; Kabir, Ahmad, Greenberg, Wang, & Rustchenko, 2005; Yang, Kravets, Bethlendy, Welle, & Rustchenko, 2013). Chromosome 5 copy number loss is also important during mating as a major mechanism to create MTL homozygous strains (Wu et al., 2005).

Mutations are the driving force in evolution and again, *C. albicans* rapidly accumulates mutations. The genome of *C. albicans* laboratory strain SC5314 harbors approximately 60,000 single nucleotide polymorphisms, and loss of heterozygosity (LOH) rates are high with $\sim 10^{-6}$ to 10^{-7} events/cell division under nonstress conditions (Forche et al., 2011; Jones et al., 2004). The capability of establishing chromosomal and genetic changes may be one of

the most important prerequisites for the adaptation of *C. albicans* to its human host and adaptation to different host niches (recently reviewed in Fischer et al., 2014). Indeed, persistence of *C. albicans* in patients and during colonization of mucosal surfaces is associated with genomic microvariations, e.g., genome rearrangements, extensive LOH, chromosome length polymorphisms, or chromosome copy number variations (Bougnoux et al., 2006; Fischer et al., 2014; Odds & Jacobsen, 2008; Sampaio et al., 2005; Schroppel, Rotman, Galask, Mac, & Soll, 1994). *Candida albicans* exhibits chromosomal features that are so-called "hot spots" for mutations, e.g., the repetitive sequences of minisatellites, telomeric regions, and tRNA genes (Fischer et al., 2014). These "hot spots" also include genes whose alteration confers resistance to antifungal drugs. For example, a "hot spot" for development of echinocandin resistance is the *FKS1* gene encoding a beta-1,3-glucan synthase, the direct target of echinocandins (Ben-Ami et al., 2011; Cowen, Kohn, & Anderson, 2001). Such mutations are rare and come with initial fitness costs if not balanced by compensatory mutations (Fischer et al., 2014). Resistance emergence in such "hot spots" has also been described for azoles (e.g., in *ERG* genes, *CDR1*, *MDR1* multidrug transporters) and polyenes (especially *ERG*-gene mutations) (Coste et al., 2006, 2007; Morio, Loge, Besse, Hennequin, & Le Pape, 2010; Morschhauser et al., 2007; Shapiro et al., 2011; Vandeputte et al., 2008). Hsp90 stress can induce aneuploidy by enhanced chromosome instability, since important Hsp90 clients will no longer be stabilized. Such stresses are string inducers of LOH events in *C. albicans*, coupling low fitness to increased evolution rates (Fischer et al., 2014).

Furthermore, a range of mobile elements were found in the *C. albicans* chromosome (Jiang et al., 2014; Zhang et al., 2014). Retrotransposons, remainders of viral genetic elements, are capable of transposition within the genome via RNA intermediates, which is normally repressed by defense mechanisms, such as DNA methylation and histone modifications, preventing deleterious insertions (Arkhipova, 2005; Hua-Van, Le Rouzic, Maisonhaute, & Capy, 2005; Jiang et al., 2014; Johnson, 2007). By translocation these mobile elements provide dispersed sites of sequence similarity at which recombination might occur (Pickeral, Makalowski, Boguski, & Boeke, 2000). Thereby retrotransposons can cause mutations and promote genomic rearrangements (Jiang et al., 2014). Important examples include several non-long terminal repeat (LTR) retrotransposons, e.g., Zorro elements. These mobile elements are affected by population density and ecological aspects, e.g., stress (Arkhipova, 2005; Johnson, 2007). For example, the non-LTR

retro-transposon Zorro2 is activated by miconazole treatment (Zhu et al., 2014). Activation of these transposons leads to additional copies in the *C. albicans* genome. Although the exact role of these additional copies remains to be elucidated, Zhu et al. hypothesized that these retro-transposons can patch together broken chromosome ends and provide dispersed regions of homology that facilitate chromosomal rearrangements (Dunham et al., 2002; Umezu, Hiraoka, Mori, & Maki, 2002; Zhu et al., 2014). This promotes the repair of ROS-induced DNA damage and chromosomal loss, which are consequences of miconazole treatment (Zhu et al., 2014).

4.2 The CUG Codon and the Establishment of Proteome Diversity

An important mechanism for diversity on a postgenomic level, is the described mistranslation of the CUG codon in *C. albicans*. In several *Candida* spp., this codon is largely translated into serine instead of leucine, as it would be in most other organisms (Gomes et al., 2007; Santos, Gomes, Santos, Carreto, & Moura, 2011). However, CUG translation is ambiguous, and partial reversion from serine to leucine identity is possible (Gomes et al., 2007), thereby leading to an exponential expansion of the *C. albicans* proteome. This in turn increases phenotypic variation—one of the most important survival skills of *C. albicans* (Gomes et al., 2007; Miranda et al., 2013). An important example is the variability in cell surface molecules, which is created by CUG mistranslation and which might have a major impact on the fungus–host interaction (Miranda et al., 2013).

Taken together, short- and long-term reactions/adaptations to stress or other environmental conditions are regulated by chromosomal changes. A *C. albicans* population at any given time point in its host is composed of individually unique cells. Each single cell might vary significantly from its neighbor on genomic, transcriptome, and proteome levels. This is the basis for all further virulence and fitness attributes the fungus sustains, and it enables *C. albicans* to survive successfully in a constantly changing environment of the human host.

5. METABOLIC ADAPTATION—NUTRIENT AND MICRONUTRIENT ACQUISITION IN THE HUMAN HOST

Nutrition is fundamental for life and metabolic adaptation is an essential process for *C. albicans* survival. In order to live and proliferate in certain host niches the fungus has to gain access to nutrients and micronutrients.

The availability of nutrients differs dramatically between various host niches the fungus might encounter during infection. In healthy individuals, *C. albicans* is predominantly found as part of the GI microbiome, a niche which is considered to be exceptionally high in nutrients. Still the fungus has to compete with other members of the microbiota for nutrients and specific types of carbon and nitrogen sources might be scarce in intestinal microniches (Perez & Johnson, 2013). Nutrients within the host tissue clearly differ from those on mucosal surfaces and free availability is often limited. Furthermore, micronutrients, such as iron, are available only in very low amounts within the host. Thus, *C. albicans* needs to be able to adapt to available nutrients and to actively recruit micronutrients during infection.

Sugars, especially glucose, are carbon sources efficiently used by *C. albicans* and common laboratory media supply glucose to promote *C. albicans* growth. In comparison to standard in vitro growth media, glucose levels in the bloodstream are much lower (0.06–0.1%) (Barelle et al., 2006). After phagocytosis, *C. albicans* encounters even lower sugar concentrations in the phagolysosome. Adaptation responses that reflect the nutritional conditions during infection include the induction of the glycolytic, tricarboxylic acid cycle, and fatty acid β-oxidation genes during mucosal invasion (Barelle et al., 2006; Owen & Katz, 1999); following ingestion by macrophages or neutrophils *C. albicans* switches from glycolysis to gluconeogenesis, activates fatty acid β-oxidation and the glyoxylate cycle as a starvation response within the phagolysosome (Fradin et al., 2005; Lorenz et al., 2004; Rubin-Bejerano, Fraser, Grisafi, & Fink, 2003). In addition to the reprogramming of metabolism in response to starvation, *C. albicans* possesses various transport mechanisms for peptides, amino acids, etc. that can be upregulated to acquire nutrients from degraded complex host molecules (Dunkel et al., 2013; Kraidlova, Van Zeebroeck, Van Dijck, & Sychrova, 2011; Ramachandra et al., 2014). The impact of metabolism upon *Candida* pathogenicity has recently been reviewed in detail (Brown, Budge, et al., 2014; Ene & Brown, 2014); thus we will only shortly summarize the current knowledge.

First, *C. albicans* secretes a range of hydrolases (e.g., Saps) that provide the fungus with effective tools to liberate nutrients such as oligopeptides and amino acids from the digested tissue (Brunke & Hube, 2013; Naglik, Albrecht, Bader, & Hube, 2004; Naglik et al., 2003). In turn, the expression of *SAP* genes is regulated by the available carbon and nitrogen sources (Hube, Monod, Schofield, Brown, & Gow, 1994). *Candida albicans* can metabolize a wide range of different sugars and amino acids and thus is able to use the liberated nutrients efficiently (Brunke & Hube, 2013).

Second, efficient nutrient acquisition is essential for production of effector and signaling molecules and energy (Brown, Brown, Netea, & Gow, 2014). Thereby metabolic adaptation contributes to a variety of virulence and fitness attributes of *C. albicans* e.g., stress resistance, cell wall remodeling, and interaction with the host immune system (Brown, Brown, et al., 2014; Lee, Morrow, & Fraser, 2013). Not surprisingly, mutations that disrupt key metabolic functions have an impact on *C. albicans* survival and virulence (Barelle et al., 2006; Martinez & Ljungdahl, 2005; Nobile et al., 2012; Noble et al., 2010). Furthermore, the presence of glucose is a trigger for morphogenesis, and glucose induces *C. albicans* genes involved in oxidative stress resistance (Brown, Brown, et al., 2014; Brown, Budge, et al., 2014). Likewise ammonium, the preferred nitrogen source of *C. albicans*, influences morphogenesis (Dunkel et al., 2014; Holmes & Shepherd, 1988). Different carbon sources substantially affect *C. albicans* adhesion and biofilm formation (Critchley & Douglas, 1985; Jin, Samaranayake, Samaranayake, & Yip, 2004; Samaranayake & MacFarlane, 1982). Also, the adaptation to different carbon sources influences cell wall architecture and functionality, and subsequently influences stress responses and antifungal resistance (Brown, Haynes, et al., 2012; Brown, Brown, et al., 2014; Ene, Adya, et al., 2012; Ene, Heilmann, et al., 2012; Mandal et al., 2014). The generation of molecules such as the osmolyte glycerol, antioxidants e.g., glutathione, and the stress protectant trehalose by metabolic pathways may furthermore contribute to the regulation of stress resistance (Brown, Brown, et al., 2014). It is not surprising that changes in the cell wall organization also influence the interaction of *C. albicans* with immune effector mechanisms. Cell wall components are major microbe-associated molecular patterns (MAMPs), which are recognized by a range of pattern recognition receptors (PRRs) on host cells triggering antimicrobial mechanisms and the release of cytokines/chemokines that modulate the resulting immune response (Lewis et al., 2012; Lowman et al., 2014; Netea et al., 2006). Alteration of the cell wall architecture will therefore ultimately lead to altered recognition responses, and indeed it has been shown that, for example, the growth of *C. albicans* on lactate stimulates the production of an anti-inflammatory IL-10 rather than proinflammatory IL-17 response by human peripheral blood mononuclear cells (PBMCs) (Ene, Cheng, Netea, & Brown, 2013). Metabolic adaptation is controlled by complex transcriptional networks in *C. albicans*, and a tight coregulation of metabolism and certain fitness or virulence attributes of *C albicans* during host colonization, commensalism, and pathogenicity has been described (Brown, Brown, et al., 2014; Brown,

Odds, & Gow, 2007; Gow & Hube, 2012; Sabina & Brown, 2009; Whittington, Gow, & Hube, 2014). Thereby nutrient availability shapes the behavior of *C. albicans* and provides an environmental clue that might trigger invasion and infection. *Candida albicans* furthermore has the ability to use metabolic adaptation to actively change its surrounding environment in favor of its own survival. In the absence of glucose, *C. albicans* has the ability to use amino acids or polyamine as a carbon source which will ultimately lead to the production and excretion of nitrogen in the form of ammonia. This, in turn, raises the surrounding extracellular pH, supplying a strong trigger for morphogenesis (Mayer, Wilson, Jacobsen, Miramon, Grosse, et al., 2012; Vylkova et al., 2011). This mechanism is thought to contribute to the escape of *C. albicans* from the phagosome of macrophages (Vylkova & Lorenz, 2014).

Third, during its long coevolution with the mammalian host *C. albicans* has gained the ability to effectively acquire micronutrients i.e., iron, zinc, copper, and manganese from the human host by a wide range of mechanisms. These trace metals are essential cofactors of many proteins and enzymes and thus indispensable for fungal growth. The availability of these micronutrients in the human host is tightly regulated to avoid toxicity and to limit availability for microorganisms as part of "nutritional immunity" (Johnson & Wessling-Resnick, 2012; Radisky & Kaplan, 1999). In turn microorganisms have developed multiple strategies to acquire these micronutrients from host molecules. *Candida albicans* has developed several iron acquisition strategies, including a reductive system, a siderophore-uptake system and a heme-iron uptake system and is thereby able to use nearly all sources of iron within the human host (Brunke & Hube, 2013; Mayer et al., 2013a). The reductive system enables the fungus to gather iron from host ferritin, transferrin, or free iron from the environment (Mayer et al., 2013a). Als3 has been shown to be the receptor for ferritin binding (Almeida et al., 2008). *Candida albicans* does not produce siderophores but possesses the siderophore transporter system Sit1/Arn1 that can utilize siderophores from other microorganisms (Heymann et al., 2002). Furthermore *C. albicans* can express hemolysins that lyse red blood cells, thereby liberating hemoglobin and other heme-proteins (Watanabe et al., 1999). The heme-receptor gene family members *RBT5*, *RBT51*, *CSA1*, *CSA2*, and *PGA7* may then gather iron from these molecules (Almeida, Wilson, & Hube, 2009; Weissman & Kornitzer, 2004; Weissman, Shemer, Conibear, & Kornitzer, 2008). Iron uptake and utilization mechanisms are controlled by three major transcription factors, namely *SFU1*, *SEF1*, and *HAP43*

(Chen, Pande, French, Tuch, & Noble, 2011). Interestingly *C. albicans* uses these iron-responsive regulators to alternate between programs needed for virulence or commensal lifestyle. While Sef1 activates iron uptake genes and promotes virulence in a bloodstream infection model, Sfu1 represses iron uptake and promotes colonization of the GI tract (Chen et al., 2011). Another interesting link is the coregulation of iron uptake and biofilm development via Als3 and Rbt5, that are involved in both iron acquisition and biofilm formation (Chaffin, 2008; Finkel & Mitchell, 2011; Perez et al., 2006, 2011). Iron is furthermore important for the expression of many adhesion genes, thereby affecting the initial step of biofilm formation (Puri, Lai, Rizzo, Buck, & Edgerton, 2014). Finally, Kronstad et al. speculate that biofilm-associated traits such as the production of an extracellular matrix might help fungal cells to sequester iron and other limited nutrients (Kronstad, Cadieux, & Jung, 2013).

The second most abundant metal in the human host is zinc (Hood & Skaar, 2012). Zinc acquisition in *C. albicans* is mediated by Pra1, a zinc-binding protein, which is thought to deliver Zinc from the environment to transporters in the *C. albicans* cell wall, i.e., Zrt1 (Citiulo et al., 2012; Wilson, Citiulo, & Hube, 2012). Other uptake mechanisms have been described for copper and manganese (Hodgkinson & Petris, 2012; Kehl-Fie & Skaar, 2010).

In summary, *C. albicans* is able to use a broad range of general nutrients and nearly all sources of natural iron and other trace metals available in the human host. This likely eases the adaptation of the fungus to a broad range of host niches and provides one explanation for the flexibility of *C. albicans*. Furthermore, the variations in the metabolic environment and fungal adaptation contributing to the heterogeneity of *C. albicans* (e.g., cell wall alterations) will positively promote fungal survival by influencing the efficacy or failure of local immune surveillance mechanisms and antifungal therapy (Brown, Brown, et al., 2014).

6. FROM ATTACHMENT TO DISEASE: ADHESION, INVASION, AND DAMAGE

Of the five major human pathogenic *Candida* species, *C. albicans*, *C. glabrata*, *C. krusei*, and *C. parapsilosis* (but not *C. tropicalis*) are normally commensals of human mucosal surfaces, including the oral and vaginal cavity, and the GI tract, which is the main reservoir for these fungi and the main source of systemic *Candida* infections (Nucci & Anaissie, 2001).

Under certain circumstances, the fungus can alter its commensal lifestyle and turn into a pathogen. The trigger for this transition is not clearly defined, but can be supported by the removal of the bacterial microbiota via antibiotic treatment, by T-cell defects (as in the case of HIV infections and AIDS), or due to even mild changes, such as hormonal alterations (Sobel, 2007). The transition from commensalism to pathogenicity would include three major steps: adhesion, invasion, and damage. It is not clear whether the fungus has the tendency to initiate these steps all the time or whether this transition is a regulated process (Hube, 2004). Only recently, a commensal transcriptional program of *C. albicans* was discovered (Pande et al., 2013), indicating that the fungus may in fact regulate its own lifestyle.

The processes from attachment to invasion and damage are dynamic processes with a smooth transition, however, it is possible to identify genes which are responsible for the distinct stages adhesion, invasion, and damage (Wachtler, Wilson, Haedicke, Dalle, & Hube, 2011).

6.1 Attachment to Epithelial Cell Surfaces

Attachment to epithelial cells is a strong trigger for hyphal formation, at least in vitro, which in turn accelerates adhesion. Yeast cells also attach to epithelial surfaces via yeast cell-expressed adhesins and are thought to build the first layer of biofilms (de Groot, Bader, de Boer, Weig, & Chauhan, 2013). However, the most powerful adhesins seem to be hypha-associated and include the major adhesins Als3 and Hwp1. This is supported by the fact that mutants unable to form hyphae on epithelial cells (e.g., *ras1*Δ) are virtually nonadhesive and mutants lacking either Als3 or Hwp1 are approximately 70% reduced in adhesion (Wachtler, Wilson, Haedicke, et al., 2011).

The hypha-associated protein Als3 is a member of a large protein family originally discovered by Hoyer and colleagues (Hoyer, Green, Oh, & Zhao, 2008) with a prototypic structure similar to many other microbial adhesins: a glycosylphosphatidylinositol-anchor, a stalk-like region, rich in threonine and serine, and a tandem repeat region, followed by the N-terminal end which is responsible for the adhesive properties (Liu & Filler, 2011). These adhesins bind to a wide variety of proteins, peptides, and host molecules (Klotz et al., 2004; Liu & Filler, 2011; Nobbs, Vickerman, & Jenkinson, 2010). Adhesion through members of the Als family can be dramatically increased by amyloid nanodomain formation (Alsteens, Garcia, Lipke, & Dufrene, 2010; Lipke et al., 2012; Otoo, Lee, Qiu, & Lipke, 2008; Ramsook et al., 2010).

Hwp1, also predominantly expressed in the hyphal form, has a unique feature, as the protein is a substrate for (a) mammalian transglutaminase(s), which leads to a covalent link between the fungus and the host cells (Staab et al., 1999). However, Hwp1 must have adhesion properties independent from the covalent binding since Hwp1 is also involved in cell–cell aggregation and biofilm formation on artificial material/surfaces (Nobile, Schneider, et al., 2008). Multiple further *Candida* adhesins have been described (reviewed in de Groot et al., 2013).

The fact that *C. albicans* provides so many different adhesins may be one of the fundamental reasons, why the fungus is such a flexible pathogen. The arsenal of adhesins is likely required during commensal growth, at the different stages of infection and for contact with different cell types (other fungal cells, bacteria, epithelial cells, endothelial cells, etc.), thus promoting attachment at multiple niches within the host.

6.2 Invasion into Epithelial Cells

Although a few studies have reported that yeast cells of *C. albicans* can invade epithelial cells to some extent ("cavitation") (Ray & Payne, 1988), invasion usually is an attribute of hyphae. Attached hyphae can grow along surfaces guided by "contact sensing" (thigmotropism, see above) (Brand et al., 2007). This may allow the fungus to search for weak points in the epithelial barrier, for example, damaged epithelial cells and ruptured layers, or guide the hyphae to cell–cell contacts to facilitate invasion between cells. However, most in vitro data suggest that invasion of hyphae occurs directly into cells by two different routes (Zakikhany et al., 2007): induced endocytosis and active penetration (schematically illustrated in Figure 2).

6.2.1 Induced Endocytosis versus Active Penetration

Induced endocytosis of *C. albicans* hyphae was first discovered by the Filler Laboratory (Phan et al., 2007). This route is entirely host driven and no fungal activity is required, even killed hyphae are engulfed. However, the uptake of fungal elements needs a distinct trigger, initiated by a fungal surface protein (the "invasin") and recognized by a receptor on the host cell surface, similar to the processes during bacterial invasion. Phan et al. identified the adhesin Als3 as the main *C. albicans* invasin and host cell–cell contact proteins, the cadherins (E-cadherin on oral epithelial cells and N-cadherin on endothelial cells), as the main receptors on host cells (Phan et al., 2007). The hypha-associated expression of Als3 explains why almost exclusively hyphae are endocytosed, but not yeast cells. However,

endocytosis of yeast cells, even yeast cells from *Saccharomyces cerevisiae* or *C. glabrata* is induced when Als3 is artificially expressed after genetic manipulation (Fu et al., 2013; Phan et al., 2007). Considering that Als3 is also involved in iron acquisition via Als3-mediated binding of the iron-storage protein ferritin, this protein can truly be described as a multifunctional surface protein (Almeida et al., 2008; Liu & Filler, 2011). A second invasin, the Hsp Ssa1, and further host receptors, including the EGF receptor and human epidermal growth factor receptor 2 (HER2), which cooperatively induce endocytosis of *C. albicans* hyphae, have been identified (Sun et al., 2010; Zhu et al., 2012). Our own data suggest that induced endocytosis occurs during the early stages of invasion and contributes to approximately 25% of the total invasion activity in vitro. Of note, induced endocytosis is not observed for all host cell types. For example, intestinal cells (Caco-2) are not invaded via induced endocytosis (Dalle et al., 2010). However, it seems possible that invasion of *C. albicans* via induced endocytosis in the gut occurs, similar to enteropathogenic bacteria, via distinct cell types, such as M cells.

In contrast to induced endocytosis, the second route of *C. albicans* invasion, active penetration is entirely fungal driven and even inactivated or dead host cells are invaded via this route (Wachtler et al., 2012). All cell types investigated, including intestinal cells, are invaded via active penetration, which seems to be the major route of invasion (Wachtler et al., 2012). Like it is the case for induced endocytosis, only hyphae are able to invade via active penetration. Consequently, *C. albicans* mutant cells with reduced ability to form hyphae are incapable to invade via both routes (Wachtler, Wilson, Haedicke, et al., 2011). The mechanisms of active penetration are not clear. It seems that a mixture of physical forces, probably based on turgor pressure, and hydrolytic activity is required. While the contribution of specific members of the Saps family for epithelial invasion is controversially discussed (Naglik et al., 2003, 2008), invasion is clearly inhibited by the aspartic protease inhibitor pepstatin A suggesting a significant contribution of Saps at some stage (Dalle et al., 2010). Although invasion seems to be a true pathogenic behavior, it may also occur in the commensal stage, potentially contributing to attachment of a constantly proliferating epithelial layer. Of note, initial invasion does neither cause damage nor inflammation or attraction of immune cells such as neutrophils (Wachtler, Wilson, & Hube, 2011; Wilson et al., 2013). Therefore, such a scenario is in agreement with a commensal phase.

Naturally, one would expect that invasion into host cells causes damage, which can, for example, be measured via the release of host lactate

dehydrogenase activity. However, uptake of killed hyphae via induced endocytosis does not induce any damage at all and even invasion by viable hyphae does not cause significant damage within the first hours of contact with epithelial cells. In fact, the host membrane is not disrupted after initial invasion and hyphae are surrounded by a host membrane (Wachtler et al., 2012; Zakikhany et al., 2007). Similar to plant pathogenic fungi, where a defined extracellular space between the invading fungus and the host membrane is observed, *C. albicans* also forms such a "pocket" and this membrane can cover the fungus even when the hyphae have fully penetrated through the host cell and have already invaded into a neighboring cell, a phenomenon which we have described as interepithelial dissemination (Wachtler et al., 2012; Wachtler, Wilson, Haedicke, et al., 2011; Zakikhany et al., 2007). Finally, this membrane will be ruptured by physical forces and intracellular components are released.

Although adhesion, invasion, and damage are processes which are linked, we identified mutants which show normal adhesion and invasion, but which caused significantly reduced damage. These are mutants lacking either Eed1, Dur31, Hsp21, or Ece1.

Eed1 was the first protein discovered, which can be described as a damage factor (Zakikhany et al., 2007). Mutants lacking the *EED1* gene are able to form short hyphae on epithelial cells, which are sufficient to adhere and invade at similar rates as the wild type. However, once invaded into the epithelial host cell, the *eed1*Δ mutant cells switch to yeast cell growth. Consequently, these cells are trapped, unable to escape from their host cell and disseminate further within the epithelial tissue. The reason for this is the general inability of this mutant to maintain hyphal elongation after initiation of hyphal formation (see above) (Martin, Moran, et al., 2011).

Another mutant with normal adhesion and invasion attributes, but reduced damage is a mutant lacking Dur31 (Mayer, Wilson, Jacobsen, Miramon, Grosse, et al., 2012). *DUR31* is not only involved in oral epithelial damage, but also in multiple stages of candidiasis, including surviving attack by human neutrophils, endothelial damage, and virulence in vivo. The *DUR31* gene encodes a sodium/substrate symporter which transports the polyamine spermidine into the fungal cell. By doing so, Dur31 contributes to the extracellular alkalinization hyphae autoinduction pathway and, consequently, is required for pH-induced hyphae formation. Therefore, the reduced hyphae formation is the cause of reduced damage. Of note, Dur31 also transports histatin 5, a highly cytotoxic AMP, into the fungal cell, thereby committing a suicide-like process (Mayer, Wilson, Jacobsen,

Miramon, Grosse, et al., 2012). Thus, Dur31 is both a virulence and avirulence factor.

The third mutant with unaltered potential for adhesion and invasion, but reduced damage is a mutant lacking the small heat shock protein Hsp21 (Mayer, Wilson, Jacobsen, Miramon, Slesiona, et al., 2012). Again, this mutant formed significantly shorter filaments compared to the wild type under various filament-inducing conditions, although sufficient hyphae were produced upon contact with epithelial cells to allow wild type levels of adhesion and invasion. Measurements of intracellular levels of stress protective molecules demonstrated that Hsp21 is involved in glycerol and glycogen regulation and plays a major role in trehalose homeostasis. Mutants defective in trehalose and, to a lesser extent, glycerol synthesis phenocopied *HSP21* deletion and had strongly impaired capacity to damage epithelial cells.

All these examples confirm the relevance of elongated hypha formation for damage, while short hyphae are sufficient for adhesion and invasion. Finally, we found one mutant, which had normal adhesion and invasion properties, but which also produced normal elongated hyphae. This mutant lacked the hypha-associated gene *ECE1*, one of the first genes identified as being hypha-associated and a member of the *C. albicans* hyphae core-response genes (Birse, Irwin, Fonzi, & Sypherd, 1993; Martin et al., 2013). Our own preliminary data show that this protein is the first *C. albicans* toxin, which directly causes damage of host cells (unpublished data). A detailed investigation of this damaging process is currently in progress.

Taken together, the existence of large adhesin gene families, their high variability and both overlapping and specific functions provide *C. albicans* with an important toolbox to adhere and invade various tissues and organs. This enables the fungus to drive infection when it changes from commensal to the pathogenic state.

7. INTERACTION WITH THE IMMUNE SYSTEM—EVADING ELIMINATION

One important function of the immune system is the detection and elimination of microorganisms that invade host tissue. Thus, development and outcome of *Candida* infections depend on the efficacy of immune defense mechanisms. Candidiasis usually only develops when the immune system is impaired and/or is overwhelmed by *Candida* outgrowth on mucosal surfaces, for example, after antibiotic treatment. While it appears obvious

that successful establishment and progression of infection involves interaction of *Candida* with the host immune system, it should be noted that immune responses extend beyond mucosal surface barriers and also occur as reaction to microbiota. In turn, mucosal immunity is influenced by the composition of the microbiome and responses toward a mucosal pathogen might affect other members of the mucosal community. Therefore interaction of *Candida* with the immune system is not limited to infections but also occurs during colonization and likely shapes mucosal host responses (Kumamoto, 2011; Kumamoto & Pierce, 2011).

The immune system consists of humoral and cellular components and can be divided into innate and acquired immunity. Cross-talk via cytokines and chemokines connects innate and acquired immunity and several humoral (soluble) and cellular immune components contribute to both. Consequently, the immune response to infection, including candidiasis, is the result of a complex network of interactions that not only include professional immune cells but also epithelial cells. In the following section, we will only briefly discuss a few selected aspects of the interaction of *C. albicans* with the immune system, referring to recent reviews for more comprehensive information.

7.1 Humoral Defenses: Antimicrobial Peptides and Complement

The complement system and AMPs are evolutionary old systems that present two of the first lines of defense against invading microbes. Both can exhibit direct antimicrobial functions; additionally, the complement system is important for opsonization of pathogens and directing phagocytic cells to the site of infection.

Activation of the complement system is triggered by binding of antibodies to fungal surfaces (classical pathway), binding of mannan-binding lectin on mannosylated *C. albicans* surface proteins (lectin pathway) and the deposition of C3 by the alternative pathway. All three pathways lead to the deposition of complement fragments on the surface that activates downstream parts of the complement cascade, mediating opsonization and assembly of the membrane attack complex. Due to the relatively robust fungal cell wall it appears unlikely that the membrane attack complex affects *Candida* viability; however, complement-mediated opsonization enhances phagocytosis of fungal cells and killing by macrophages and neutrophils (reviewed in Rambach & Speth, 2009). The relevance of complement for the anti-*Candida* response has been clearly demonstrated in mice, where strains with complement defects show a higher susceptibility to systemic candidiasis

(Ashman et al., 2003; Mullick et al., 2004; Peltz et al., 2011; Tsoni et al., 2009). Like many bacterial pathogens, *C. albicans* has developed several mechanisms to interfere with complement activation (recently reviewed in Cheng, Joosten, Kullberg, & Netea, 2012; Luo, Skerka, Kurzai, & Zipfel, 2013). These include degradation of C3b by Saps and binding of different regulatory host proteins that inhibit complement activation (Gropp et al., 2009). The latter is mediated by a variety of fungal proteins, including Pra1, Gpm1, Hgt1, and Gpd2 (Luo et al., 2011; Luo, Hoffmann, Skerka, & Zipfel, 2013; Luo, Poltermann, Kunert, Rupp, & Zipfel, 2009; Luo, Skerka, et al., 2013; Meri et al., 2002; Poltermann et al., 2007).

AMPs are produced not only by immune cells but also by epithelial cells, especially on mucosal surfaces. In addition to exerting direct antifungal activity by pore formation or interference with cellular ATP metabolism, several AMPs also act as chemoattractants promoting the influx of phagocytic immune cells and T cells, thereby modulating the immune response (Swidergall & Ernst, 2014). Several human AMPs, the cathelicidin LL-37, histatin 5, and β-defensins can kill *C. albicans* in vitro (Chang et al., 2012; den Hertog et al., 2005; Vylkova, Nayyar, Li, & Edgerton, 2007). While the extent to which AMPs contribute to antifungal protection in vivo remains unknown, it appears plausible that AMPs are involved both in host—fungal homeostasis on mucosal surfaces during colonization and in the response to infection. In this context, it was recently proposed that the reduced levels of histatin 5 in the saliva of HIV^+ patients contribute to the increased susceptibility to oral candidiasis (Khan et al., 2013). Given the ubiquitous presence of AMPs on mucosal surfaces, it is not surprising that many bacteria have developed AMP resistance mechanisms, likely as a consequence of coevolution with the host (Peschel & Sahl, 2006). The necessity to tolerate basal levels of AMPs on mucosal surfaces during colonization probably also led to the development of AMP resistance mechanisms in *C. albicans* (recently reviewed in Swidergall & Ernst, 2014): *C. albicans* inactivates AMPs through proteolytic cleavage by Sap9 and Sap10 (Meiller et al., 2009). Saps might also be involved in the generation of the secreted glycodomain of Msb2, that inactivates a wide range of AMPs extracellularly (Puri, Kumar, Chadha, Tati, Conti, Hube, et al., 2012; Swidergall, Ernst, & Ernst, 2013; Szafranski-Schneider et al., 2012). Efflux transporters can extrude AMPs that reach the cytoplasm of *C. albicans*, as it has been shown for the MDR transporter Flu1 which mediates efflux of histatin 5 in *C. albicans* (Li, Kumar, Tati, Puri, & Edgerton, 2013). Less specifically, the HOG1 stress response pathway involving Pbs2 contributes to tolerance to basal AMP levels by inducing compensatory

responses (Argimon, Fanning, Blankenship, & Mitchell, 2011). Similarly, Ssd1 and Bcr1, enhance AMP resistance by maintaining mitochondrial integrity in the presence of AMPs (Jung et al., 2013).

7.2 Facing the Foe: Neutrophils and Macrophages

As professional phagocytic cells that are either present in the tissue (tissue macrophages) or rapidly recruited to sites of infection (neutrophils and monocytes differentiating into macrophages), neutrophils and macrophages are the most important constituents of the first line of cellular defense against *C. albicans*. Neutrophils are recruited in large numbers to the site of infection in murine models of candidiasis and the importance of these cells for the host defense has been established in various animal experiments: direct depletion of neutrophils, indirect depletion and interference with neutrophil recruitment by deletion of Interleukin 6 (IL-6) or other cytokines render mice hypersusceptible to systemic infection (Basu, Quilici, Zhang, Grail, & Dunn, 2008; van Enckevort et al., 1999; Han & Cutler, 1997; Kullberg, Netea, Vonk, & van der Meer, 1999; Netea et al., 1999; Romani et al., 1996; van 't Wout, Linde, Leijh, & van Furth, 1988). Recently, mathematical modeling based on mouse experiments suggested that neutrophils can mediate a logarithmic decline of fungal burden in vivo (Hope et al., 2007). Consistently, neutropenia is a risk factor for disseminated candidiasis in human patients (Perlroth et al., 2007). The contribution of macrophages and monocytes to the anti-*Candida* response is less clear; splenic macrophages were found to contribute to resistance in a murine systemic infection model in one study, while another study found no difference between immunocompetent and monocytopenic mice (Qian, Jutila, Van Rooijen, & Cutler, 1994; van 't Wout et al., 1988). It should however be noted that monocytes, macrophages, and their subpopulations have distinct functions, including recruitment of other immune cells. Consistently, a subpopulation of renal resident macrophages has recently been identified that is important for early fungal control and outcome of systemic candidiasis (Lionakis et al., 2013). The following examples will describe some of the interactions between *C. albicans* and neutrophils/macrophages; for more comprehensive information we refer to recent reviews (Cheng et al., 2012; Miramon et al., 2013).

In order to exhibit antifungal functions, phagocytes have to recognize *Candida*. This recognition is mediated by a multitude of pattern recognition receptors (PRRs) on the immune cell surface that can bind different microbial components, so-called MAMPs, which are mainly constituents of the fungal cell wall. These complex interactions have been reviewed

comprehensively by Netea et al. (Netea, Brown, Kullberg, & Gow, 2008). Here, we would like to highlight that the exposure of some MAMPs by *C. albicans* can change during infection. β-glucan on the fungal surface is, among others, recognized by the PRR dectin-1 and this interaction is important for the initiation of phagocytosis and other antifungal activities. While β-glucan exposure of *C. albicans* grown in vitro is relatively low, it increases during systemic infection of mice and after exposure to caspofungin (Wheeler & Fink, 2006; Wheeler, Kombe, Agarwala, & Fink, 2008). However, the host protective effect of dectin-1 is masked by high levels of chitin in the *C. albicans* cell wall. In vivo, the role of dectin-1 in systemic infection of mice differs depending on the *C. albicans* strain used. Marakalala et al. could demonstrate that the dectin-1 dependency correlated with the chitin levels of the cell wall. Importantly, these differences only became obvious during in vivo infection but not in in vitro experiments (Marakalala et al., 2013). Chitin furthermore exhibits anti-inflammatory effects via NOD2, Toll-like receptor 9 (TLR9), and the mannose receptor, suggesting that chitin contents also directly influence the immune response (Wagener et al., 2014). These examples demonstrate that dramatic changes in the cell wall proteome can occur in vivo in response to growth conditions and environmental signals without obvious changes in gross morphology. Thus recognition by the immune system and subsequent anti-*Candida* responses might differ depending on the anatomical site (Gow, 2013). It should also be noted that *C. albicans* recognition is influenced by the morphology and we refer to a recent review for more details (Gow & Hube, 2012).

Phagocytosis of pathogens serves two aims: Inactivation of the pathogen by intracellular killing and, in the case of antigen-presenting cells, stimulation and coordination of the adaptive immune response. Intracellular killing is mediated by release of AMPs, ROS, RNS, reduction of the phagolysosomal pH, and nutrient starvation. Thus, the stress resistance mechanisms and metabolic flexibility of *C. albicans* discussed in detail above counteract intracellular killing to a certain extend. In addition, phagocytosed *C. albicans* cells are able to escape from macrophage phagolysosomes, a process which can furthermore results in macrophage death (see illustrated in Figure 2). Escape is associated with the formation of hyphae and requires modulation of the phagosomal pH (Ghosh et al., 2009; McKenzie et al., 2010; Vylkova & Lorenz, 2014). While it was generally believed that hyphae physically rupture macrophages, recent research strongly suggests that *C. albicans* also triggers pyroptosis (Uwamahoro et al., 2014; Wellington, Koselny, Sutterwala, & Krysan, 2014). Pyroptosis is a host-cell programmed death

pathway that leads to macrophage death and *C. albicans* escape, associated with inflammasome activation and release of proinflammatory cytokines. To which extend this and other kinds of programmed cell death contribute to *Candida* survival within the host, still remains to be elucidated.

7.3 Dendritic and Natural Killer Cells: Cross-Talk Matters

Dendritic cells (DCs) are professional antigen-presenting cells, that are strategically located at the primary entry sites of *C. albicans*, mucosal surfaces (Roy & Klein, 2012). They are responsible for the sampling and processing of antigens from the environment, and the subsequent shaping of T-cell responses by the secretion of cytokines and the presentation of antigen-fragments on their surface (Ramirez-Ortiz & Means, 2012; Roy & Klein, 2012). Both *C. albicans* yeast and hyphae are efficiently phagocytosed by DCs, but DCs kill *C. albicans* less efficiently than monocytes and macrophages. It has therefore been suggested that the main role of DCs is antigen presentation and coordination of subsequent immune responses (Netea et al., 2004; Ramirez-Ortiz & Means, 2012). DCs are able to differentiate between the different morphological growth forms of *C. albicans* resulting in differential cytokine patterns and T-cell responses (Kurzai, Schmitt, Brocker, Frosch, & Kolb-Maurer, 2005; d'Ostiani et al., 2000; Romani, Bistoni, & Puccetti, 2002; Romani et al., 2004). Interestingly, fungal factors like cell wall components have been shown to influence DC maturation (Kikuchi, Ohno, & Ohno, 2002; Nisini et al., 2007; Ramirez-Ortiz & Means, 2012; Roy & Klein, 2012; Torosantucci et al., 2004), and thereby *C. albicans* may modulate the subsequent immune reaction. Interaction with DCs not only occurs during infection but also during colonization of the gut, where they probably play an important role in the in vivo tolerance of *C. albicans* (Bonifazi et al., 2009). Similarly, natural killer (NK) cells can kill *C. albicans* but the dominant role is likely their influence on other parts of the immune system via production of cytokines (Voigt et al., 2014). This hypothesis is supported by the observation that the role of NK cells in *C. albicans* infection depends on the overall immune status of the host (Quintin et al., 2014). Thus, NK cells and DCs likely modulate the immune response toward *Candida* both during infection and commensalism by coordinating complex responses and by providing a connection between innate and adaptive immunity (Ramirez-Ortiz & Means, 2012).

7.4 Epithelial Cells and Mucosal Immunity

Mucosal responses to microbes are not only important during infection but also for homeostasis between the host and its microbiota (Bevins & Salzman,

2011). The interactions on mucosal surfaces are complex and differ between anatomical sites: T-cell responses, for example, significantly contribute to interactions on the oral mucosa, as exemplified by the high susceptibility of AIDS patients to OPC (Cassone & Cauda, 2012; Fidel, 2011). In contrast, AIDS does not predispose patients for vaginal or systemic *Candida* infections (Perlroth et al., 2007). Epithelial cells play a crucial role during commensalism and mucosal candidiasis, as their response stimulates recruitment of professional immune cells (Li, Chen, Tang, Shen, & Liu, 2011; Naglik, Moyes, Wachtler, & Hube, 2011; Weindl, Wagener, & Schaller, 2010, 2011). Thus, we will briefly summarize how oral epithelial cells respond immunologically to *C. albicans*.

Epithelial cells recognize *C. albicans* cell wall components, thereby triggering activation of NF-κB and the first MAPK phase, including c-Jun activation. This initial activation is independent of morphology and does not result in the production of cytokines. Cytokine production depends on the presence of hyphae and fungal load, which activate c-MKP1 and c-Fos signaling, and might constitute a "danger response" pathway (Moyes et al., 2010, 2011, 2012, 2014; Moyes & Naglik, 2011). The "danger response" results in the secretion of immune-stimulatory chemokines and cytokines, e.g., granulocyte-macrophage colony stimulating factor (GM-CSF), which modulate the subsequent immune response and mediate cross-talk with neutrophils (Weindl et al., 2007). Neutrophil signaling, likely via Tumor necrosis factor α (TNF-α), in turn induces epithelial responses that include the upregulation of TLR4 and production of antimicrobial molecules (Li et al., 2011; Lopez-Garcia, Lee, Yamasaki, & Gallo, 2005; Moyes et al., 2010, 2012; Netea & Kullberg, 2010; Vylkova et al., 2007; Wagener et al., 2013; Weindl et al., 2007). Together with the recruitment of immune cells, the epithelial response thus enhances the antifungal resistance of the mucosal barrier. With regard of the survival of *C. albicans* in the host the mechanisms described above nicely demonstrate that *C. albicans* is tolerated as long as it behaves as a "benign" commensal— the epithelial cells sense the presence of *Candida* but only react if fungal load and invasion-associated filamentation indicate that the commensal has turned to a pathogenic lifestyle.

8. LIVING WITHIN A COMMUNITY

The sections above describe how single *C. albicans* cells sense and react to diverse environmental stimuli. However, in the recent years it became

more and more evident that planktonic growth in vivo is rather the exception than the rule, which makes the discussion of survival mechanisms of *C. albicans* on single cell level insufficient. It has been estimated that up to 80% of all microorganisms grow as sessile communities attached to a surface rather than as planktonic cells and a significant portion of all human microbial infections arise from pathogenic biofilms (Bonhomme & d'Enfert, 2013; Douglas, 2003; Ramage et al., 2009; Shirtliff, Peters, et al., 2009). Medical devices routinely used in clinical settings such as central venous or urinary catheters, artificial heart valves, prosthetic joints or dentures, provide an ideal point for biofilm formation and subsequent infection (Fanning & Mitchell, 2012; Kojic & Darouiche, 2004).

Furthermore, under most in vivo situations *C. albicans* will encounter other members of the natural human microbiota and/or might get in contact with coinfecting pathogens. Therefore, *C. albicans* must not only cope with host defense mechanisms, but also compete with other microorganisms for host niches, adhesion sites, and nutrients and must deal with toxins and metabolic byproducts of its neighbors in order to successfully colonize and survive within the human host.

Therefore, the following section will summarize the recent insights into multicellular behavior, highlighting the importance of biofilm formation for *Candida* survival and pathogenicity and discuss the ability of intra- and interspecies communication and its role in *C. albicans* survival within the human host.

8.1 Biofilms—A Strong Community Facing the Host

Biofilms are three-dimensional structures formed by microbes on abiotic and biotic surfaces wherein single cells might differentiate to gain specialized properties or functions (Costerton, Montanaro, & Arciola, 2005; Douglas, 2003; Lynch & Robertson, 2008). Mediated by cell—cell contacts and the secretion of a variety of signaling molecules single cells adapt their behavior acting as a multicellular community (Williams, 2007). Fungal biofilms are problematic in the clinical context because of their intrinsic tolerance to many commonly used antifungals and their increased resistance to physical forces, diverse stresses and host immune defense mechanisms (Chandra et al., 2001; Hall-Stoodley & Stoodley, 2009; Ning et al., 2013). The ability of clinical isolates to form biofilms has furthermore been associated with increased pathogenicity and higher mortality rates in patients with candidemia (Sherry et al., 2014; Tumbarello et al., 2007; Wenzel, 1995). *Candida albicans*, the leading pathogen among the *Candida* clade, forms the most robust biofilms on abiotic and biotic surfaces, but other *Candida* spp. have

also been associated with biofilm formation in human infections, i.e., *Candida dubliniensis*, *C. glabrata*, *C. tropicalis*, and *C. parapsilosis* (Bonhomme & d'Enfert, 2013; Dongari-Bagtzoglou, Kashleva, Dwivedi, Diaz, & Vasilakos, 2009; Douglas, 2002, 2003; Kojic & Darouiche, 2004; Silva, Henriques, Oliveira, Williams, & Azeredo, 2010).

8.1.1 Candida albicans *Biofilm Formation is a Sequential Process*

The efficiency of biofilm formation of *C. albicans* on a surface depends on the texture of the surface (e.g., roughness, hydrophobicity), the environmental conditions (e.g., nutrient availability, shear forces, O_2 availability, pH, and CO_2 levels), the intrinsic ability of the fungus to form biofilms and the presence and activity of host immune cells (Chandra et al., 2001; de Vasconcellos, Goncalves, Del Bel Cury, & da Silva, 2014; Finkel & Mitchell, 2011). *Candida* biofilm formation is a multistep process which starts with the settlement and adhesion of single cells to a surface (adherence step) (Blankenship & Mitchell, 2006; Chandra et al., 2001; Finkel & Mitchell, 2011). This initial contact is mediated by cell surface adhesins that have already been described in context of adhesion to host tissues, e.g., Als3 and Hwp1 (Liu & Filler, 2011; Nobile, Nett, Andes, & Mitchell, 2006). Adhesion in biofilms involves cell−substrate, as well as cell−cell adherence during biofilm growth. The attached cells will start to proliferate to a thin layer of cells that provides the basis for further biofilm development (initiation step) (Chandra et al., 2001; Finkel & Mitchell, 2011). Subsequently the biofilm grows and matures, which is associated with the formation of pseudohyphae and hyphae, and the secretion of extracellular matrix (ECM) components by biofilm-associated cells (maturation step) (Baillie & Douglas, 1999; Finkel & Mitchell, 2011). The surface of the mature biofilm provides then the basis for dispersal and dissemination by releasing cells, preferably yeast, into the environment (dispersal step) (Finkel & Mitchell, 2011; Uppuluri, Chaturvedi, et al., 2010).

The mature biofilm is a very complex structure including microniches with particular properties, wherein cells will adopt specialized features to survive. Cells growing in deeper biofilm layers, for example, face decreased oxygen levels (Bonhomme & d'Enfert, 2013). Consequently, cells in different parts of the biofilm show different gene expression profiles regarding starvation and oxidative stress, e.g., upregulation of glycolysis, fatty acid metabolism, and ergosterol synthesis (Bonhomme et al., 2011; Bonhomme & d'Enfert, 2013; Garcia-Sanchez et al., 2004). Different cell types within a biofilm were furthermore shown to be subject to genetic

and epigenetic changes (Bonhomme & d'Enfert, 2013; Finkel & Mitchell, 2011; Stewart & Franklin, 2008).

8.1.2 Regulation of Biofilm Formation and Dispersal

Many of the genes that are involved in biofilm formation also have a role during morphogenesis. However, the initial adhesion step is independent of the morphological growth form of *C. albicans* (Finkel & Mitchell, 2011). Eap1 and Als1, both surface proteins present on yeast and hyphal cells are therefore thought to mediate the initial contact with the surface (Finkel & Mitchell, 2011; Hoyer, 2001; Li & Palecek, 2003). Upon surface contact *C. albicans* activates MAPK signaling responses associated with hypha formation (Mkc1 and Cek1 pathways) to initiate adherence to the surface (Kumamoto, 2005; Kumamoto & Vinces, 2005a; Zucchi, Davis, & Kumamoto, 2010). Components of the cAMP/PKA-signaling pathway were also shown to affect biofilm formation (Giacometti, Kronberg, Biondi, & Passeron, 2011; Yi et al., 2011). The primal surface contact is a strong trigger for filamentation and expression of hypha-associated adhesins, i.e., Als3 and Hwp1. These will further promote cell-surface adherence and cell−cell attachment (Chaffin, 2008; Finkel & Mitchell, 2011; Liu & Filler, 2011; Nobile et al., 2006; Nobile, Schneider, et al., 2008).

In 2012, Nobile and colleagues identified the transcriptional network that orchestrates *C. albicans* biofilm development: Six master regulators, namely Bcr1, Tec1, Efg1, Ndt80, Rob1, and Brg1, are arranged in a complex circuit, directly or indirectly regulating each other and the expression of approximately 1000 target genes (Fox & Nobile, 2012; Nobile et al., 2012). The expression of most master regulators is regulated by the Set3 histone deacetylase complex, which was recently shown to promote biofilm cell dispersal and drug resistance (Nobile et al., 2014). The complexity and partial redundancy of the network enables the fungus to sense and respond to multiple environmental inputs and to fine-tune its gene expression according to these stimuli (Fox & Nobile, 2012). Important target genes include adhesins (e.g., *ALS1*, *ALS3*, *HWP1*), nutrient transporters (e.g., *CAN2*, encoding an amino acid permease), genes involved in hyphal growth and virulence (e.g., *TEC1*, *UME6*), zinc uptake genes and genes involved in biofilm matrix regulation (e.g., *ZAP1*, *FUS1*, *XOG1*) (Fox & Nobile, 2012; Nobile et al., 2012).

Dispersed cells from biofilms have the potential to initiate infection in other body sites. The mechanisms leading to dispersal are however only poorly understood. One key factor controlling dispersion seems to be the morphogenic regulator Hsp90, which also regulates matrix glucan level

and resistance to antifungals (Robbins et al., 2011; Singh et al., 2009). Dispersion requires the downregulation of important filament-associated genes, e.g., *UME6*, as well as the upregulation of the yeast growth promoting genes *NRG1* and *PES1* (Finkel & Mitchell, 2011; Uppuluri, Chaturvedi, et al., 2010; Uppuluri, Pierce, et al., 2010). Nrg1 protein, but not transcriptional levels are regulated by the QSM farnesol (Lu et al., 2011; Lu, Su, Unoje, & Liu, 2014). Farnesol appears to regulate both, biofilm adhesion (negatively) and dispersal (positively) (Deveau & Hogan, 2011). This is likely mediated by the inhibitory effects of farnesol on hyphae formation and by promoting the switch back to yeast growth (Hornby et al., 2001; Lindsay, Deveau, Piispanen, & Hogan, 2012). Interestingly, dispersed cells show enhanced filamentation, adhesion, biofilm formation, and virulence properties compared to their planktonic counterparts (Uppuluri, Chaturvedi, et al., 2010). These changes might be mediated by long-term epigenetic alterations that are retained upon dispersal of yeast cells from the biofilm.

8.1.3 Biofilm Resistance Mechanisms and Their Relevance during Infection

Candida albicans biofilms are intrinsically resistant to a wide variety of stresses, including several antifungal drugs, mechanical stresses, and immune defense mechanisms. The mechanisms of biofilm resistance to antifungal drugs have recently been reviewed in detail (Bonhomme & d'Enfert, 2013; Mathe & Van Dijck, 2013; Taff, Mitchell, Edward, & Andes, 2013). Briefly, the complex architecture of the biofilm itself, the trapping of passing molecules or cells by components of the ECM, the upregulation of efflux pumps and stress defense mechanisms, as well as the phenotypic and metabolic plasticity of biofilms contribute to their resistance (for illustration see Figure 3):

1. The biofilm environment promotes the formation of phenotypic variants that are specialized for a specific niche within a biofilm. Such adaptation responses may include epigenetic changes or transient aneuploidy that confers increased resistance (persister cells) or adaptation to the host (Selmecki, Forche, & Berman, 2010). Persister cells define a small fraction of phenotypic variants that have gained the intrinsic ability to survive antifungal concentrations well above the normal minimal inhibitory concentrations (MICs) (Bonhomme & d'Enfert, 2013; LaFleur, Kumamoto, & Lewis, 2006; Lafleur, Qi, & Lewis, 2010; Lewis, 2010, 2012; Taff et al., 2013). These cells comprise about 1% of all biofilm cells and lie deep within the structure (Mathe & Van Dijck, 2013). Although

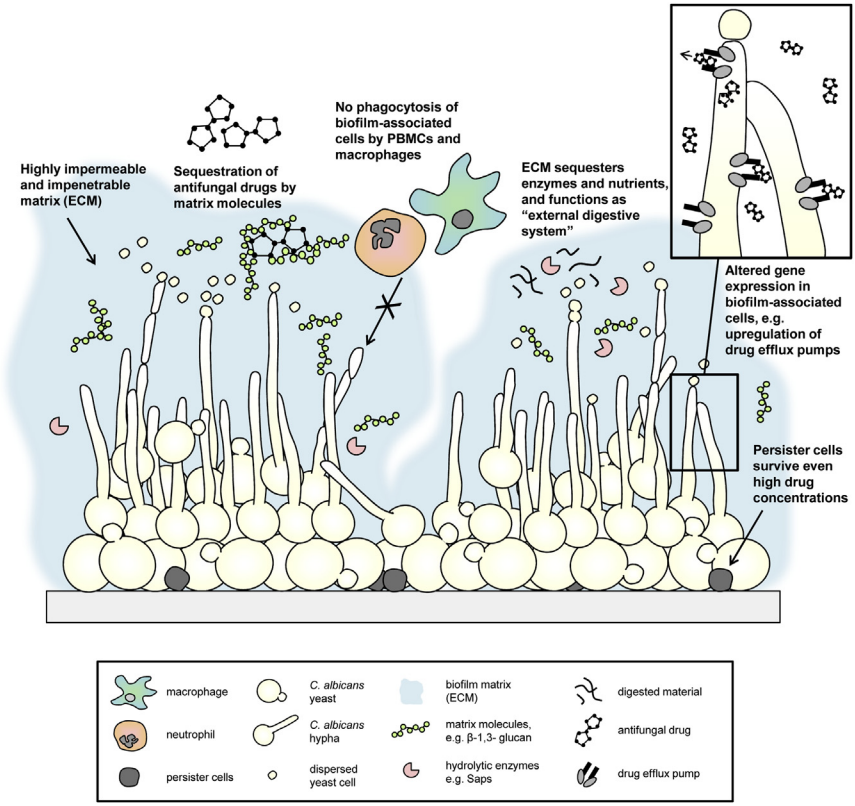

Figure 3 Biofilm resistance mechanisms. The growth in form of biofilms, multicellular communities with a complex three-dimensional ultrastructure, is associated with a high intrinsic tolerance to several stressful conditions, e.g., antifungal treatment, immune defense mechanisms, physical and chemical stresses. Some important resistance mechanisms are represented in this figure. ECM, extracellular matrix; PBMCs, peripheral blood mononuclear cells.

not much is known about their origin, there is evidence that ROS signaling may play a role in persister cell formation and that the formation is species- and strain-specific (Al-Dhaheri & Douglas, 2008; Bink et al., 2011; Lafleur et al., 2010; Vandenbosch, Braeckmans, Nelis, & Coenye, 2010). During treatment with antifungals these cells can provide a population that survives and recolonizes the host.

2. Cells within a biofilm are embedded within a matrix (ECM) that primarily consists of cell wall carbohydrates, proteins, lipids, hexosamine, phosphorus, uranic acid, and extracellular DNA (eDNA) (Al-Fattani & Douglas, 2006; Costerton, 1995; Lal, Sharma, Pruthi, & Pruthi, 2010;

Martins et al., 2010; Mathe & Van Dijck, 2013; Zarnowski et al., 2014). The eDNA contributes to the structure and stability of the mature biofilm, promotes hyphal growth and increases drug resistance (Martins, Henriques, Lopez-Ribot, & Oliveira, 2012; Martins et al., 2010; Sapaar et al., 2014). The matrix material is also thought to contribute to the retention of water, nutrients, and enzymes (Taff et al., 2013). The importance of the ECM for resistance mechanisms is supported by the observation that biofilm resistance directly correlates with the amount of matrix material present (Al-Fattani & Douglas, 2006). What makes the ECM such a good "shield" against potential threats? First, it was shown that diffusion of antifungals is slower within biofilms, but without a dramatic difference in overall diffusion rates (Al-Fattani & Douglas, 2004). Second, a higher amount of β-1,3-glucan is produced and shed by biofilm-associated cells (Nett et al., 2007; Nett, Sanchez, Cain, & Andes, 2010). β-1,3-glucan has the ability to bind fluconazole, which reduces the potential of the drug to reach and control biofilm-associated cells (Nett et al., 2007; Nett, Sanchez, Cain, & Andes, 2010). Similar resistance mechanisms have also been described for other antifungal drugs (Nett, Crawford, Marchillo, & Andes, 2010; Vediyappan, Rossignol, & d'Enfert, 2010) and non-*albicans Candida* species (Mitchell et al., 2013). Very recently, a unique branched-mannan-β-1,6-glucan conjugate was discovered as the major carbohydrate matrix component, while β-1,3-glucan was more prominent in the cell wall (Zarnowski et al., 2014). The matrix therefore appears to be a specialized structure rather than a simple agglomeration of released cell wall components (Zarnowski et al., 2014). Not surprisingly, ECM production is controlled by different regulators that target cell wall carbohydrate synthesis and excretion. Zap1, a zinc-responsive transcription factor, is a major negative regulator of β-1,3-glucan synthesis, whereas the glucoamylases Gca1 and Gca2, the glucan transferases Bgl2 and Phr2, and the exoglucanase Xog1 have a positive effect on β-1,3-glucan excretion and biofilm matrix formation (Bonhomme & d'Enfert, 2013; Mathe & Van Dijck, 2013; Taff et al., 2012, 2013). Upstream elements of genes regulating the cell wall β-1,3-glucan content and matrix production include genes encoding members of the protein kinase C cell wall integrity pathways, i.e., *SMI1* and *RLM1* (Mathe & Van Dijck, 2013; Nett, Sanchez, Cain, Ross, & Andes, 2011). Matrix formation in MTL-heterozygous cells is furthermore Ras1/cAMP-dependent and requires Efg1, Tec1, and Brg1 activation (Lassak et al., 2011; Nobile & Mitchell, 2005; Ramage,

VandeWalle, Lopez-Ribot, & Wickes, 2002; Stichternoth & Ernst, 2009). Interestingly, a recent analysis of the matrix composition of *C. albicans* biofilms in vitro and in vivo by Zarnowski et al. found proteins as a major component of the biofilm matrix (Zarnowski et al., 2014). This did not include cell wall-associated proteins, but rather factors involved in carbohydrate and amino acid metabolism, suggesting that the biofilm matrix might also function as an external digestive system which breaks down extracellular molecules as energy source for the biofilm-associated cells.

3. Cells within a biofilm show altered gene expression in comparison to their planktonic counterparts. Differentially regulated genes include genes encoding antifungal targets or genes involved in ergosterol or cell wall biosynthesis (e.g., *ERG* genes mediating ergosterol synthesis, *SKN1* and *FKS1* and *KRE* genes mediating β-1,3-glucan synthesis) (Borecka-Melkusova, Moran, Sullivan, Kucharikova, Chorvat, & Bujdakova, 2009; Garcia-Sanchez et al., 2004; Khot, Suci, Miller, Nelson, & Tyler, 2006; Mathe & Van Dijck, 2013; Murillo, Newport, Lan, Habelitz, Dungan & Agabian, 2005; Nailis, Vandenbosch, Deforce, Nelis, & Coenye, 2010; Nett, Crawford, et al., 2010; Nett, Lepak, Marchillo, & Andes, 2009; White, 1997). The upregulation of ergosterol synthesis genes may contribute to resistance of biofilm-associated cells to fluconazole and polyenes (Mathe & Van Dijck, 2013). In contrast, the ergosterol content of cells during late biofilm growth is reduced, thereby potentially limiting the efficacy of ergosterol-targeting drugs (Mukherjee, Chandra, Kuhn, & Ghannoum, 2003). Furthermore during early biofilm formation an increased expression of drug efflux pump genes including *CDR1*, *CDR2*, *MDR1*, and *FLU1* has been described, which seems to play a role in fluconazole resistance (Mukherjee et al., 2003; Nett et al., 2009; Ramage, VandeWalle, Bachmann, Wickes, & Lopez-Ribot, 2002; Sanglard, 2002; White, 1997). However, the presence of those transporters is not essential for drug resistance in mature and aging biofilms where these genes are again downregulated (Mathe & Van Dijck, 2013). In addition, expression of several *C. albicans* genes driving resistance against oxidative stress (e.g., *CAT1*), cell wall stress (e.g., *MKC1*), and general stress responses (e.g., *HSP90*, *HSP104*, *CNB1*, *CRZ1*) is increased in biofilms (Mathe & Van Dijck, 2013; Taff et al., 2013).

Taken together, life in a biofilm renders *C. albicans* extremely resistant to antifungals and host defenses, thereby presenting a "safe haven" for the fungus. The complexity of the biofilm promotes escape from the immune system

as phagocytes and PBMCs are not able to phagocytose biofilm-associated cells (Chandra, McCormick, Imamura, Mukherjee, & Ghannoum, 2007; Katragkou et al., 2010). Furthermore, the presence of PBMCs and the proinflammatory cytokine IL-17A within a biofilm enhance biofilm formation and mass (Chandra et al., 2007; Zelante et al., 2012). Components of the matrix in mature *C. albicans* biofilms furthermore interfere with ROS production by neutrophils (Xie et al., 2012). Last but not least, the "inherited" increased virulence potential of dispersed biofilm cells furthers colonization and establishment of biofilms on new sites of infection, thereby promoting the populating of new host niches (Uppuluri, Chaturvedi, et al., 2010).

8.2 Quorum Sensing and Beyond—Talking in Molecules

Quorum sensing is a mechanism of microbial communication wherein microorganisms synchronize their behavior depending on the population density (Miller & Bassler, 2001). The mediating factors are so-called autoinducers or QSMs that are released by the cells. QSMs trigger intracellular responses once a critical threshold is reached (Bassler, 2002; Williams, 2007). The existence of cooperative behavioral patterns has first been described for *Streptococcus pneumoniae* and the marine luminescent bacterium *Vibrio fischeri* (Bassler & Losick, 2006). In 2001, the first QSM in a eukaryotic organism was described: farnesol in *C. albicans* (Hornby et al., 2001). Since then, a range of other (putative) QSMs have been identified in *C. albicans* (Albuquerque & Casadevall, 2012; De Sordi & Muhlschlegel, 2009; Kruppa, 2009). Quorum sensing plays a pivotal role during biofilm development by providing the communication necessary to build, maintain, and regulate such a complex multicellular structure (Molloy, 2010; Nadell, Xavier, Levin, & Foster, 2008; Peleg, Hogan, & Mylonakis, 2010).

8.2.1 The Quorum Sensing Molecule Farnesol and Cellular Signaling Pathways

Farnesol is an isoprenoid alcohol which is produced as a byproduct of the ergosterol biosynthesis pathway (Hornby et al., 2001; Hornby & Nickerson, 2004; Nickerson et al., 2006). Within the *Candida* clade, *C. albicans* produces the highest amounts of farnesol, with levels reaching up to 55 μM in dense *C. albicans* cultures (Weber, Schulz, & Ruhnke, 2010; Weber, Sohr, Schulz, Fleischhacker, & Ruhnke, 2008). Farnesol has several effects on *C. albicans*. Probably the most prominent action is the inhibition of the yeast-to-hyphae transition (Hornby et al., 2001; Mosel, Dumitru, Hornby, Atkin, & Nickerson, 2005; Oh, Miyazawa, Naito, & Matsuoka, 2001). Very recently

Lindsay et al. discovered that farnesol also triggers the switch back from filamentous to budding yeast cell growth (Lindsay et al., 2012), which makes farnesol an important candidate for regulation of yeast cell dispersal from the surface of mature biofilms. Furthermore farnesol is a potent inhibitor of the initial adhesion step of biofilm formation (Alem, Oteef, Flowers, & Douglas, 2006; Cao et al., 2005; Deveau & Hogan, 2011; Martins et al., 2007; Nickerson et al., 2006; Ramage, Saville, Wickes, & Lopez-Ribot, 2002). In contrast, farnesol has no effect on biofilm maturation, only cells in mature biofilms become sensitive to farnesol again (Ramage, Saville, et al., 2002). Besides its effect on yeast-to-hyphae and hyphae-to-yeast transition, farnesol affects genes involved in drug resistance, cell wall maintenance, phagocytic response, surface hydrophobicity, iron transport, and a range of Hsps (Cao et al., 2005; Cho et al., 2007; Enjalbert & Whiteway, 2005; Uppuluri, Mekala, & Chaffin, 2007).

Considering the diverse modes of action, it is not surprising that farnesol affects several signaling pathways, including the Ras1/Cyr1/cAMP cascade (Davis-Hanna, Piispanen, Stateva, & Hogan, 2008; Lindsay et al., 2012). Farnesol exerts a direct effect on Cyr1 adenylyl cyclase function by binding to the cyclase domain, thereby reducing intracellular cAMP levels (Hall et al., 2011). Furthermore, farnesol promotes the cleavage of Ras1, resulting in a soluble Ras1 form that has a reduced ability to activate Cyr1, thereby supporting the formation of yeast cells (Piispanen, Grahl, Hollomon, & Hogan, 2013). The inhibition of cAMP signaling by farnesol has been shown to mediate a protective effect against oxidative stress by promoting the activation of the general stress MAPK Hog1 (Brown, Budge, et al., 2014; Deveau, Piispanen, Jackson, & Hogan, 2010; Smith et al., 2004). Farnesol also influences other signaling pathways, e.g., the morphogenic MAPK signaling pathway via Cek1p, two-component signaling via Chk1p and important regulators of morphogenesis (Kruppa et al., 2004; Roman et al., 2009; Sato et al., 2004): Tup1, a major negative regulator of filamentation; as well as Czf1 and Efg1, both important regulators of filamentation in *C. albicans* under different growth conditions (Braun & Johnson, 1997; Kebaara et al., 2008; Langford et al., 2013), have been implicated in farnesol signaling. *NRG1* expression is affected indirectly via cAMP signaling but farnesol also exerts an effect on Nrg1 protein levels (Braun, Kadosh, & Johnson, 2001; Lu et al., 2011, 2014). The latter is mediated via inhibition of the E3 ubiquitin ligase Ubr1, which in turn stabilizes the Cup9 transcriptional repressor of *SOK1* (Lu et al., 2014). Subsequently *SOK1* expression, a kinase required for Nrg1 degradation, is downregulated,

the Nrg1 protein becomes stabilized and finally inhibits hyphal initiation (Lu et al., 2014). It is also possible that farnesol has indirect effects on the cells by interacting with the cell membrane, since it is a highly hydrophobic molecule. This could lead to pleiotropic signals throughout the cell (Langford, Atkin, & Nickerson, 2009).

8.2.2 Differences in Farnesol Sensitivity May Account for Diversification in Certain Host Niches

The farnesol effects described above occur in white cells, the major phenotypic form of *C. albicans* in vitro. Opaque cells, in contrast, are adversely affected by farnesol. Under aerobic conditions opaque cells are killed by farnesol concentrations that are sublethal to white cells (Dumitru et al., 2007). Under anaerobic concentrations, when white cells become unresponsive to farnesol, opaque cells stay unharmed (Dumitru et al., 2007; Dumitru, Hornby, & Nickerson, 2004). Thereby farnesol may reduce mating efficiency in certain human body niches and restrict mating to anaerobic sites in the host, e.g., the GI tract (Dumitru et al., 2007). Under normal growth conditions the MIC of farnesol is >250 μM for white cells, a concentration which easily inhibits or kills other cell types or species (Albuquerque & Casadevall, 2012; Jabra-Rizk, Meiller, James, & Shirtliff, 2006; Langford et al., 2009). How *C. albicans* white cells withstand these high farnesol concentrations is largely unknown, but it appears to be an energy- and growth-phase dependent process as log-phase cells are more susceptible to killing than stationary phase cells (Langford et al., 2009; Shirtliff, Krom, et al., 2009; Uppuluri et al., 2007). Similarly, the exact mechanisms by which farnesol induces apoptotic and necrotic cell death are poorly understood (Dumitru et al., 2007; Shirtliff, Krom, et al., 2009). Farnesol might exert inhibitory effects on the mitochondrial function leading to perturbations in respiration and ROS production, which are either tolerated (white cells) or induce cell death (opaque cells) (Langford et al., 2009). Furthermore, the lipophilic nature of farnesol may disrupt membrane function leading to nonspecific necrosis (Langford et al., 2009). As the overall reactivity of cells to farnesol is very diverse and dose-dependent, Hogan and Muhlschlegel suggested that farnesol may allow diversification of cells within a population (Hogan & Muhlschlegel, 2011).

8.2.3 Other Important Signaling Molecules Involved in Intraspecies Communication

Another important QSM of *C. albicans* is tyrosol (Chen, Fujita, Feng, Clardy, & Fink, 2004). In contrast to farnesol, tyrosol stimulates

filamentation and biofilm formation by decreasing the lag-phase of cell growth under environmental conditions that favor filamentation (Alem et al., 2006; Chen, Fujita, et al., 2004). In biofilms, the production of tyrosol is exceptionally high and tyrosol can overcome the inhibitory effect of farnesol to a certain extent (Alem et al., 2006). In vitro, tyrosol also elicits inhibitory effects on neutrophils by interfering with the oxidative burst (Cremer, Vatou, & Braveny, 1999). However, it is still unknown if tyrosol is in fact produced during infection in the human host (Albuquerque & Casadevall, 2012).

More molecules have been proposed to be quorum sensing or likewise signaling molecules in *C. albicans* (Albuquerque & Casadevall, 2012; Shareck & Belhumeur, 2011). Many of them are aromatic alcohols, e.g., tryptophol and phenylethanol (Chen & Fink, 2006; Ghosh, Kebaara, Atkin, & Nickerson, 2008). The production of these molecules depends on the environmental conditions and is supported by anaerobic growth, alkaline pH, and amino acid availability (Ghosh et al., 2008). Fusel alcohols also exert a morphogenic autoregulatory function, but are only active at very high concentrations (Albuquerque & Casadevall, 2012). Although they do not play a major role as QSM under most in vitro conditions, they may play a role under certain in vivo conditions (Albuquerque & Casadevall, 2012; Chen & Fink, 2006). Dodecanol inhibits hypha formation via the transcription factor Sfl1, independent of the cAMP/PKA pathway (Hall et al., 2011). A farnesol related molecule, farnesoic acid, also inhibits the yeast-to-hyphae transition, although at much higher concentrations than farnesol (Kruppa, 2009).

In addition, *C. albicans* might utilize arachidonic acid released by infected host cell membranes to synthesize extracellular prostaglandins during infection. Prostaglandins, e.g., PGE_2, play an important role for the induction of hypha formation and as potent local messenger molecules during biofilm formation (Erb-Downward & Noverr, 2007; Noverr & Huffnagle, 2004; Zarnowski et al., 2014). ROS have also been implicated in signaling, especially in biofilms (Cap et al., 2012). Low doses of ROS, e.g., induced by farnesol signaling, can have a beneficial effect by provoking hormesis (see chapter *How to deal with stress—Lessons from Candida albicans*) (Deveau et al., 2010; Pan, 2011; Ristow & Schmeisser, 2011; Westwater, Balish, & Schofield, 2005).

8.2.4 Effects of Quorum Sensing during Infection

Because of its hyphae-inhibitory function farnesol was thought to have a protective effect against *Candida* infection (Nickerson et al., 2006).

However, studies using systemic infections of mice suggest that it rather functions as an important virulence factor (Navarathna, Hornby, et al., 2007; Navarathna, Nickerson, Duhamel, Jerrels, & Petro, 2007). Farnesol modulates the immune response toward a nonprotective Th2 response rather than a protective Th1 response, inducing IL-5 rather than Interferon gamma (IFNγ) and IL-12 (Navarathna et al., 2007). It furthermore inhibits IL-6 cytokine production in murine macrophages, a key cytokine in mucosal and systemic *C. albicans* infections, and induces apoptosis of macrophages (Abe et al., 2009; Conti et al., 2009; Ghosh et al., 2010). In contrast to its function as a virulence factor in a systemic model, exogenously added farnesol exerted a protective effect on cells in a reconstituted human epithelial (RHE) model and in a mouse model of oral candidiasis (Hisajima et al., 2008; Saidi, Luitaud, & Rouabhia, 2006). Here, farnesol increased TLR2 expression levels, promoted IL-6 secretion and increased the production of the AMP β-defensin 2 (Decanis, Savignac, & Rouabhia, 2009). This highlights that the effects of farnesol on *C. albicans* survival in the host may be niche-dependent. As farnesol is not produced, nor are cells farnesol-responsive, under anaerobic growth conditions, it appears unlikely that farnesol affects colonization of the GI tract (Dumitru et al., 2004; Kumamoto & Vinces, 2005a). Other QSMs, such as tyrosol, phenylethanol, or pentanol, which are favorably produced under anaerobic growth, may be relevant in anaerobic niches (Albuquerque & Casadevall, 2012; Ghosh et al., 2008). Moreover, it is still unknown what effect farnesol has on biofilms growing on central venous catheters, where cells get in contact with human blood components and serum. Usage of 10% fetal calf serum (FCS) in vitro has been shown to reduce farnesol production about 18-fold (Mosel et al., 2005; Weber et al., 2008). Therefore, one may speculate that host systems can counteract the farnesol effect of biofilm dispersal under certain in vivo circumstances.

8.3 Are You Friend or Foe?—Interkingdom Communication between *C. albicans*, Microbiota, and Opportunistic Bacterial Pathogens

As a commensal, *C. albicans* colonizes niches that are co-colonized by a wide range of other microbes forming the natural microbiome of the human host. Therefore, interactions between *C. albicans* and commensal bacteria have evolved during coevolution in the human host (De Sordi & Muhlschlegel, 2009; Harriott & Noverr, 2011; Mallick & Bennett, 2013; Morales & Hogan, 2010; Peleg et al., 2010; Shirtliff, Peters, et al., 2009; Wang,

2013; Wargo & Hogan, 2006). Such interactions can be mutualistic or competitive, and can occur via direct contact of cells, the secretion of signaling molecules or toxins, competition for nutrients/metabolites, or simply via alteration of the environment in a beneficial or detrimental way for one or all interaction partners (Lynch & Robertson, 2008; Peleg et al., 2010; Wargo & Hogan, 2006). In fact, it becomes more and more clear that the most common situation in nature are polymicrobial multispecies communities rather than planktonic or single-species growth (Harriott & Noverr, 2011). In addition, coinfections are not uncommon: 4–8% of all *Candida*-associated bloodstream infections involve more than one *Candida* spp. and it was estimated that >20% of *C. albicans* bloodstream infections also involve bacteria (Bouza et al., 2013; Klotz, Chasin, et al., 2007; Nace, Horn, & Neofytos, 2009; Pulimood et al., 2002). The most often coisolated species are *Staphylococcus epidermidis*, *Enterococcus* spp. and *Staphylococcus aureus* (Klotz, Chasin, et al., 2007; Shirtliff, Peters, et al., 2009). In order to survive bacterial attacks or gain beneficial effects from these coinfections, *C. albicans* has to sense and communicate with its neighbors. Some important fungal–bacterial interactions are illustrated in Figure 4.

8.3.1 Interkingdom Signaling

Interkingdom signaling allows the communication across the borders of species and even genera. Farnesol, which is important for the coordinated behavior of single-species *C. albicans* biofilms, probably also plays a major role in interkingdom signaling. Farnesol has strong antifungal and antibacterial activities against a range of different organisms, partly by inducing high levels of ROS production (Albuquerque & Casadevall, 2012; Langford et al., 2009; Machida & Tanaka, 1999). It induces apoptosis in several species, inhibits cell growth and germination, and inhibits *S. aureus* biofilm formation (Albuquerque & Casadevall, 2012; Jabra-Rizk et al., 2006). In turn, bacteria have been shown to inhibit *C. albicans* filamentation by producing small signaling molecules, e.g., dodecanol and 3-oxo-C12-HSL from *Pseudomonas aeruginosa*, butyric acid from *Lactobacillus rhamnosus*, capric acid from *Saccharomyces boulardii*, *cis*-2-dodecenoic acid from *Burkholderia cenocepacia*, diffusible signal factor (DSF) from *Xanthomonas campestris*, and QSMs from *Acinetobacter baumannii*, *Salmonella enterica* Serovar Typhimurium, and *Streptococcus mutans* (Boon et al., 2008; Krasowska, Murzyn, Dyjankiewicz, Lukaszewicz, & Dziadkowiec, 2009; Murzyn, Krasowska, Stefanowicz, Dziadkowiec, & Lukaszewicz, 2010; Noverr & Huffnagle, 2004; Peleg et al., 2008; Tampakakis, Peleg, & Mylonakis, 2009; Wang et al., 2004).

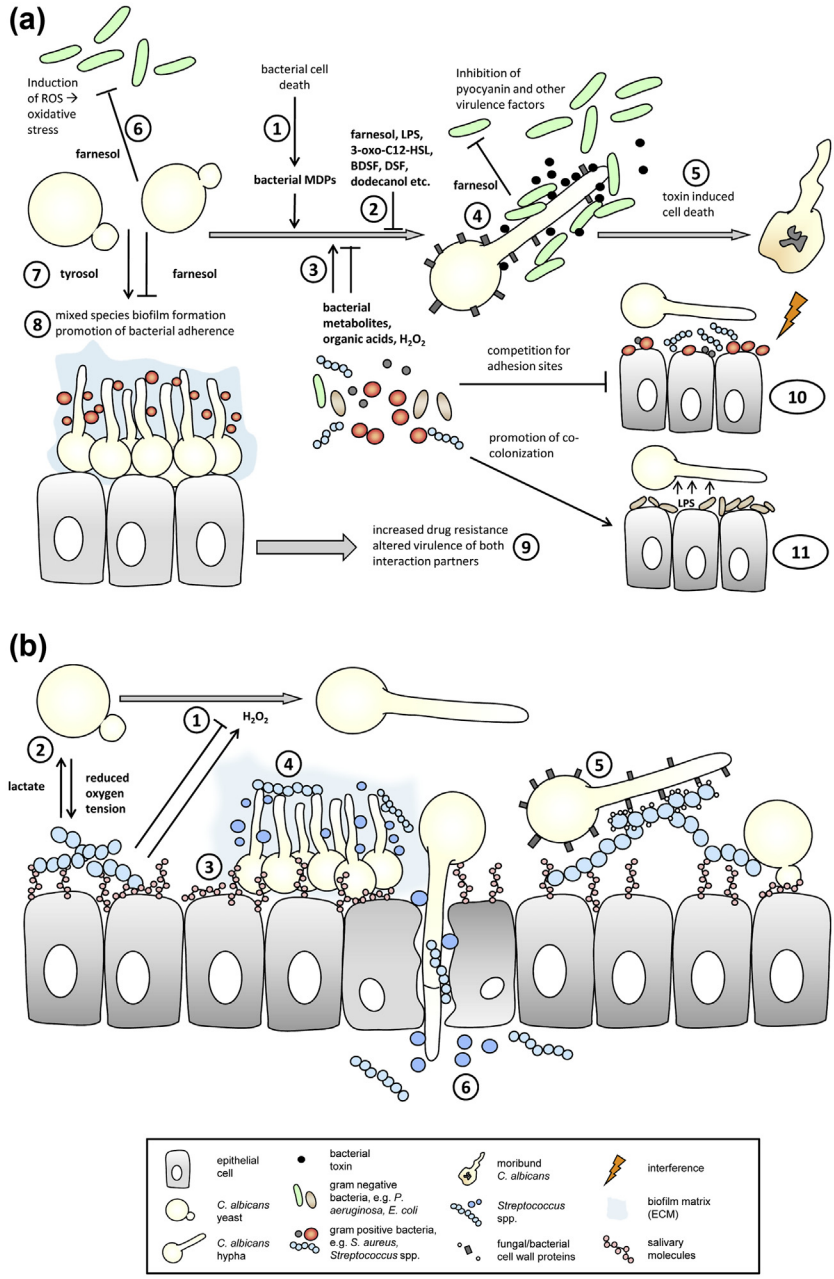

Figure 4 ***Quorum sensing and fungal—bacterial interactions.*** (a) Mechanisms and consequences of fungal—bacterial interactions. (1) Induction of *Candida albicans* filamentation by bacterial cell wall components (muramyl dipeptides, MDPs); (2) Inhibition of the yeast-to-hyphae transition by fungal and bacterial quorum sensing molecules

Lactobacillus spp., the most prevalent bacterial group in the female reproductive tract, as well as *Enterococcus faecalis*, a member of the GI-tract microbiota and an opportunistic pathogen, produce signaling molecules as metabolic byproducts (e.g., lactic acid, butyric acid), H_2O_2 or organic acids that interfere with fungal adhesion, growth, adaptation or might influence the host immune response in an unfavorable way for *C. albicans* (Boris & Barbes, 2000; Braun, Hector, Kamark, Hart, & Cihlar, 1987; Cruz, Graham, Gagliano, Lorenz, & Garsin, 2013; Harriott & Noverr, 2011; Hoberg, Cihlar, & Calderone, 1983; Morales & Hogan, 2010; Noverr & Huffnagle, 2004; Shirtliff, Peters, et al., 2009; Strus et al., 2005; Wargo & Hogan, 2006). The necessary adaptations of *C. albicans* to these conditions are reflected in substantial changes in gene expression profiles when *C. albicans* is co-cultured with certain bacteria. These include genes involved in adhesion, cell wall composition, cell cycle progression, enzymatic activity, signaling, transcription, and substance transport (Fox, Shelton, & Kruppa, 2013).

Nevertheless, fungal–bacterial interactions can also be beneficial for *C. albicans*. *Candida albicans* can directly react to lipopolysaccharide (LPS) molecules from bacterial cell membranes, which is an important modulator of *C. albicans* virulence contributing to coinfection of *C. albicans* and *Escherichia coli* (Akagawa, Abe, & Yamaguchi, 1995; Bandara, Yau, Watt, Jin, & Samaranayake, 2009; Rogers, Williams, Feng, Lewis, & Wei, 2013).

(QSM); (3) influence of bacterial metabolites on *C. albicans* filamentation; (4) inhibition of *Pseudomonas aeruginosa* virulence factors and toxin production by the *C. albicans* QSM farnesol; (5) binding of *P. aeruginosa* to *C. albicans* hyphae and induction of cell death, countered by farnesol-promoted yeast growth (2); (6) inhibition of bacterial growth by farnesol via induction of reactive oxygen species (ROS); (7) regulation of biofilm formation by *C. albicans* QSM; (8) adhesion of co-colonizing bacteria to hyphae promoting mixed-species biofilm formation; (9) increased drug resistance in co-species biofilms and altered virulence; (10) inhibition of *C. albicans* adhesion and colonization by commensal bacteria; (11) promotion of *C. albicans* urinary bladder colonization by *Escherichia coli*. (b) Fungal–bacterial interactions in the oral cavity. (1) Species-dependent promotion or inhibition of *C. albicans* filamentation by streptococci, e.g., H_2O_2 production promotes hyphal growth; (2) lactate secreted by streptococci is used by *C. albicans* as alternative carbon source while reduction of local oxygen tension by *C. albicans* promotes bacterial growth; (3) adhesion of *C. albicans* to salivary molecules promotes niche colonization; (4) adhesion of streptococci to *C. albicans* hyphae leading to co-species biofilms; (5) adherence of *C. albicans* to pre-adhered bacteria supports fungal colonization; (6) epithelial damage by *C. albicans* promotes bacterial invasion. LPS, lipopolysaccharides; DSF, diffusible signal factor; BDSF, *cis*-2-dodecenoic acid; HSL, homoserine lactone.

Escherichia coli infection in the urinary tract furthermore enhances *C. albicans* adherence to the bladder mucosa enabling *C. albicans* to colonize and infect this niche (Levison & Pitsakis, 1987). *Candida albicans* also recognizes muramyl dipeptides (MDPs), subunits of peptidoglycan that directly stimulate yeast-to-hyphae formation (Xu et al., 2008). MDPs are found in high amounts in human serum, likely derived from the cell walls of the human microbiome (O'Hara & Shanahan, 2006; Xu et al., 2008). These molecules directly interact with the leucine-rich repeat domain of the adenylyl cyclase Cyr1 promoting cAMP production (Xu et al., 2008). *Candida albicans* is commonly in contact with masses of MDPs from the normal flora in certain niches e.g., the GI tract. Under healthy conditions, filamentation is probably inhibited via further mechanisms, whereas the use of broad-spectrum antibiotics depletes the bacterial flora, leading to an increase in MDPs, but failure to control fungal outgrowth and filamentation, thereby increasing the risk of systemic infection (Wang, 2013).

In the following, some more complex interactions between *C. albicans* and the clinically relevant bacteria, *P. aeruginosa*, *Streptococcus* spp., and *S. aureus*, will be described in more detail (see Figure 4).

8.3.2 *Interactions between* P. aeruginosa *and* C. albicans

Both *C. albicans* and *P. aeruginosa* are commonly found in mixed opportunistic infections in lung isolates of cystic fibrosis (CF) patients, in burn wounds, or on catheter-related biofilms (De Sordi & Muhlschlegel, 2009; Pierce, 2005; Williams & Camara, 2009). Both microbes can also be found in joined mucosal niches and this is the likely site where the mechanisms of interaction between these two species have evolved (Hogan & Kolter, 2002; Shirtliff, Peters, et al., 2009; Williams & Camara, 2009). *Pseudomonas aeruginosa* is able to form biofilms in the presence of *C. albicans*, adheres to and kills specifically *C. albicans* hyphae whereas yeast cells remain unharmed (Brand, Barnes, Mackenzie, Odds, & Gow, 2008; Hogan & Kolter, 2002). In turn, *C. albicans* has gained several mechanisms to survive killing by *P. aeruginosa*. *Candida albicans* controls yeast-to-hyphae transition in response to the *P. aeruginosa* QSM 3-oxo-C12-HSL (Hogan, Vik, & Kolter, 2004; McAlester, O'Gara, & Morrissey, 2008). HSL is able to inhibit *C. albicans* filamentation via the same route as farnesol by directly inhibiting the activity of Cyr1 (Hall et al., 2011). LPS from *P. aeruginosa* also inhibits filamentation of *C. albicans*, probably by targeting glycolysis-associated mechanisms during filamental growth (Bandara, K Cheung, Watt, Jin, & Samaranayake, 2013; Hogan et al., 2004; Pires et al., 2013). Thereby the presence of *P. aeruginosa* favors

yeast growth of *C. albicans* under conditions that normally promote hyphal formation (Shirtliff, Peters, et al., 2009). On the other hand, farnesol inhibits the production of *P. aeruginosa* quinolone signaling which is necessary for the induction of pyocyanin, an important virulence factor and toxin against other microbes (Cugini et al., 2007). Other important virulence factors of *P. aeruginosa* that are suppressed by farnesol include the hemolytic phospholipase C, phenazines, GacA, LasR, RhlR, and RpoN, which in turn limit *C. albicans* growth, biofilm formation, and virulence (Hogan & Kolter, 2002; Kerr et al., 1999; McAlester et al., 2008; Park, Han, Park, Choi, & Lee, 2014). Furthermore farnesol inhibits the swarming activity of *P. aeruginosa* (Cugini et al., 2007; McAlester et al., 2008; Williams & Camara, 2009). A recent comparison of the proteome of both species in mixed-species biofilms revealed that the coexistence of both species leads to enhanced production of virulence factors and increased mutability in both species, potentially altering host–pathogen interactions (Trejo-Hernandez, Andrade-Dominguez, Hernandez, & Encarnacion, 2014). Although the relationship between *P. aeruginosa* and *C. albicans* is generally antagonistic, *P. aeruginosa* can also promote *C. albicans* virulence under distinct conditions, e.g., in patients with severe burn wounds (Branski et al., 2009; Sun et al., 2012). Under these conditions, the virulence factor LasB, a pseudolysine, from *P. aeruginosa* probably generates an amino acid signal that increases biofilm formation of *C. albicans* (Mallick & Bennett, 2013; Roux et al., 2009). Taken together the complex interactions that occur between the fungus and the opportunistic bacterial pathogen *P. aeruginosa* very likely have clinical implications, especially for CF-patients (Kerr, 1994).

8.3.3 *Biofilm Interaction of* C. albicans *with Microbes in the Oral Cavity*

Oral infections are often polymicrobial, since the oral cavity harbors a wide range of commensal bacteria and opportunistic pathogens. Important interactions between *C. albicans* and bacteria in the oral cavity are schematically depicted in Figure 4(b). Streptococci are major components of the oral microbiome and *C. albicans* forms aggregates with different oral *Streptococcus* spp. such as *Streptococcus gordonii*, *Streptococcus oralis*, *Streptococcus sanguinis*, and *S. mutans* (Bagg & Silverwood, 1986; Harriott & Noverr, 2011; Holmes, McNab, & Jenkinson, 1996; Jenkinson, Lala, & Shepherd, 1990; Metwalli, Khan, Krom, & Jabra-Rizk, 2013; Shirtliff, Peters, et al., 2009). The underlying mechanisms include interaction of the streptococcal AgI/II adhesins SspA and SspB with fungal adhesins such as Als3, Eap1, and Hwp1 (Bamford

et al., 2009; Brady et al., 2010; El-Sabaeny, Demuth, Park, & Lamont, 2000; Holmes et al., 1996; Klotz, Gaur, et al., 2007; Silverman, Nobbs, Vickerman, Barbour, & Jenkinson, 2010; Wright et al., 2013). For *S. gordonii* stabilization of fungal adhesins, such as Als3, by O-mannosylation furthermore seems to be essential to mediate protrusion of the N-terminal domain of the fungal adhesin from the cell surface (Dutton et al., 2014). These domains are subsequently recognized by bacterial adhesins, allowing clustering and nanodomain formation and ultimately leading to localized attachment, accumulation, and microcolony formation of *S. gordonii* on *C. albicans* cells (Dutton et al., 2014). Furthermore, indirect binding and promotion of filamentation is mediated by saliva components that bind to both streptococci and *C. albicans* (Holmes, Cannon, & Jenkinson, 1995; O'Sullivan, Jenkinson, & Cannon, 2000). Soluble glucans produced by streptococcal glucosyltransferases and *C. albicans* further promote production of biofilm matrix and intergeneric adhesive interactions (Falsetta et al., 2014; Ricker, Vickerman, & Dongari-Bagtzoglou, 2014). These interactions are crucial for *C. albicans* colonization and persistence in the oral cavity, since they promote establishment on the oral mucosa even when *C. albicans* cell numbers or the affinity for the mucosal surface are too low (Cannon & Chaffin, 2001; Shirtliff, Peters, et al., 2009). Interestingly, interactions between *Streptococcus* spp. and *C. albicans* can either promote or inhibit filamentation in the fungus. While *S. mutans* inhibits hypha formation via the QSM *trans*-2-decenoic acid, *S. gordonii* stimulates filamentation and promotes dual biofilm formation by secretion of autoinducer 2 (AI-2) (Bamford et al., 2009; Vilchez et al., 2010). AI-2 may function by inducing low levels of H_2O_2 (Bamford et al., 2009). Nontoxic levels of H_2O_2 can in turn induce hypha formation by activating Cek1 (Nasution et al., 2008; Srinivasa, Kim, Yee, Kim, & Choi, 2012). H_2O_2 at low levels is also produced by other bacteria and might promote *C. albicans* growth and filamentation while inhibiting and killing other competing bacterial species (Holmberg & Hallander, 1973; Kreth, Merritt, Shi, & Qi, 2005). Mixed *Streptococcus* spp.-*Candida* biofilms are likely beneficial for both species as the formed ECM is more viscous and highly impenetrable to drugs such as fluconazole (Bamford et al., 2009; Dongari-Bagtzoglou et al., 2009). In these biofilms, lactate produced by the bacteria can be used as an alternative carbon source by *C. albicans* (Holmes, van der Wielen, Cannon, Ruske, & Dawes, 2006; Jenkinson et al., 1990; Ramsey, Rumbaugh, & Whiteley, 2011). In turn, *C. albicans* reduces the oxygen tension by respiration in its surrounding which is a stimulatory factor for streptococcal growth (Shirtliff, Peters, et al., 2009).

Interestingly, *S. oralis* alone is not able to form biofilms on the oral mucosa but the quorum sensing system is activated by the presence of *C. albicans* hyphae which increases multispecies biofilm formation (Whitmore & Lamont, 2011; Xu, Jenkinson, & Dongari-Bagtzoglou, 2014). *Candida albicans* filamentation furthermore provides adhesion sites for the bacteria, damages the oral mucosa releasing nutrients for bacterial growth and clears the way for disseminated infection for both species. Coinfections with *Streptococcus* spp. and *C. albicans* during OPC show pathogenic synergy wherein the bacteria act as "accessory" pathogens (Baena-Monroy et al., 2005; Ramsey et al., 2011; Whitmore & Lamont, 2011; Xu, Jenkinson, et al., 2014; Xu, Sobue, et al., 2014). In summary, *C. albicans* synergistic interactions with oral bacteria not only promote colonization but can also enhance severity of oral diseases.

8.3.4 Staphylococcus aureus *and* C. albicans *Interactions*

Candida albicans and *S. aureus* are two leading causes of bloodstream infections in hospitalized patients and coinfections with both pathogens can occur (Klotz, Chasin, et al., 2007; Perlroth et al., 2007; Shirtliff, Peters, et al., 2009). In a mouse model of intra-abdominal infection coinfection with both species lead to mortality, while mono infections were avirulent (Carlson, 1982, 1983a,b; Nash, Peters, Palmer, Fidel, & Noverr, 2014). This effect was independent of the morphological growth form of *C. albicans*, but probably due to increased local and systemic induction of proinflammatory cytokines (Nash et al., 2014). Furthermore, bacteria penetrated the surrounding organs more easily in the presence of *C. albicans*, probably driven by filamentation induced damage promoted by *C. albicans* (Shirtliff, Peters, et al., 2009). *Candida albicans* and *S. aureus* are also commonly found together in oral infections (Baena-Monroy et al., 2005). Both organisms seem to interact physically in mixed biofilms leading to increased biofilm mass and resistance rates to drugs (Baena-Monroy et al., 2005; Harriott & Noverr, 2011; Shirtliff, Peters, et al., 2009). *Staphylococcus aureus* normally adheres very poorly to abiotic surfaces, but attaches to hyphae, leading to co-species biofilm formation (Baena-Monroy et al., 2005; Cassat, Lee, & Smeltzer, 2007; Harriott & Noverr, 2011; Shirtliff, Peters, et al., 2009). However, the interaction is not solely mutualistic as under certain conditions *C. albicans* inhibits *S. aureus* biofilm formation and lipase activity, and farnesol interferes with *S. aureus* viability and antibiotic susceptibility (Jabra-Rizk et al., 2006; Kuroda, Nagasaki, Ito, & Ohta, 2007). Thus, although the coexistence of both organisms seems to be beneficial for both, *C. albicans*

has developed mechanisms to control bacterial growth (Shirtliff, Peters, et al., 2009).

In conclusion, the interaction of *C. albicans* with members of the human microbiome and bacterial pathogens affect *Candida* survival, colonization, and infection in the human host. Bacteria–fungal interactions can lead to enhanced virulence of one or both microbes, but bacteria may also limit the growth, survival, and colonization abilities of *C. albicans* in certain host niches. Therefore, disturbance of the microbial community will influence *C. albicans* lifestyle and signaling under almost all in vivo conditions, with important clinical implications: In fact, antibiotic treatment is one of the most prominent risk factors for candidiasis and the possibility of coinfections or increased resistance of mixed-species biofilms should be considered in the selection of antimicrobial therapy (Harriott & Noverr, 2011; Samonis et al., 1994; Shirtliff, Peters, et al., 2009; Yan, Yang, & Tang, 2013).

9. CONCLUSION

Fungal infections are a major health problem worldwide and *C. albicans* is one of the leading fungal pathogens (Brown, Denning, et al., 2012). Especially mucosal infections are extremely common; in fact, 50–70% of women in the childbearing ages suffer from at least one episode of vulvovaginal candidiasis, and 5–8% will undergo reoccurring clinical infection (Sobel, 2007). The genetic, phenotypic, and physiologic plasticity described in this chapter enables *C. albicans* to rapidly adapt to the changing environments in the human host and allow the colonization and infection of a wide variety of organs. The redundancy in many signaling pathways involved in infection, such as the induction of morphogenesis, adhesion, and antifungal resistance mechanisms, probably supports rapid adaptation. Even though most research focuses on the role of *C. albicans* as a pathogen, it should be noted that *C. albicans* is nearly exclusively found in association with a warm-blooded host. Furthermore, host-to-host transmission is rare and most individuals will likely only be colonized by *C. albicans* strains that they acquired early in life. Together with the absence of an environmental reservoir, survival of *C. albicans* populations thus relies on long-term persistence in the host. Clinical infections, especially life-threatening disseminated disease, would be detrimental rather than beneficial and might be an accidental side effect of the traits that *C. albicans* acquired to successfully compete and survive as a commensal. This hypothesis is supported by the observations that regulators of virulence also

mediate commensalism, and the circuits that regulate either lifestyle are tightly interknit (Perez & Johnson, 2013; Perez, Kumamoto, & Johnson, 2013; Pierce et al., 2013; Pierce & Kumamoto, 2012). This highlights once again the fine line that separates the roles of *C. albicans* as harmless colonizer and harmful pathogen.

In addition, *Candida* spp. coexist with numerous microbial species on the host mucosa. The interactions between bacteria and *Candida* are complex and only poorly understood; future research will hopefully shed some light on the role of microbial interactions during colonization, disease, and dissemination to aid our ability to effectively prevent and treat both candidiasis and polymicrobial infections. Another issue to be addressed is the lack of data on non-albicans *Candida* spp. Most studies so far concentrated on *C. albicans*; however, considering the rising incidence of infections caused by other *Candida* species, increased efforts to understand the species-specific differences in physiology and interaction with the host are warranted. In closing, a better understanding of the mechanisms that allow *Candida* spp. to survive, persist, and cause disease within the human host will likely be crucial for the development of novel antifungal strategies which are desperately needed to reduce the unacceptably high mortality rates of systemic candidiasis.

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REFERENCES

Abe, S., Tsunashima, R., Iijima, R., Yamada, T., Maruyama, N., Hisajima, T., et al. (2009). Suppression of anti-*Candida* activity of macrophages by a quorum-sensing molecule, farnesol, through induction of oxidative stress. *Microbiology and Immunology, 53*, 323–330.

Akagawa, G., Abe, S., & Yamaguchi, H. (1995). Mortality of *Candida albicans*-infected mice is facilitated by superinfection of *Escherichia coli* or administration of its lipopolysaccharide. *Journal of Infectious Diseases, 171*, 1539–1544.

Al-Dhaheri, R. S., & Douglas, L. J. (2008). Absence of amphotericin B-tolerant persister cells in biofilms of some *Candida* species. *Antimicrobial Agents and Chemotherapy, 52*, 1884–1887.

Al-Fattani, M. A., & Douglas, L. J. (2004). Penetration of *Candida* biofilms by antifungal agents. *Antimicrobial Agents and Chemotherapy, 48*, 3291–3297.

Al-Fattani, M. A., & Douglas, L. J. (2006). Biofilm matrix of *Candida albicans* and *Candida tropicalis*: chemical composition and role in drug resistance. *Journal of Medical Microbiology, 55*, 999–1008.

Alarco, A. M., & Raymond, M. (1999). The bZip transcription factor Cap1p is involved in multidrug resistance and oxidative stress response in *Candida albicans*. *Journal of Bacteriology, 181*, 700–708.

Albuquerque, P., & Casadevall, A. (2012). Quorum sensing in fungi–a review. *Medical Mycology, 50*, 337–345.

Alem, M. A., Oteef, M. D., Flowers, T. H., & Douglas, L. J. (2006). Production of tyrosol by *Candida albicans* biofilms and its role in quorum sensing and biofilm development. *Eukaryotic Cell, 5*, 1770–1779.

Almeida, R. S., Brunke, S., Albrecht, A., Thewes, S., Laue, M., Edwards, J. E., et al. (2008). the hyphal-associated adhesin and invasin Als3 of *Candida albicans* mediates iron acquisition from host ferritin. *PLoS Pathogens, 4*, e1000217.

Almeida, R. S., Wilson, D., & Hube, B. (2009). *Candida albicans* iron acquisition within the host. *FEMS Yeast Research, 9*, 1000–1012.

Alonso-Monge, R., Navarro-Garcia, F., Molero, G., Diez-Orejas, R., Gustin, M., Pla, J., et al. (1999). Role of the mitogen-activated protein kinase Hog1p in morphogenesis and virulence of *Candida albicans*. *Journal of Bacteriology, 181*, 3058–3068.

Alonso-Monge, R., Roman, E., Arana, D. M., Pla, J., & Nombela, C. (2009). Fungi sensing environmental stress. *Clinical Microbiology and Infection, 15*(Suppl. 1), 17–19.

Alsteens, D., Garcia, M. C., Lipke, P. N., & Dufrene, Y. F. (2010). Force-induced formation and propagation of adhesion nanodomains in living fungal cells. *Proceedings of the National Academy of Sciences of the United States of America, 107*, 20744–20749.

Aoki, S., Ito-Kuwa, S., Nakamura, K., Vidotto, V., & Takeo, K. (1998). Oxygen as a possible tropic factor in hyphal growth of *Candida albicans*. *Mycoscience, 39*, 231–238.

Argimon, S., Fanning, S., Blankenship, J. R., & Mitchell, A. P. (2011). Interaction between the *Candida albicans* high-osmolarity glycerol (HOG) pathway and the response to human beta-defensins 2 and 3. *Eukaryotic Cell, 10*, 272–275.

Arguelles, J. C. (1997). Thermotolerance and trehalose accumulation induced by heat shock in yeast cells of *Candida albicans*. *FEMS Microbiology Letters, 146*, 65–71.

Arkhipova, I. R. (2005). Mobile genetic elements and sexual reproduction. *Cytogenetic and Genome Research, 110*, 372–382.

Arkowitz, R. A., & Bassilana, M. (2011). Polarized growth in fungi: symmetry breaking and hyphal formation. *Seminars in Cell and Developmental Biology, 22*, 806–815.

van Asbeck, E. C., Clemons, K. V., & Stevens, D. A. (2009). *Candida parapsilosis*: a review of its epidemiology, pathogenesis, clinical aspects, typing and antimicrobial susceptibility. *Critical Reviews in Microbiology, 35*, 283–309.

Ashman, R. B., Papadimitriou, J. M., Fulurija, A., Drysdale, K. E., Farah, C. S., Naidoo, O., et al. (2003). Role of complement C5 and T lymphocytes in pathogenesis of disseminated and mucosal candidiasis in susceptible DBA/2 mice. *Microbial Pathogenesis, 34*, 103–113.

Baena-Monroy, T., Moreno-Maldonado, V., Franco-Martinez, F., Aldape-Barrios, B., Quindos, G., & Sanchez-Vargas, L. O. (2005). *Candida albicans, Staphylococcus aureus* and *Streptococcus mutans* colonization in patients wearing dental prosthesis. *Medicina Oral Patologia Oral y Cirugia Bucal, 10*(Suppl. 1), E27–E39.

Bagg, J., & Silverwood, R. W. (1986). Coagglutination reactions between *Candida albicans* and oral bacteria. *Journal of Medical Microbiology, 22*, 165–169.

Baillie, G. S., & Douglas, L. J. (1999). Role of dimorphism in the development of *Candida albicans* biofilms. *Journal of Medical Microbiology, 48*, 671–679.

Bamford, C. V., d'Mello, A., Nobbs, A. H., Dutton, L. C., Vickerman, M. M., & Jenkinson, H. F. (2009). *Streptococcus gordonii* modulates *Candida albicans* biofilm formation through intergeneric communication. *Infection and Immunity, 77*, 3696–3704.

Bandara, H. M., K Cheung, B. P., Watt, R. M., Jin, L. J., & Samaranayake, L. P. (2013). *Pseudomonas aeruginosa* lipopolysaccharide inhibits *Candida albicans* hyphae formation and alters gene expression during biofilm development. *Molecular Oral Microbiology, 28*, 54–69.

Bandara, H. M., Yau, J. Y., Watt, R. M., Jin, L. J., & Samaranayake, L. P. (2009). *Escherichia coli* and its lipopolysaccharide modulate in vitro *Candida* biofilm formation. *Journal of Medical Microbiology, 58*, 1623–1631.

Banerjee, M., Thompson, D. S., Lazzell, A., Carlisle, P. L., Pierce, C., Monteagudo, C., et al. (2008). *UME6*, a novel filament-specific regulator of *Candida albicans* hyphal extension and virulence. *Molecular Biology of the Cell, 19*, 1354–1365.

Banerjee, M., Uppuluri, P., Zhao, X. R., Carlisle, P. L., Vipulanandan, G., Villar, C. C., et al. (2013). Expression of *UME6*, a key regulator of *Candida albicans* hyphal development, enhances biofilm formation via Hgc1- and Sun41-dependent mechanisms. *Eukaryotic Cell, 12*, 224–232.

Barelle, C. J., Priest, C. L., Maccallum, D. M., Gow, N. A., Odds, F. C., & Brown, A. J. (2006). Niche-specific regulation of central metabolic pathways in a fungal pathogen. *Cellular Microbiology, 8*, 961–971.

Barton, R. C., & Scherer, S. (1994). Induced chromosome rearrangements and morphologic variation in *Candida albicans*. *Journal of Bacteriology, 176*, 756–763.

Barwell, K. J., Boysen, J. H., Xu, W., & Mitchell, A. P. (2005). Relationship of *DFG16* to the Rim101p pH response pathway in *Saccharomyces cerevisiae* and *Candida albicans*. *Eukaryotic Cell, 4*, 890–899.

Bassetti, M., Merelli, M., Righi, E., Diaz-Martin, A., Rosello, E. M., Luzzati, R., et al. (2013). Epidemiology, species distribution, antifungal susceptibility, and outcome of candidemia across five sites in Italy and Spain. *Journal of Clinical Microbiology, 51*, 4167–4172.

Bassler, B. L. (2002). Small talk. Cell-to-cell communication in bacteria. *Cell, 109*, 421–424.

Bassler, B. L., & Losick, R. (2006). Bacterially speaking. *Cell, 125*, 237–246.

Basu, S., Quilici, C., Zhang, H. H., Grail, D., & Dunn, A. R. (2008). Mice lacking both G-CSF and IL-6 are more susceptible to *Candida albicans* infection: critical role of neutrophils in defense against *Candida albicans*. *Growth Factors, 26*, 23–34.

Ben-Ami, R., Garcia-Effron, G., Lewis, R. E., Gamarra, S., Leventakos, K., Perlin, D. S., et al. (2011). Fitness and virulence costs of *Candida albicans FKS1* hot spot mutations associated with echinocandin resistance. *Journal of Infectious Diseases, 204*, 626–635.

Bennett, R. J. (2009). A *Candida*-based view of fungal sex and pathogenesis. *Genome Biology, 10*, 230.

Bennett, R. J., & Johnson, A. D. (2003). Completion of a parasexual cycle in *Candida albicans* by induced chromosome loss in tetraploid strains. *The EMBO Journal, 22*, 2505–2515.

Bennett, R. J., & Johnson, A. D. (2005). Mating in *Candida albicans* and the search for a sexual cycle. *Annual Review of Microbiology, 59*, 233–255.

Bennett, R. J., Miller, M. G., Chua, P. R., Maxon, M. E., & Johnson, A. D. (2005). Nuclear fusion occurs during mating in *Candida albicans* and is dependent on the *KAR3* gene. *Molecular Microbiology, 55*, 1046–1059.

Bennett, R. J., Uhl, M. A., Miller, M. G., & Johnson, A. D. (2003). Identification and characterization of a *Candida albicans* mating pheromone. *Molecular and Cellular Biology, 23*, 8189–8201.

Bensen, E. S., Martin, S. J., Li, M., Berman, J., & Davis, D. A. (2004). Transcriptional profiling in *Candida albicans* reveals new adaptive responses to extracellular pH and functions for Rim101p. *Molecular Microbiology, 54*, 1335–1351.

Berman, J., & Sudbery, P. E. (2002). *Candida Albicans*: a molecular revolution built on lessons from budding yeast. *Nature Reviews Genetics, 3*, 918–930.

Bevins, C. L., & Salzman, N. H. (2011). The potter's wheel: the host's role in sculpting its microbiota. *Cellular and Molecular Life Sciences, 68*, 3675–3685.

Bink, A., Vandenbosch, D., Coenye, T., Nelis, H., Cammue, B. P., & Thevissen, K. (2011). Superoxide dismutases are involved in *Candida albicans* biofilm persistence against miconazole. *Antimicrobial Agents and Chemotherapy, 55*, 4033–4037.

Birse, C. E., Irwin, M. Y., Fonzi, W. A., & Sypherd, P. S. (1993). Cloning and characterization of *ECE1*, a gene expressed in association with cell elongation of the dimorphic pathogen *Candida albicans*. *Infection and Immunity, 61*, 3648–3655.

Blankenship, J. R., & Mitchell, A. P. (2006). How to build a biofilm: a fungal perspective. *Current Opinion in Microbiology, 9*, 588–594.

Bliska, J. B., & Casadevall, A. (2009). Intracellular pathogenic bacteria and fungi–a case of convergent evolution? *Nature Reviews Microbiology, 7*, 165–171.

Bogdan, C., Rollinghoff, M., & Diefenbach, A. (2000). The role of nitric oxide in innate immunity. *Immunological Reviews, 173*, 17–26.

Bonhomme, J., Chauvel, M., Goyard, S., Roux, P., Rossignol, T., & d'Enfert, C. (2011). Contribution of the glycolytic flux and hypoxia adaptation to efficient biofilm formation by *Candida albicans*. *Molecular Microbiology, 80*, 995–1013.

Bonhomme, J., & d'Enfert, C. (2013). *Candida albicans* biofilms: building a heterogeneous, drug-tolerant environment. *Current Opinion in Microbiology, 16*, 398–403.

Bonifazi, P., Zelante, T., D'Angelo, C., De Luca, A., Moretti, S., Bozza, S., et al. (2009). Balancing inflammation and tolerance in vivo through dendritic cells by the commensal *Candida albicans*. *Mucosal Immunology, 2*, 362–374.

Boon, C., Deng, Y., Wang, L. H., He, Y., Xu, J. L., Fan, Y., et al. (2008). A novel DSF-like signal from *Burkholderia cenocepacia* interferes with *Candida albicans* morphological transition. *ISME Journal, 2*, 27–36.

Borecka-Melkusova, S., Moran, G. P., Sullivan, D. J., Kucharikova, S., Chorvat, D., Jr., & Bujdakova, H. (2009). The expression of genes involved in the ergosterol biosynthesis pathway in *Candida albicans* and *Candida dubliniensis* biofilms exposed to fluconazole. *Mycoses, 52*, 118–128.

Boris, S., & Barbes, C. (2000). Role played by lactobacilli in controlling the population of vaginal pathogens. *Microbes and Infection, 2*, 543–546.

Bougnoux, M. E., Diogo, D., Francois, N., Sendid, B., Veirmeire, S., Colombel, J. F., et al. (2006). Multilocus sequence typing reveals intrafamilial transmission and microevolutions of *Candida albicans* isolates from the human digestive tract. *Journal of Clinical Microbiology, 44*, 1810–1820.

Bouza, E., Burillo, A., Munoz, P., Guinea, J., Marin, M., & Rodriguez-Creixems, M. (2013). Mixed bloodstream infections involving bacteria and *Candida* spp. *Journal of Antimicrobial Chemotherapy, 68*, 1881–1888.

Brady, L. J., Maddocks, S. E., Larson, M. R., Forsgren, N., Persson, K., Deivanayagam, C. C., et al. (2010). The changing faces of *Streptococcus* antigen I/II polypeptide family adhesins. *Molecular Microbiology, 77*, 276–286.

Brand, A., Barnes, J. D., Mackenzie, K. S., Odds, F. C., & Gow, N. A. (2008). Cell wall glycans and soluble factors determine the interactions between the hyphae of *Candida albicans* and *Pseudomonas aeruginosa*. *FEMS Microbiology Letters, 287*, 48–55.

Brand, A., & Gow, N. A. (2009). Mechanisms of hypha orientation of fungi. *Current Opinion in Microbiology, 12*, 350–357.

Brand, A., Lee, K., Veses, V., & Gow, N. A. (2009). Calcium homeostasis is required for contact-dependent helical and sinusoidal tip growth in *Candida albicans* hyphae. *Molecular Microbiology, 71*, 1155–1164.

Brand, A., Shanks, S., Duncan, V. M., Yang, M., Mackenzie, K., & Gow, N. A. (2007). Hyphal orientation of *Candida albicans* is regulated by a calcium-dependent mechanism. *Current Biology, 17*, 347–352.

Brand, A., Vacharaksa, A., Bendel, C., Norton, J., Haynes, P., Henry-Stanley, M., et al. (2008). An internal polarity landmark is important for externally induced hyphal behaviors in *Candida albicans*. *Eukaryotic Cell, 7*, 712–720.

Branski, L. K., Al-Mousawi, A., Rivero, H., Jeschke, M. G., Sanford, A. P., & Herndon, D. N. (2009). Emerging infections in burns. *Surgical Infections (Larchmt), 10*, 389–397.

Braun, B. R., Head, W. S., Wang, M. X., & Johnson, A. D. (2000). Identification and characterization of *TUP1*-regulated genes in *Candida albicans*. *Genetics, 156*, 31–44.

Braun, B. R., & Johnson, A. D. (1997). Control of filament formation in *Candida albicans* by the transcriptional repressor *TUP1*. *Science, 277*, 105–109.

Braun, B. R., Kadosh, D., & Johnson, A. D. (2001). *NRG1*, a repressor of filamentous growth in *C. albicans*, is down-regulated during filament induction. *The EMBO Journal, 20*, 4753–4761.

Braun, P. C., Hector, R. F., Kamark, M. E., Hart, J. T., & Cihlar, R. L. (1987). Effect of cerulenin and sodium butyrate on chitin synthesis in *Candida albicans*. *Canadian Journal of Microbiology, 33*, 546–550.

Brown, D. H., Jr., Giusani, A. D., Chen, X., & Kumamoto, C. A. (1999). Filamentous growth of *Candida albicans* in response to physical environmental cues and its regulation by the unique *CZF1* gene. *Molecular Microbiology, 34*, 651–662.

Brown, A. J., Brown, G. D., Netea, M. G., & Gow, N. A. (2014). Metabolism impacts upon *Candida* immunogenicity and pathogenicity at multiple levels. *Trends in Microbiology, 22*(11), 614–622. http://dx.doi.org/10.1016/j.tim.2014.07.001.

Brown, A. J., Budge, S., Kaloriti, D., Tillmann, A., Jacobsen, M. D., Yin, Z., et al. (2014). Stress adaptation in a pathogenic fungus. *Journal of Experimental Biology, 217*, 144–155.

Brown, A. J., Haynes, K., & Quinn, J. (2009). Nitrosative and oxidative stress responses in fungal pathogenicity. *Current Opinion in Microbiology, 12*, 384–391.

Brown, A. J., Odds, F. C., & Gow, N. A. (2007). Infection-related gene expression in *Candida albicans*. *Current Opinion in Microbiology, 10*, 307–313.

Brown, A. J. P., Haynes, K., Gow, N. A. R., & Quinn, J. (2012). Stress responses in *Candida*. In R. A. Calderone, & C. J. Clancy (Eds.), Candida *and Candidiasis* (pp. 225–242). ASM Press.

Brown, G. D., Denning, D. W., Gow, N. A., Levitz, S. M., Netea, M. G., & White, T. C. (2012). Hidden killers: human fungal infections. *Science Translational Medicine, 4*, 165rv13.

Brunke, S., & Hube, B. (2013). Two unlike cousins: *Candida albicans* and *C. glabrata* infection strategies. *Cellular Microbiology, 15*, 701–708.

Brunke, S., & Hube, B. (2014). Adaptive prediction as a strategy in microbial infections. *PLoS Pathogens, 10*, e1004356.

Calderone, R. A. (2002). Candida *and Candidiasis*. ASM Press.

Cannon, R. D., & Chaffin, W. L. (2001). Colonization is a crucial factor in oral candidiasis. *Journal of Dental Education, 65*, 785–787.

Cao, Y. Y., Cao, Y. B., Xu, Z., Ying, K., Li, Y., Xie, Y., et al. (2005). cDNA microarray analysis of differential gene expression in *Candida albicans* biofilm exposed to farnesol. *Antimicrobial Agents and Chemotherapy, 49*, 584–589.

Cao, F., Lane, S., Raniga, P. P., Lu, Y., Zhou, Z., Ramon, K., et al. (2006). The Flo8 transcription factor is essential for hyphal development and virulence in *Candida albicans*. *Molecular Biology of the Cell, 17*, 295–307.

Cap, M., Vachova, L., & Palkova, Z. (2012). Reactive oxygen species in the signaling and adaptation of multicellular microbial communities. *Oxidative Medicine and Cellular Longevity, 2012*, 976753.

Carlisle, P. L., Banerjee, M., Lazzell, A., Monteagudo, C., Lopez-Ribot, J. L., & Kadosh, D. (2009). Expression levels of a filament-specific transcriptional regulator are sufficient to determine *Candida albicans* morphology and virulence. *Proceedings of the National Academy of Sciences of the United States of America, 106*, 599–604.

Carlisle, P. L., & Kadosh, D. (2010). *Candida albicans* Ume6, a filament-specific transcriptional regulator, directs hyphal growth via a pathway involving Hgc1 cyclin-related protein. *Eukaryotic Cell, 9*, 1320–1328.

Carlson, E. (1982). Synergistic effect of *Candida albicans* and *Staphylococcus aureus* on mouse mortality. *Infection and Immunity, 38*, 921–924.

Carlson, E. (1983a). Effect of strain of *Staphylococcus aureus* on synergism with *Candida albicans* resulting in mouse mortality and morbidity. *Infection and Immunity, 42*, 285–292.

Carlson, E. (1983b). Enhancement by *Candida albicans* of *Staphylococcus aureus, Serratia marcescens*, and *Streptococcus faecalis* in the establishment of infection in mice. *Infection and Immunity, 39*, 193–197.

Casadevall, A. (2008). Evolution of intracellular pathogens. *Annual Review of Microbiology, 62*, 19–33.

Cassat, J. E., Lee, C. Y., & Smeltzer, M. S. (2007). Investigation of biofilm formation in clinical isolates of *Staphylococcus aureus*. *Methods in Molecular Biology, 391*, 127–144.

Cassone, A., & Cauda, R. (2012). *Candida* and candidiasis in HIV-infected patients: where commensalism, opportunistic behavior and frank pathogenicity lose their borders. *AIDS, 26*, 1457–1472.

Castrejon, F., Gomez, A., Sanz, M., Duran, A., & Roncero, C. (2006). The *RIM101* pathway contributes to yeast cell wall assembly and its function becomes essential in the absence of mitogen-activated protein kinase Slt2p. *Eukaryotic Cell, 5*, 507–517.

Chaffin, W. L. (2008). *Candida albicans* cell wall proteins. *Microbiology and Molecular Biology Reviews, 72*, 495–544.

Chandra, J., Kuhn, D. M., Mukherjee, P. K., Hoyer, L. L., McCormick, T., & Ghannoum, M. A. (2001). Biofilm formation by the fungal pathogen *Candida albicans*: development, architecture, and drug resistance. *Journal of Bacteriology, 183*, 5385–5394.

Chandra, J., McCormick, T. S., Imamura, Y., Mukherjee, P. K., & Ghannoum, M. A. (2007). Interaction of *Candida albicans* with adherent human peripheral blood mononuclear cells increases *C. albicans* biofilm formation and results in differential expression of pro- and anti-inflammatory cytokines. *Infection and Immunity, 75*, 2612–2620.

Chang, H. T., Tsai, P. W., Huang, H. H., Liu, Y. S., Chien, T. S., & Lan, C. Y. (2012). LL37 and hBD-3 elevate the beta-1,3-exoglucanase activity of *Candida albicans* Xog1p, resulting in reduced fungal adhesion to plastic. *Biochemical Journal, 441*, 963–970.

Chaves, G. M., Bates, S., Maccallum, D. M., & Odds, F. C. (2007). *Candida albicans GRX2*, encoding a putative glutaredoxin, is required for virulence in a murine model. *Genetics and Molecular Research, 6*, 1051–1063.

Chen, C., Pande, K., French, S. D., Tuch, B. B., & Noble, S. M. (2011). An iron homeostasis regulatory circuit with reciprocal roles in *Candida albicans* commensalism and pathogenesis. *Cell Host & Microbe, 10*, 118–135.

Chen, H., & Fink, G. R. (2006). Feedback control of morphogenesis in fungi by aromatic alcohols. *Genes & Development, 20*, 1150–1161.

Chen, H., Fujita, M., Feng, Q., Clardy, J., & Fink, G. R. (2004). Tyrosol is a quorum-sensing molecule in *Candida albicans*. *Proceedings of the National Academy of Sciences of the United States of America, 101*, 5048–5052.

Chen, J., Chen, J., Lane, S., & Liu, H. (2002). A conserved mitogen-activated protein kinase pathway is required for mating in *Candida albicans*. *Molecular Microbiology, 46*, 1335–1344.

Chen, J., Wang, Q., & Chen, J. Y. (2000). *CEK2*, a novel MAPK from *Candida albicans* complement the mating defect of fus3/kss1 mutant. *Sheng Wu Hua Xue Yu Sheng Wu Wu Li Xue Bao (Shanghai), 32*, 299–304.

Chen, X., Magee, B. B., Dawson, D., Magee, P. T., & Kumamoto, C. A. (2004). Chromosome 1 trisomy compromises the virulence of *Candida albicans*. *Molecular Microbiology, 51*, 551–565.

Cheng, S. C., Joosten, L. A., Kullberg, B. J., & Netea, M. G. (2012). Interplay between *Candida albicans* and the mammalian innate host defense. *Infection and Immunity, 80*, 1304–1313.

Childers, D. S., Mundodi, V., Banerjee, M., & Kadosh, D. (2014). A 5' UTR-mediated translational efficiency mechanism inhibits the *Candida albicans* morphological transition. *Molecular Microbiology, 92*, 570–585.

Chiranand, W., McLeod, I., Zhou, H., Lynn, J. J., Vega, L. A., Myers, H., et al. (2008). *CTA4* transcription factor mediates induction of nitrosative stress response in *Candida albicans*. *Eukaryotic Cell, 7*, 268–278.

Cho, T., Aoyama, T., Toyoda, M., Nakayama, H., Chibana, H., & Kaminishi, H. (2007). Transcriptional changes in *Candida albicans* Genes by both farnesol and high cell density at an early stage of morphogenesis in N-acetyl-D-glucosamine medium. *Nihon Ishinkin Gakkai Zasshi, 48*, 159–167.

Citiulo, F., Jacobsen, I. D., Miramon, P., Schild, L., Brunke, S., Zipfel, P., et al. (2012). *Candida albicans* scavenges host zinc via Pra1 during endothelial invasion. *PLoS Pathogens, 8*, e1002777.

Conti, H. R., Shen, F., Nayyar, N., Stocum, E., Sun, J. N., Lindemann, M. J., et al. (2009). Th17 cells and IL-17 receptor signaling are essential for mucosal host defense against oral candidiasis. *Journal of Experimental Medicine, 206*, 299–311.

Coste, A., Selmecki, A., Forche, A., Diogo, D., Bougnoux, M. E., d'Enfert, C., et al. (2007). Genotypic evolution of azole resistance mechanisms in sequential *Candida albicans* isolates. *Eukaryotic Cell, 6*, 1889–1904.

Coste, A., Turner, V., Ischer, F., Morschhauser, J., Forche, A., Selmecki, A., et al. (2006). A mutation in Tac1p, a transcription factor regulating *CDR1* and *CDR2*, is coupled with loss of heterozygosity at chromosome 5 to mediate antifungal resistance in *Candida albicans*. *Genetics, 172*, 2139–2156.

Costerton, J. W. (1995). Overview of microbial biofilms. *Journal of Industrial Microbiology, 15*, 137–140.

Costerton, J. W., Montanaro, L., & Arciola, C. R. (2005). Biofilm in implant infections: its production and regulation. *International Journal of Artificial Organs, 28*, 1062–1068.

Cottier, F., & Muhlschlegel, F. A. (2009). Sensing the environment: response of *Candida albicans* to the X factor. *FEMS Microbiology Letters, 295*, 1–9.

Cowen, L. E., Kohn, L. M., & Anderson, J. B. (2001). Divergence in fitness and evolution of drug resistance in experimental populations of *Candida albicans*. *Journal of Bacteriology, 183*, 2971–2978.

Cremer, J., Vatou, V., & Braveny, I. (1999). 2,4-(hydroxyphenyl)-ethanol, an antioxidative agent produced by *Candida* spp., impairs neutrophilic yeast killing in vitro. *FEMS Microbiology Letters, 170*, 319–325.

Critchley, I. A., & Douglas, L. J. (1985). Differential adhesion of pathogenic *Candida species* to epithelial and inert surfaces. *FEMS Microbiology Letters, 28*, 199–203.

Crombie, T., Gow, N. A., & Gooday, G. W. (1990). Influence of applied electrical fields on yeast and hyphal growth of *Candida albicans*. *Journal of General Microbiology, 136*, 311–317.

Cruz, M. R., Graham, C. E., Gagliano, B. C., Lorenz, M. C., & Garsin, D. A. (2013). *Enterococcus faecalis* inhibits hyphal morphogenesis and virulence of *Candida albicans*. *Infection and Immunity, 81*, 189–200.

Csank, C., Schroppel, K., Leberer, E., Harcus, D., Mohamed, O., Meloche, S., et al. (1998). Roles of the *Candida albicans* mitogen-activated protein kinase homolog, Cek1p, in hyphal development and systemic candidiasis. *Infection and Immunity, 66*, 2713–2721.

Cugini, C., Calfee, M. W., Farrow, J. M., 3rd, Morales, D. K., Pesci, E. C., & Hogan, D. A. (2007). Farnesol, a common sesquiterpene, inhibits PQS production in *Pseudomonas aeruginosa*. *Molecular Microbiology, 65*, 896–906.

Dalle, F., Wachtler, B., L'Ollivier, C., Holland, G., Bannert, N., Wilson, D., et al. (2010). Cellular interactions of *Candida albicans* with human oral epithelial cells and enterocytes. *Cellular Microbiology, 12*, 248–271.

Daniels, K. J., Srikantha, T., Lockhart, S. R., Pujol, C., & Soll, D. R. (2006). Opaque cells signal white cells to form biofilms in *Candida albicans*. *The EMBO Journal, 25*, 2240–2252.

Davies, J. M., Stacey, A. J., & Gilligan, C. A. (1999). *Candida albicans* hyphal invasion: thigmotropism or chemotropism? *FEMS Microbiology Letters, 171*, 245–249.

Davis-Hanna, A., Piispanen, A. E., Stateva, L. I., & Hogan, D. A. (2008). Farnesol and dodecanol effects on the *Candida albicans* Ras1-cAMP signalling pathway and the regulation of morphogenesis. *Molecular Microbiology, 67*, 47–62.

Davis, D., Edwards, J. E., Jr., Mitchell, A. P., & Ibrahim, A. S. (2000). *Candida albicans* RIM101 pH response pathway is required for host-pathogen interactions. *Infection and Immunity, 68*, 5953–5959.

Davis, D., Wilson, R. B., & Mitchell, A. P. (2000). RIM101-dependent and-independent pathways govern pH responses in *Candida albicans*. *Molecular and Cellular Biology, 20*, 971–978.

Davis, D. A. (2009). How human pathogenic fungi sense and adapt to pH: the link to virulence. *Current Opinion in Microbiology, 12*, 365–370.

Davis, D. A., Bruno, V. M., Loza, L., Filler, S. G., & Mitchell, A. P. (2002). *Candida albicans* Mds3p, a conserved regulator of pH responses and virulence identified through insertional mutagenesis. *Genetics, 162*, 1573–1581.

De Bernardis, F., Muhlschlegel, F. A., Cassone, A., & Fonzi, W. A. (1998). The pH of the host niche controls gene expression in and virulence of *Candida albicans*. *Infection and Immunity, 66*, 3317–3325.

De Sordi, L., & Muhlschlegel, F. A. (2009). Quorum sensing and fungal-bacterial interactions in *Candida albicans*: a communicative network regulating microbial coexistence and virulence. *FEMS Yeast Research, 9*, 990–999.

Decanis, N., Savignac, K., & Rouabhia, M. (2009). Farnesol promotes epithelial cell defense against *Candida albicans* through Toll-like receptor 2 expression, interleukin-6 and human beta-defensin 2 production. *Cytokine, 45*, 132–140.

Deveau, A., & Hogan, D. A. (2011). Linking quorum sensing regulation and biofilm formation by *Candida albicans*. *Methods in Molecular Biology, 692*, 219–233.

Deveau, A., Piispanen, A. E., Jackson, A. A., & Hogan, D. A. (2010). Farnesol induces hydrogen peroxide resistance in *Candida albicans* yeast by inhibiting the Ras-cyclic AMP signaling pathway. *Eukaryotic Cell, 9*, 569–577.

Diekema, D., Arbefeville, S., Boyken, L., Kroeger, J., & Pfaller, M. (2012). The changing epidemiology of healthcare-associated candidemia over three decades. *Diagnostic Microbiology and Infectious Disease, 73*, 45–48.

Diez-Orejas, R., Molero, G., Navarro-Garcia, F., Pla, J., Nombela, C., & Sanchez-Perez, M. (1997). Reduced virulence of *Candida albicans MKC1* mutants: a role for mitogen-activated protein kinase in pathogenesis. *Infection and Immunity, 65*, 833–837.

Diezmann, S., Michaut, M., Shapiro, R. S., Bader, G. D., & Cowen, L. E. (2012). Mapping the Hsp90 genetic interaction network in *Candida albicans* reveals environmental contingency and rewired circuitry. *PLoS Genetics, 8*, e1002562.

Doedt, T., Krishnamurthy, S., Bockmuhl, D. P., Tebarth, B., Stempel, C., Russell, C. L., et al. (2004). APSES proteins regulate morphogenesis and metabolism in *Candida albicans*. *Molecular Biology of the Cell, 15*, 3167–3180.

Dongari-Bagtzoglou, A., Kashleva, H., Dwivedi, P., Diaz, P., & Vasilakos, J. (2009). Characterization of mucosal *Candida albicans* biofilms. *PLoS One, 4*, e7967.

Douglas, L. J. (2002). Medical importance of biofilms in *Candida* infections. *Revista Iberoamericana De Micologia, 19*, 139–143.

Douglas, L. J. (2003). *Candida* biofilms and their role in infection. *Trends in Microbiology, 11*, 30–36.

Dubin, A., & Estenssoro, E. (2008). Mechanisms of tissue hypercarbia in sepsis. *Frontiers in Bioscience, 13*, 1340–1351.

Dumitru, R., Hornby, J. M., & Nickerson, K. W. (2004). Defined anaerobic growth medium for studying *Candida albicans* basic biology and resistance to eight antifungal drugs. *Antimicrobial Agents and Chemotherapy, 48*, 2350–2354.

Dumitru, R., Navarathna, D. H., Semighini, C. P., Elowsky, C. G., Dumitru, R. V., Dignard, D., et al. (2007). In vivo and in vitro anaerobic mating in *Candida albicans*. *Eukaryotic Cell, 6*, 465–472.

Dunham, M. J., Badrane, H., Ferea, T., Adams, J., Brown, P. O., Rosenzweig, F., et al. (2002). Characteristic genome rearrangements in experimental evolution of *Saccharomyces cerevisiae*. *Proceedings of the National Academy of Sciences of the United States of America, 99*, 16144–16149.

Dunkel, N., Biswas, K., Hiller, E., Fellenberg, K., Satheesh, S. V., Rupp, S., et al. (2014). Control of morphogenesis, protease secretion and gene expression in *Candida albicans* by the preferred nitrogen source ammonium. *Microbiology, 160*(Pt 8), 1599–1608. http://dx.doi.org/10.1099/mic.0.078238-0.

Dunkel, N., Hertlein, T., Franz, R., Reuss, O., Sasse, C., Schafer, T., et al. (2013). Roles of different peptide transporters in nutrient acquisition in *Candida albicans*. *Eukaryotic Cell, 12*, 520–528.

Dutton, L. C., Nobbs, A. H., Jepson, K., Jepson, M. A., Vickerman, M. M., Aqeel Alawfi, S., et al. (2014). O-mannosylation in *Candida albicans* enables development of interkingdom biofilm communities. *MBio, 5*, e00911.

Eisman, B., Alonso-Monge, R., Roman, E., Arana, D., Nombela, C., & Pla, J. (2006). The Cek1 and Hog1 mitogen-activated protein kinases play complementary roles in cell wall biogenesis and chlamydospore formation in the fungal pathogen *Candida albicans*. *Eukaryotic Cell, 5*, 347–358.

El-Sabaeny, A., Demuth, D. R., Park, Y., & Lamont, R. J. (2000). Environmental conditions modulate the expression of the *sspA* and *sspB* genes in *Streptococcus gordonii*. *Microbial Pathogenesis, 29*, 101–113.

van Enckevort, F. H., Netea, M. G., Hermus, A. R., Sweep, C. G., Meis, J. F., Van der Meer, J. W., et al. (1999). Increased susceptibility to systemic candidiasis in interleukin-6 deficient mice. *Medical Mycology, 37*, 419–426.

Ene, I., & Brown, A. P. (2014). Integration of metabolism with virulence in *Candida albicans*. In M. Nowrousian (Ed.), *Fungal genomics* (Vol. 13, pp. 349–370). Springer Berlin Heidelberg.

Ene, I. V., Adya, A. K., Wehmeier, S., Brand, A. C., MacCallum, D. M., Gow, N. A., et al. (2012). Host carbon sources modulate cell wall architecture, drug resistance and virulence in a fungal pathogen. *Cellular Microbiology, 14*, 1319–1335.

Ene, I. V., Cheng, S. C., Netea, M. G., & Brown, A. J. (2013). Growth of *Candida albicans* cells on the physiologically relevant carbon source lactate affects their recognition and phagocytosis by immune cells. *Infection and Immunity, 81*, 238–248.

Ene, I. V., Heilmann, C. J., Sorgo, A. G., Walker, L. A., de Koster, C. G., Munro, C. A., et al. (2012). Carbon source-induced reprogramming of the cell wall proteome and

secretome modulates the adherence and drug resistance of the fungal pathogen *Candida albicans*. *Proteomics, 12*, 3164–3179.
d'Enfert, C. (2009). Hidden killers: persistence of opportunistic fungal pathogens in the human host. *Current Opinion in Microbiology, 12*, 358–364.
Enjalbert, B., MacCallum, D. M., Odds, F. C., & Brown, A. J. (2007). Niche-specific activation of the oxidative stress response by the pathogenic fungus *Candida albicans*. *Infection and Immunity, 75*, 2143–2151.
Enjalbert, B., Smith, D. A., Cornell, M. J., Alam, I., Nicholls, S., Brown, A. J., et al. (2006). Role of the Hog1 stress-activated protein kinase in the global transcriptional response to stress in the fungal pathogen *Candida albicans*. *Molecular Biology of the Cell, 17*, 1018–1032.
Enjalbert, B., & Whiteway, M. (2005). Release from quorum-sensing molecules triggers hyphal formation during *Candida albicans* resumption of growth. *Eukaryotic Cell, 4*, 1203–1210.
Erb-Downward, J. R., & Noverr, M. C. (2007). Characterization of prostaglandin E_2 production by *Candida albicans*. *Infection and Immunity, 75*, 3498–3505.
Ernst, J. F., & Tielker, D. (2009). Responses to hypoxia in fungal pathogens. *Cellular Microbiology, 11*, 183–190.
Falsetta, M. L., Klein, M. I., Colonne, P. M., Scott-Anne, K., Gregoire, S., Pai, C. H., et al. (2014). Symbiotic relationship between *Streptococcus mutans* and *Candida albicans* synergizes virulence of plaque biofilms in vivo. *Infection and Immunity, 82*, 1968–1981.
Feng, Q., Summers, E., Guo, B., & Fink, G. (1999). Ras signaling is required for serum-induced hyphal differentiation in *Candida albicans*. *Journal of Bacteriology, 181*, 6339–6346.
Fanning, S., & Mitchell, A. P. (2012). Fungal biofilms. *PLoS Pathogens, 8*, e1002585.
Felk, A., Kretschmar, M., Albrecht, A., Schaller, M., Beinhauer, S., Nichterlein, T., et al. (2002). *Candida albicans* hyphal formation and the expression of the Efg1-regulated proteinases Sap4 to Sap6 are required for the invasion of parenchymal organs. *Infection and Immunity, 70*, 3689–3700.
Fidel, P. L., Jr. (2011). *Candida*-host interactions in HIV disease: implications for oropharyngeal candidiasis. *Advances in Dental Research, 23*, 45–49.
Finkel, J. S., & Mitchell, A. P. (2011). Genetic control of *Candida albicans* biofilm development. *Nature Reviews Microbiology, 9*, 109–118.
Fischer, D., Hube, B., & Brunke, S. (2014). Fine-Scale chromosomal changes in fungal fitness. *Current Fungal Infection Reports, 8*, 171–178.
Fonzi, W. A. (1999). *PHR1* and *PHR2* of *Candida albicans* encode putative glycosidases required for proper cross-linking of beta-1,3- and beta-1,6-glucans. *Journal of Bacteriology, 181*, 7070–7079.
Forche, A., Abbey, D., Pisithkul, T., Weinzierl, M. A., Ringstrom, T., Bruck, D., et al. (2011). Stress alters rates and types of loss of heterozygosity in *Candida albicans*. *MBio, 2*.
Forche, A., Alby, K., Schaefer, D., Johnson, A. D., Berman, J., & Bennett, R. J. (2008). The parasexual cycle in *Candida albicans* provides an alternative pathway to meiosis for the formation of recombinant strains. *PLoS Biology, 6*, e110.
Fox, E. P., & Nobile, C. J. (2012). A sticky situation: untangling the transcriptional network controlling biofilm development in *Candida albicans*. *Transcription, 3*, 315–322.
Fox, S. J., Shelton, B. T., & Kruppa, M. D. (2013). Characterization of genetic determinants that modulate *Candida albicans* filamentation in the presence of bacteria. *PLoS One, 8*, e71939.
Fradin, C., De Groot, P., MacCallum, D., Schaller, M., Klis, F., Odds, F. C., et al. (2005). Granulocytes govern the transcriptional response, morphology and proliferation of *Candida albicans* in human blood. *Molecular Microbiology, 56*, 397–415.

Frohner, I. E., Bourgeois, C., Yatsyk, K., Majer, O., & Kuchler, K. (2009). *Candida albicans* cell surface superoxide dismutases degrade host-derived reactive oxygen species to escape innate immune surveillance. *Molecular Microbiology, 71*, 240–252.

Fu, M. S., De Sordi, L., & Muhlschlegel, F. A. (2012). Functional characterization of the small heat shock protein Hsp12p from *Candida albicans*. *PLoS One, 7*, e42894.

Fu, Y., Phan, Q. T., Luo, G., Solis, N. V., Liu, Y., Cormack, B. P., et al. (2013). Investigation of the function of *Candida albicans* Als3 by heterologous expression in *Candida glabrata*. *Infection and Immunity, 81*, 2528–2535.

Garcia-Sanchez, S., Aubert, S., Iraqui, I., Janbon, G., Ghigo, J. M., & d'Enfert, C. (2004). *Candida albicans* biofilms: a developmental state associated with specific and stable gene expression patterns. *Eukaryotic Cell, 3*, 536–545.

Geiger, J., Wessels, D., Lockhart, S. R., & Soll, D. R. (2004). Release of a potent polymorphonuclear leukocyte chemoattractant is regulated by white-opaque switching in *Candida albicans*. *Infection and Immunity, 72*, 667–677.

Ghosh, S., Howe, N., Volk, K., Tati, S., Nickerson, K. W., & Petro, T. M. (2010). *Candida albicans* cell wall components and farnesol stimulate the expression of both inflammatory and regulatory cytokines in the murine RAW264.7 macrophage cell line. *FEMS Immunology and Medical Microbiology, 60*, 63–73.

Ghosh, S., Kebaara, B. W., Atkin, A. L., & Nickerson, K. W. (2008). Regulation of aromatic alcohol production in *Candida albicans*. *Applied and Environmental Microbiology, 74*, 7211–7218.

Ghosh, S., Navarathna, D. H., Roberts, D. D., Cooper, J. T., Atkin, A. L., Petro, T. M., et al. (2009). Arginine-induced germ tube formation in *Candida albicans* is essential for escape from murine macrophage line RAW 264.7. *Infection and Immunity, 77*, 1596–1605.

Giacometti, R., Kronberg, F., Biondi, R. M., & Passeron, S. (2011). *Candida albicans* Tpk1p and Tpk2p isoforms differentially regulate pseudohyphal development, biofilm structure, cell aggregation and adhesins expression. *Yeast, 28*, 293–308.

Giusani, A. D., Vinces, M., & Kumamoto, C. A. (2002). Invasive filamentous growth of *Candida albicans* is promoted by Czf1p-dependent relief of Efg1p-mediated repression. *Genetics, 160*, 1749–1753.

Gleason, J. E., Galaleldeen, A., Peterson, R. L., Taylor, A. B., Holloway, S. P., Waninger-Saroni, J., et al. (2014). *Candida albicans SOD5* represents the prototype of an unprecedented class of Cu-only superoxide dismutases required for pathogen defense. *Proceedings of the National Academy of Sciences of the United States of America, 111*, 5866–5871.

Gleason, J. E., Li, C. X., Odeh, H. M., & Culotta, V. C. (2014). Species-specific activation of Cu/Zn SOD by its CCS copper chaperone in the pathogenic yeast *Candida albicans*. *Journal of Biological Inorganic Chemistry, 19*, 595–603.

Gomes, A. C., Miranda, I., Silva, R. M., Moura, G. R., Thomas, B., Akoulitchev, A., et al. (2007). A genetic code alteration generates a proteome of high diversity in the human pathogen *Candida albicans*. *Genome Biology, 8*, R206.

Gooday, G. W., & Adams, D. J. (1993). Sex hormones and fungi. *Advances in Microbial Physiology, 34*, 69–145.

Gow, N. A. (2009). Fungal morphogenesis: some like it hot. *Current Biology, 19*, R333–R334.

Gow, N. A. (2013). A developmental program for *Candida* commensalism. *Nature Genetics, 45*, 967–968.

Gow, N. A., Brown, A. J., & Odds, F. C. (2002). Fungal morphogenesis and host invasion. *Current Opinion in Microbiology, 5*, 366–371.

Gow, N. A., & Hube, B. (2012). Importance of the *Candida albicans* cell wall during commensalism and infection. *Current Opinion in Microbiology, 15*, 406–412.

Gow, N. A., van de Veerdonk, F. L., Brown, A. J., & Netea, M. G. (2012). *Candida albicans* morphogenesis and host defence: discriminating invasion from colonization. *Nature Reviews Microbiology, 10*, 112–122.

Grahl, N., Shepardson, K. M., Chung, D., & Cramer, R. A. (2012). Hypoxia and fungal pathogenesis: to air or not to air? *Eukaryotic Cell, 11*, 560–570.

de Groot, P. W., Bader, O., de Boer, A. D., Weig, M., & Chauhan, N. (2013). Adhesins in human fungal pathogens: glue with plenty of stick. *Eukaryotic Cell, 12*, 470–481.

Gropp, K., Schild, L., Schindler, S., Hube, B., Zipfel, P. F., & Skerka, C. (2009). The yeast *Candida albicans* evades human complement attack by secretion of aspartic proteases. *Molecular Immunology, 47*, 465–475.

Guan, G., Xie, J., Tao, L., Nobile, C. J., Sun, Y., Cao, C., et al. (2013). Bcr1 plays a central role in the regulation of opaque cell filamentation in *Candida albicans*. *Molecular Microbiology, 89*, 732–750.

Guinea, J. (2014). Global trends in the distribution of *Candida* species causing candidemia. *Clinical Microbiology and Infection, 20*(Suppl. 6), 5–10.

Hall-Stoodley, L., & Stoodley, P. (2009). Evolving concepts in biofilm infections. *Cellular Microbiology, 11*, 1034–1043.

Hall, R. A., Turner, K. J., Chaloupka, J., Cottier, F., De Sordi, L., Sanglard, D., et al. (2011). The quorum-sensing molecules farnesol/homoserine lactone and dodecanol operate via distinct modes of action in *Candida albicans*. *Eukaryotic Cell, 10*, 1034–1042.

Han, Y., & Cutler, J. E. (1997). Assessment of a mouse model of neutropenia and the effect of an anti-candidiasis monoclonal antibody in these animals. *Journal of Infectious Diseases, 175*, 1169–1175.

Harriott, M. M., & Noverr, M. C. (2011). Importance of *Candida*-bacterial polymicrobial biofilms in disease. *Trends in Microbiology, 19*, 557–563.

Harrison, B. D., Hashemi, J., Bibi, M., Pulver, R., Bavli, D., Nahmias, Y., et al. (2014). A tetraploid intermediate precedes aneuploid formation in yeasts exposed to fluconazole. *PLoS Biology, 12*, e1001815.

He, G., Shankar, R. A., Chzhan, M., Samouilov, A., Kuppusamy, P., & Zweier, J. L. (1999). Noninvasive measurement of anatomic structure and intraluminal oxygenation in the gastrointestinal tract of living mice with spatial and spectral EPR imaging. *Proceedings of the National Academy of Sciences of the United States of America, 96*, 4586–4591.

Heilmann, C. J., Sorgo, A. G., Siliakus, A. R., Dekker, H. L., Brul, S., de Koster, C. G., et al. (2011). Hyphal induction in the human fungal pathogen *Candida albicans* reveals a characteristic wall protein profile. *Microbiology, 157*, 2297–2307.

Hernday, A. D., Lohse, M. B., Fordyce, P. M., Nobile, C. J., DeRisi, J. L., & Johnson, A. D. (2013). Structure of the transcriptional network controlling white-opaque switching in *Candida albicans*. *Molecular Microbiology, 90*, 22–35.

Herrero-de-Dios, C., Alonso-Monge, R., & Pla, J. (2014). The lack of upstream elements of the Cek1 and Hog1 mediated pathways leads to a synthetic lethal phenotype upon osmotic stress in *Candida albicans*. *Fungal Genetics and Biology, 69*, 31–42. http://dx.doi.org/10.1016/j.fgb.2014.05.010.

den Hertog, A. L., van Marle, J., van Veen, H. A., Van't Hof, W., Bolscher, J. G., Veerman, E. C., et al. (2005). Candidacidal effects of two antimicrobial peptides: histatin 5 causes small membrane defects, but LL-37 causes massive disruption of the cell membrane. *Biochemical Journal, 388*, 689–695.

Heymann, P., Gerads, M., Schaller, M., Dromer, F., Winkelmann, G., & Ernst, J. F. (2002). The siderophore iron transporter of *Candida albicans* (Sit1p/Arn1p) mediates uptake of ferrichrome-type siderophores and is required for epithelial invasion. *Infection and Immunity, 70*, 5246–5255.

Hickman, M. A., Zeng, G., Forche, A., Hirakawa, M. P., Abbey, D., Harrison, B. D., et al. (2013). The 'obligate diploid' *Candida albicans* forms mating-competent haploids. *Nature, 494*, 55–59.

Hisajima, T., Maruyama, N., Tanabe, Y., Ishibashi, H., Yamada, T., Makimura, K., et al. (2008). Protective effects of farnesol against oral candidiasis in mice. *Microbiology and Immunology, 52*, 327–333.

Hoberg, K. A., Cihlar, R. L., & Calderone, R. A. (1983). Inhibitory effect of cerulenin and sodium butyrate on germination of *Candida albicans*. *Antimicrobial Agents and Chemotherapy, 24*, 401–408.

Hodgkinson, V., & Petris, M. J. (2012). Copper homeostasis at the host-pathogen interface. *Journal of Biological Chemistry, 287*, 13549–13555.

Hogan, D. A. (2006). Talking to themselves: autoregulation and quorum sensing in fungi. *Eukaryotic Cell, 5*, 613–619.

Hogan, D. A., & Kolter, R. (2002). *Pseudomonas-Candida* interactions: an ecological role for virulence factors. *Science, 296*, 2229–2232.

Hogan, D. A., & Muhlschlegel, F. A. (2011). *Candida albicans* developmental regulation: adenylyl cyclase as a coincidence detector of parallel signals. *Current Opinion in Microbiology, 14*, 682–686.

Hogan, D. A., Vik, A., & Kolter, R. (2004). A *Pseudomonas aeruginosa* quorum-sensing molecule influences *Candida albicans* morphology. *Molecular Microbiology, 54*, 1212–1223.

Holmberg, K., & Hallander, H. O. (1973). Production of bactericidal concentrations of hydrogen peroxide by *Streptococcus sanguis*. *Archives of Oral Biology, 18*, 423–434.

Holmes, A. R., Cannon, R. D., & Jenkinson, H. F. (1995). Interactions of *Candida albicans* with bacteria and salivary molecules in oral biofilms. *Journal of Industrial Microbiology, 15*, 208–213.

Holmes, A. R., McNab, R., & Jenkinson, H. F. (1996). *Candida albicans* binding to the oral bacterium *Streptococcus gordonii* involves multiple adhesin-receptor interactions. *Infection and Immunity, 64*, 4680–4685.

Holmes, A. R., & Shepherd, M. G. (1988). Nutritional factors determine germ tube formation in *Candida albicans*. *Journal of Medical and Veterinary Mycology, 26*, 127–131.

Holmes, A. R., van der Wielen, P., Cannon, R. D., Ruske, D., & Dawes, P. (2006). *Candida albicans* binds to saliva proteins selectively adsorbed to silicone. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology, 102*, 488–494.

Hood, M. I., & Skaar, E. P. (2012). Nutritional immunity: transition metals at the pathogen-host interface. *Nature Reviews Microbiology, 10*, 525–537.

Hope, W. W., Drusano, G. L., Moore, C. B., Sharp, A., Louie, A., Walsh, T. J., et al. (2007). Effect of neutropenia and treatment delay on the response to antifungal agents in experimental disseminated candidiasis. *Antimicrobial Agents and Chemotherapy, 51*, 285–295.

Hornby, J. M., Jensen, E. C., Lisec, A. D., Tasto, J. J., Jahnke, B., Shoemaker, R., et al. (2001). Quorum sensing in the dimorphic fungus *Candida albicans* is mediated by farnesol. *Applied and Environmental Microbiology, 67*, 2982–2992.

Hornby, J. M., & Nickerson, K. W. (2004). Enhanced production of farnesol by *Candida albicans* treated with four azoles. *Antimicrobial Agents and Chemotherapy, 48*, 2305–2307.

Hoyer, L. L. (2001). The ALS gene family of *Candida albicans*. *Trends in Microbiology, 9*, 176–180.

Hoyer, L. L., Green, C. B., Oh, S. H., & Zhao, X. (2008). Discovering the secrets of the *Candida albicans* agglutinin-like sequence (ALS) gene family–a sticky pursuit. *Medical Mycology, 46*, 1–15.

Hromatka, B. S., Noble, S. M., & Johnson, A. D. (2005). Transcriptional response of *Candida albicans* to nitric oxide and the role of the *YHB1* gene in nitrosative stress and virulence. *Molecular Biology of the Cell, 16*, 4814–4826.

Hua-Van, A., Le Rouzic, A., Maisonhaute, C., & Capy, P. (2005). Abundance, distribution and dynamics of retrotransposable elements and transposons: similarities and differences. *Cytogenetic and Genome Research, 110*, 426–440.

Huang, G. (2012). Regulation of phenotypic transitions in the fungal pathogen *Candida albicans*. *Virulence, 3*, 251–261.

Huang, G., Wang, H., Chou, S., Nie, X., Chen, J., & Liu, H. (2006). Bistable expression of *WOR1*, a master regulator of white-opaque switching in *Candida albicans*. *Proceedings of the National Academy of Sciences of the United States of America, 103*, 12813–12818.

Hube, B. (2004). From commensal to pathogen: stage- and tissue-specific gene expression of *Candida albicans*. *Current Opinion in Microbiology, 7*, 336–341.

Hube, B. (2009). Fungal adaptation to the host environment. *Current Opinion in Microbiology, 12*, 347–349.

Hube, B., Monod, M., Schofield, D. A., Brown, A. J., & Gow, N. A. (1994). Expression of seven members of the gene family encoding secretory aspartyl proteinases in *Candida albicans*. *Molecular Microbiology, 14*, 87–99.

Hull, C. M., Raisner, R. M., & Johnson, A. D. (2000). Evidence for mating of the "asexual" yeast *Candida albicans* in a mammalian host. *Science, 289*, 307–310.

Hwang, C. S., Baek, Y. U., Yim, H. S., & Kang, S. O. (2003). Protective roles of mitochondrial manganese-containing superoxide dismutase against various stresses in *Candida albicans*. *Yeast, 20*, 929–941.

Hwang, C. S., Rhie, G., Kim, S. T., Kim, Y. R., Huh, W. K., Baek, Y. U., et al. (1999). Copper- and zinc-containing superoxide dismutase and its gene from *Candida albicans*. *Biochimica et Biophysica Acta, 1427*, 245–255.

Hwang, C. S., Rhie, G. E., Oh, J. H., Huh, W. K., Yim, H. S., & Kang, S. O. (2002). Copper- and zinc-containing superoxide dismutase (Cu/ZnSOD) is required for the protection of *Candida albicans* against oxidative stresses and the expression of its full virulence. *Microbiology, 148*, 3705–3713.

Inglis, D. O., & Sherlock, G. (2013). Ras signaling gets fine-tuned: regulation of multiple pathogenic traits of *Candida albicans*. *Eukaryotic Cell, 12*, 1316–1325.

Jabra-Rizk, M. A., Meiller, T. F., James, C. E., & Shirtliff, M. E. (2006). Effect of farnesol on *Staphylococcus aureus* biofilm formation and antimicrobial susceptibility. *Antimicrobial Agents and Chemotherapy, 50*, 1463–1469.

Jacobsen, I. D., Wilson, D., Wachtler, B., Brunke, S., Naglik, J. R., & Hube, B. (2012). *Candida albicans* dimorphism as a therapeutic target. *Expert Review of Anti-infective Therapy, 10*, 85–93.

Jamieson, D. J., Stephen, D. W., & Terriere, E. C. (1996). Analysis of the adaptive oxidative stress response of *Candida albicans*. *FEMS Microbiology Letters, 138*, 83–88.

Janbon, G., Sherman, F., & Rustchenko, E. (1998). Monosomy of a specific chromosome determines L-sorbose utilization: a novel regulatory mechanism in *Candida albicans*. *Proceedings of the National Academy of Sciences of the United States of America, 95*, 5150–5155.

Jenkinson, H. F., Lala, H. C., & Shepherd, M. G. (1990). Coaggregation of *Streptococcus sanguis* and other streptococci with *Candida albicans*. *Infection and Immunity, 58*, 1429–1436.

Jiang, J., Zhao, L., Yan, L., Zhang, L., Cao, Y., Wang, Y., et al. (2014). Structural features and mechanism of translocation of non-LTR retrotransposons in *Candida albicans*. *Virulence, 5*, 245–252.

Jin, Y., Samaranayake, L. P., Samaranayake, Y., & Yip, H. K. (2004). Biofilm formation of *Candida albicans* is variably affected by saliva and dietary sugars. *Archives of Oral Biology, 49*, 789–798.

Johnson, A. (2003). The biology of mating in *Candida albicans*. *Nature Reviews Microbiology, 1*, 106–116.

Johnson, E. E., & Wessling-Resnick, M. (2012). Iron metabolism and the innate immune response to infection. *Microbes and Infection, 14*, 207–216.

Johnson, L. (2007). The genome strikes back: the evolutionary importance of defence against Mobile elements. *Evolutionary Biology, 34*, 121–129.

Jones, T., Federspiel, N. A., Chibana, H., Dungan, J., Kalman, S., Magee, B. B., et al. (2004). The diploid genome sequence of *Candida albicans*. *Proceedings of the National Academy of Sciences of the United States of America, 101*, 7329–7334.

Jung, S. I., Finkel, J. S., Solis, N. V., Chaili, S., Mitchell, A. P., Yeaman, M. R., et al. (2013). Bcr1 functions downstream of Ssd1 to mediate antimicrobial peptide resistance in *Candida albicans*. *Eukaryotic Cell, 12*, 411–419.

Kabir, M. A., Ahmad, A., Greenberg, J. R., Wang, Y. K., & Rustchenko, E. (2005). Loss and gain of chromosome 5 controls growth of *Candida albicans* on sorbose due to dispersed redundant negative regulators. *Proceedings of the National Academy of Sciences of the United States of America, 102*, 12147–12152.

Kadosh, D., & Lopez-Ribot, J. L. (2013). Candida albicans: adapting to succeed. *Cell Host & Microbe, 14*, 483–485.

Kaloriti, D., Jacobsen, M., Yin, Z., Patterson, M., Tillmann, A., Smith, D. A., et al. (2014). Mechanisms underlying the exquisite sensitivity of *Candida albicans* to combinatorial cationic and oxidative stress that enhances the potent fungicidal activity of phagocytes. *MBio, 5*.

Karhausen, J., Furuta, G. T., Tomaszewski, J. E., Johnson, R. S., Colgan, S. P., & Haase, V. H. (2004). Epithelial hypoxia-inducible factor-1 is protective in murine experimental colitis. *Journal of Clinical Investigation, 114*, 1098–1106.

Katragkou, A., Kruhlak, M. J., Simitsopoulou, M., Chatzimoschou, A., Taparkou, A., Cotten, C. J., et al. (2010). Interactions between human phagocytes and *Candida albicans* biofilms alone and in combination with antifungal agents. *Journal of Infectious Diseases, 201*, 1941–1949.

Kauffman, C. A. (2006). Fungal infections. *Proceedings of the American Thoracic Society, 3*, 35–40.

Kebaara, B. W., Langford, M. L., Navarathna, D. H., Dumitru, R., Nickerson, K. W., & Atkin, A. L. (2008). *Candida albicans* Tup1 is involved in farnesol-mediated inhibition of filamentous-growth induction. *Eukaryotic Cell, 7*, 980–987.

Kehl-Fie, T. E., & Skaar, E. P. (2010). Nutritional immunity beyond iron: a role for manganese and zinc. *Current Opinion in Chemical Biology, 14*, 218–224.

Kerr, J. (1994). Inhibition of fungal growth by *Pseudomonas aeruginosa* and *Pseudomonas cepacia* isolated from patients with cystic fibrosis. *Journal of Infection, 28*, 305–310.

Kerr, J. R., Taylor, G. W., Rutman, A., Hoiby, N., Cole, P. J., & Wilson, R. (1999). *Pseudomonas aeruginosa* pyocyanin and 1-hydroxyphenazine inhibit fungal growth. *Journal of Clinical Pathology, 52*, 385–387.

Khan, S. A., Fidel, P. L., Jr., Thunayyan, A. A., Varlotta, S., Meiller, T. F., & Jabra-Rizk, M. A. (2013). Impaired Histatin-5 levels and salivary antimicrobial activity against in HIV infected individuals. *Journal of AIDS Clinical Research, 4*.

Khot, P. D., Suci, P. A., Miller, R. L., Nelson, R. D., & Tyler, B. J. (2006). A small subpopulation of blastospores in *Candida albicans* biofilms exhibit resistance to amphotericin B associated with differential regulation of ergosterol and beta-1,6-glucan pathway genes. *Antimicrobial Agents and Chemotherapy, 50*, 3708–3716.

Kikuchi, T., Ohno, N., & Ohno, T. (2002). Maturation of dendritic cells induced by *Candida* β-D-glucan. *International Immunopharmacology, 2*, 1503–1508.

Klengel, T., Liang, W. J., Chaloupka, J., Ruoff, C., Schroppel, K., Naglik, J. R., et al. (2005). Fungal adenylyl cyclase integrates CO_2 sensing with cAMP signaling and virulence. *Current Biology, 15*, 2021–2026.

Klis, F. M., de Koster, C. G., & Brul, S. (2014). Cell wall-related bionumbers and bioestimates of *Saccharomyces cerevisiae* and *Candida albicans*. *Eukaryotic Cell, 13*, 2–9.

Klotz, S. A., Chasin, B. S., Powell, B., Gaur, N. K., & Lipke, P. N. (2007). Polymicrobial bloodstream infections involving *Candida* species: analysis of patients and review of the literature. *Diagnostic Microbiology and Infectious Disease, 59*, 401–406.

Klotz, S. A., Gaur, N. K., De Armond, R., Sheppard, D., Khardori, N., Edwards, J. E., Jr., et al. (2007). *Candida albicans* Als proteins mediate aggregation with bacteria and yeasts. *Medical Mycology, 45*, 363–370.

Klotz, S. A., Gaur, N. K., Lake, D. F., Chan, V., Rauceo, J., & Lipke, P. N. (2004). Degenerate peptide recognition by *Candida albicans* adhesins Als5p and Als1p. *Infection and Immunity, 72*, 2029–2034.

Koh, A. Y., Kohler, J. R., Coggshall, K. T., Van Rooijen, N., & Pier, G. B. (2008). Mucosal damage and neutropenia are required for *Candida albicans* dissemination. *PLoS Pathogens, 4*, e35.

Kojic, E. M., & Darouiche, R. O. (2004). *Candida* infections of medical devices. *Clinical Microbiology Reviews, 17*, 255–267.

Kraidlova, L., Van Zeebroeck, G., Van Dijck, P., & Sychrova, H. (2011). The *Candida albicans GAP* gene family encodes permeases involved in general and specific amino acid uptake and sensing. *Eukaryotic Cell, 10*, 1219–1229.

Krasowska, A., Murzyn, A., Dyjankiewicz, A., Lukaszewicz, M., & Dziadkowiec, D. (2009). The antagonistic effect of *Saccharomyces boulardii* on *Candida albicans* filamentation, adhesion and biofilm formation. *FEMS Yeast Research, 9*, 1312–1321.

Kreth, J., Merritt, J., Shi, W., & Qi, F. (2005). Competition and coexistence between *Streptococcus mutans* and *Streptococcus sanguinis* in the dental biofilm. *Journal of Bacteriology, 187*, 7193–7203.

Kronstad, J. W., Cadieux, B., & Jung, W. H. (2013). Pathogenic yeasts deploy cell surface receptors to acquire iron in vertebrate hosts. *PLoS Pathogens, 9*, e1003498.

Kruppa, M. (2009). Quorum sensing and *Candida albicans*. *Mycoses, 52*, 1–10.

Kruppa, M., Krom, B. P., Chauhan, N., Bambach, A. V., Cihlar, R. L., & Calderone, R. A. (2004). The two-component signal transduction protein Chk1p regulates quorum sensing in *Candida albicans*. *Eukaryotic Cell, 3*, 1062–1065.

Kullas, A. L., Martin, S. J., & Davis, D. (2007). Adaptation to environmental pH: integrating the Rim101 and calcineurin signal transduction pathways. *Molecular Microbiology, 66*, 858–871.

Kullberg, B. J., Netea, M. G., Vonk, A. G., & van der Meer, J. W. (1999). Modulation of neutrophil function in host defense against disseminated *Candida albicans* infection in mice. *FEMS Immunology and Medical Microbiology, 26*, 299–307.

Kumamoto, C. A. (2005). A contact-activated kinase signals *Candida albicans* invasive growth and biofilm development. *Proceedings of the National Academy of Sciences of the United States of America, 102*, 5576–5581.

Kumamoto, C. A. (2008a). Molecular mechanisms of mechanosensing and their roles in fungal contact sensing. *Nature Reviews Microbiology, 6*, 667–673.

Kumamoto, C. A. (2008b). Niche-specific gene expression during *C. albicans* infection. *Current Opinion in Microbiology, 11*, 325–330.

Kumamoto, C. A. (2011). Inflammation and gastrointestinal *Candida* colonization. *Current Opinion in Microbiology, 14*, 386–391.

Kumamoto, C. A., & Pierce, J. V. (2011). Immunosensing during colonization by *Candida albicans*: does it take a village to colonize the intestine? *Trends in Microbiology, 19*, 263–267.

Kumamoto, C. A., & Vinces, M. D. (2005a). Alternative *Candida albicans* lifestyles: growth on surfaces. *Annual Review of Microbiology, 59*, 113–133.

Kumamoto, C. A., & Vinces, M. D. (2005b). Contributions of hyphae and hypha-co-regulated genes to *Candida albicans* virulence. *Cellular Microbiology, 7*, 1546–1554.

Kuroda, M., Nagasaki, S., Ito, R., & Ohta, T. (2007). Sesquiterpene farnesol as a competitive inhibitor of lipase activity of *Staphylococcus aureus*. *FEMS Microbiology Letters, 273*, 28–34.

Kurzai, O., Schmitt, C., Brocker, E., Frosch, M., & Kolb-Maurer, A. (2005). Polymorphism of *Candida albicans* is a major factor in the interaction with human dendritic cells. *International Journal of Medical Microbiology, 295*, 121–127.

Kvaal, C., Lachke, S. A., Srikantha, T., Daniels, K., McCoy, J., & Soll, D. R. (1999). Misexpression of the opaque-phase-specific gene *PEP1 (SAP1)* in the white phase of *Candida albicans* confers increased virulence in a mouse model of cutaneous infection. *Infection and Immunity, 67*, 6652–6662.

Kvaal, C. A., Srikantha, T., & Soll, D. R. (1997). Misexpression of the white-phase-specific gene *WH11* in the opaque phase of *Candida albicans* affects switching and virulence. *Infection and Immunity, 65*, 4468–4475.

LaFleur, M. D., Kumamoto, C. A., & Lewis, K. (2006). *Candida albicans* biofilms produce antifungal-tolerant persister cells. *Antimicrobial Agents and Chemotherapy, 50*, 3839–3846.

Lafleur, M. D., Qi, Q., & Lewis, K. (2010). Patients with long-term oral carriage harbor high-persister mutants of *Candida albicans*. *Antimicrobial Agents and Chemotherapy, 54*, 39–44.

Lal, P., Sharma, D., Pruthi, P., & Pruthi, V. (2010). Exopolysaccharide analysis of biofilm-forming *Candida albicans*. *Journal of Applied Microbiology, 109*, 128–136.

Lamarre, C., LeMay, J. D., Deslauriers, N., & Bourbonnais, Y. (2001). *Candida albicans* expresses an unusual cytoplasmic manganese-containing superoxide dismutase (*SOD3* gene product) upon the entry and during the stationary phase. *Journal of Biological Chemistry, 276*, 43784–43791.

Lan, C. Y., Newport, G., Murillo, L. A., Jones, T., Scherer, S., Davis, R. W., et al. (2002). Metabolic specialization associated with phenotypic switching in *Candida albicans*. *Proceedings of the National Academy of Sciences of the United States of America, 99*, 14907–14912.

Langford, M. L., Atkin, A. L., & Nickerson, K. W. (2009). Cellular interactions of farnesol, a quorum-sensing molecule produced by *Candida albicans*. *Future Microbiology, 4*, 1353–1362.

Langford, M. L., Hargarten, J. C., Patefield, K. D., Marta, E., Blankenship, J. R., Fanning, S., et al. (2013). *Candida albicans* Czf1 and Efg1 coordinate the response to farnesol during quorum sensing, white-opaque thermal dimorphism, and cell death. *Eukaryotic Cell, 12*, 1281–1292.

Lassak, T., Schneider, E., Bussmann, M., Kurtz, D., Manak, J. R., Srikantha, T., et al. (2011). Target specificity of the *Candida albicans* Efg1 regulator. *Molecular Microbiology, 82*, 602–618.

Leach, M. D., Budge, S., Walker, L., Munro, C., Cowen, L. E., & Brown, A. J. (2012). Hsp90 orchestrates transcriptional regulation by Hsf1 and cell wall remodelling by MAPK signalling during thermal adaptation in a pathogenic yeast. *PLoS Pathogens, 8*, e1003069.

Leach, M. D., Klipp, E., Cowen, L. E., & Brown, A. J. (2012). Fungal Hsp90: a biological transistor that tunes cellular outputs to thermal inputs. *Nature Reviews Microbiology, 10*, 693–704.

Leach, M. D., Tyc, K. M., Brown, A. J., & Klipp, E. (2012). Modelling the regulation of thermal adaptation in *Candida albicans*, a major fungal pathogen of humans. *PLoS One, 7*, e32467.

Leberer, E., Harcus, D., Broadbent, I. D., Clark, K. L., Dignard, D., Ziegelbauer, K., et al. (1996). Signal transduction through homologs of the Ste20p and Ste7p protein kinases can trigger hyphal formation in the pathogenic fungus *Candida albicans*. *Proceedings of the National Academy of Sciences of the United States of America, 93*, 13217–13222.

Lee, I. R., Morrow, C. A., & Fraser, J. A. (2013). Nitrogen regulation of virulence in clinically prevalent fungal pathogens. *FEMS Microbiology Letters, 345*, 77–84.
Levison, M. E., & Pitsakis, P. G. (1987). Susceptibility to experimental *Candida albicans* urinary tract infection in the rat. *Journal of Infectious Diseases, 155*, 841–846.
Lewis, K. (2010). Persister cells. *Annual Review of Microbiology, 64*, 357–372.
Lewis, K. (2012). Persister cells: molecular mechanisms related to antibiotic tolerance. *Handbook of Experimental Pharmacology*, 121–133.
Lewis, L. E., Bain, J. M., Lowes, C., Gillespie, C., Rudkin, F. M., Gow, N. A., et al. (2012). Stage specific assessment of *Candida albicans* phagocytosis by macrophages identifies cell wall composition and morphogenesis as key determinants. *PLoS Pathogens, 8*, e1002578.
Lewis, R. E. (2009). Overview of the changing epidemiology of candidemia. *Current Medical Research and Opinion, 25*, 1732–1740.
Li, F., & Palecek, S. P. (2003). *EAP1*, a *Candida albicans* gene involved in binding human epithelial cells. *Eukaryotic Cell, 2*, 1266–1273.
Li, M., Chen, Q., Tang, R., Shen, Y., & Liu, W. D. (2011). The expression of beta-defensin-2, 3 and LL-37 induced by *Candida albicans* phospholipomannan in human keratinocytes. *Journal of Dermatological Science, 61*, 72–75.
Li, R., Kumar, R., Tati, S., Puri, S., & Edgerton, M. (2013). *Candida albicans* flu1-mediated efflux of salivary histatin 5 reduces its cytosolic concentration and fungicidal activity. *Antimicrobial Agents and Chemotherapy, 57*, 1832–1839.
Lindsay, A. K., Deveau, A., Piispanen, A. E., & Hogan, D. A. (2012). Farnesol and cyclic AMP signaling effects on the hypha-to-yeast transition in *Candida albicans*. *Eukaryotic Cell, 11*, 1219–1225.
Lionakis, M. S., Swamydas, M., Fischer, B. G., Plantinga, T. S., Johnson, M. D., Jaeger, M., et al. (2013). CX3CR1-dependent renal macrophage survival promotes *Candida* control and host survival. *Journal of Clinical Investigation, 123*, 5035–5051.
Lipke, P. N., Garcia, M. C., Alsteens, D., Ramsook, C. B., Klotz, S. A., & Dufrene, Y. F. (2012). Strengthening relationships: amyloids create adhesion nanodomains in yeasts. *Trends in Microbiology, 20*, 59–65.
Liu, H., Kohler, J., & Fink, G. R. (1994). Suppression of hyphal formation in *Candida albicans* by mutation of a *STE12* homolog. *Science, 266*, 1723–1726.
Liu, Y., & Filler, S. G. (2011). *Candida albicans* Als3, a multifunctional adhesin and invasin. *Eukaryotic Cell, 10*, 168–173.
Lo, H. J., Kohler, J. R., DiDomenico, B., Loebenberg, D., Cacciapuoti, A., & Fink, G. R. (1997). Nonfilamentous *C. albicans* mutants are avirulent. *Cell, 90*, 939–949.
Lockhart, S. R., Daniels, K. J., Zhao, R., Wessels, D., & Soll, D. R. (2003). Cell biology of mating in *Candida albicans*. *Eukaryotic Cell, 2*, 49–61.
Lockhart, S. R., Pujol, C., Daniels, K. J., Miller, M. G., Johnson, A. D., Pfaller, M. A., et al. (2002). In *Candida albicans*, white-opaque switchers are homozygous for mating type. *Genetics, 162*, 737–745.
Lockhart, S. R., Zhao, R., Daniels, K. J., & Soll, D. R. (2003). Alpha-pheromone-induced "shmooing" and gene regulation require white-opaque switching during *Candida albicans* mating. *Eukaryotic Cell, 2*, 847–855.
Lohse, M. B., Hernday, A. D., Fordyce, P. M., Noiman, L., Sorrells, T. R., Hanson-Smith, V., et al. (2013). Identification and characterization of a previously undescribed family of sequence-specific DNA-binding domains. *Proceedings of the National Academy of Sciences of the United States of America, 110*, 7660–7665.
Lohse, M. B., & Johnson, A. D. (2009). White-opaque switching in *Candida albicans*. *Current Opinion in Microbiology, 12*, 650–654.
Lopez-Garcia, B., Lee, P. H., Yamasaki, K., & Gallo, R. L. (2005). Anti-fungal activity of cathelicidins and their potential role in *Candida albicans* skin infection. *Journal of Investigative Dermatology, 125*, 108–115.

Lorenz, M. C., Bender, J. A., & Fink, G. R. (2004). Transcriptional response of *Candida albicans* upon internalization by macrophages. *Eukaryotic Cell, 3*, 1076–1087.

Lowman, D. W., Greene, R. R., Bearden, D. W., Kruppa, M. D., Pottier, M., Monteiro, M. A., et al. (2014). Novel structural features in *Candida albicans* hyphal glucan provide a basis for differential innate immune recognition of hyphae versus yeast. *Journal of Biological Chemistry, 289*, 3432–3443.

Lu, Y., Su, C., & Liu, H. (2012). A GATA transcription factor recruits Hda1 in response to reduced Tor1 signaling to establish a hyphal chromatin state in *Candida albicans*. *PLoS Pathogens, 8*, e1002663.

Lu, Y., Su, C., Solis, N. V., Filler, S. G., & Liu, H. (2013). Synergistic regulation of hyphal elongation by hypoxia, CO(2), and nutrient conditions controls the virulence of *Candida albicans*. *Cell Host & Microbe, 14*, 499–509.

Lu, Y., Su, C., Unoje, O., & Liu, H. (2014). Quorum sensing controls hyphal initiation in *Candida albicans* through Ubr1-mediated protein degradation. *Proceedings of the National Academy of Sciences of the United States of America, 111*, 1975–1980.

Lu, Y., Su, C., Wang, A., & Liu, H. (2011). Hyphal development in *Candida albicans* requires two temporally linked changes in promoter chromatin for initiation and maintenance. *PLoS Biology, 9*, e1001105.

Luo, S., Blom, A. M., Rupp, S., Hipler, U. C., Hube, B., Skerka, C., et al. (2011). The pH-regulated antigen 1 of *Candida albicans* binds the human complement inhibitor C4b-binding protein and mediates fungal complement evasion. *Journal of Biological Chemistry, 286*, 8021–8029.

Luo, S., Hoffmann, R., Skerka, C., & Zipfel, P. F. (2013). Glycerol-3-phosphate dehydrogenase 2 is a novel factor H-, factor H-like protein 1-, and plasminogen-binding surface protein of *Candida albicans*. *Journal of Infectious Diseases, 207*, 594–603.

Luo, S., Poltermann, S., Kunert, A., Rupp, S., & Zipfel, P. F. (2009). Immune evasion of the human pathogenic yeast *Candida albicans*: Pra1 is a Factor H, FHL-1 and plasminogen binding surface protein. *Molecular Immunology, 47*, 541–550.

Luo, S., Skerka, C., Kurzai, O., & Zipfel, P. F. (2013). Complement and innate immune evasion strategies of the human pathogenic fungus *Candida albicans*. *Molecular Immunology, 56*, 161–169.

Lynch, A. S., & Robertson, G. T. (2008). Bacterial and fungal biofilm infections. *Annual Review of Medicine, 59*, 415–428.

Machida, K., & Tanaka, T. (1999). Farnesol-induced generation of reactive oxygen species dependent on mitochondrial transmembrane potential hyperpolarization mediated by F(0)F(1)-ATPase in yeast. *FEBS Letters, 462*, 108–112.

Magee, B. B., Legrand, M., Alarco, A. M., Raymond, M., & Magee, P. T. (2002). Many of the genes required for mating in *Saccharomyces cerevisiae* are also required for mating in *Candida albicans*. *Molecular Microbiology, 46*, 1345–1351.

Magee, B. B., & Magee, P. T. (1997). WO-2, a stable aneuploid derivative of *Candida albicans* strain WO-1, can switch from white to opaque and form hyphae. *Microbiology, 143*(Pt 2), 289–295.

Magee, B. B., & Magee, P. T. (2000). Induction of mating in *Candida albicans* by construction of MTLa and MTLα strains. *Science, 289*, 310–313.

Magee, P. T., & Magee, B. B. (2004). Through a glass opaquely: the biological significance of mating in *Candida albicans*. *Current Opinion in Microbiology, 7*, 661–665.

Mallick, E. M., & Bennett, R. J. (2013). Sensing of the microbial neighborhood by *Candida albicans*. *PLoS Pathogens, 9*, e1003661.

Mandal, S. M., Mahata, D., Migliolo, L., Parekh, A., Addy, P. S., Mandal, M., et al. (2014). Glucose directly promotes antifungal resistance in the fungal pathogen, *Candida* spp. *Journal of Biological Chemistry, 289*(37), 25468–25473. http://dx.doi.org/10.1074/jbc.C114.571778.

Marakalala, M. J., Vautier, S., Potrykus, J., Walker, L. A., Shepardson, K. M., Hopke, A., et al. (2013). Differential adaptation of *Candida albicans* in vivo modulates immune recognition by dectin-1. *PLoS Pathogens, 9*, e1003315.

Martchenko, M., Alarco, A. M., Harcus, D., & Whiteway, M. (2004). Superoxide dismutases in *Candida albicans*: transcriptional regulation and functional characterization of the hyphal-induced *SOD5* gene. *Molecular Biology of the Cell, 15*, 456–467.

Martin, R., Moran, G. P., Jacobsen, I. D., Heyken, A., Domey, J., Sullivan, D. J., et al. (2011). The *Candida albicans*-specific gene *EED1* encodes a key regulator of hyphal extension. *PLoS One, 6*, e18394.

Martin, R., Albrecht-Eckardt, D., Brunke, S., Hube, B., Hunniger, K., & Kurzai, O. (2013). A core filamentation response network in *Candida albicans* is restricted to eight genes. *PLoS One, 8*, e58613.

Martin, R., Wachtler, B., Schaller, M., Wilson, D., & Hube, B. (2011). Host-pathogen interactions and virulence-associated genes during *Candida albicans* oral infections. *International Journal of Medical Microbiology, 301*, 417–422.

Martinez-Esparza, M., Aguinaga, A., Gonzalez-Parraga, P., Garcia-Penarrubia, P., Jouault, T., & Arguelles, J. C. (2007). Role of trehalose in resistance to macrophage killing: study with a tps1/tps1 trehalose-deficient mutant of *Candida albicans*. *Clinical Microbiology and Infection, 13*, 384–394.

Martinez, P., & Ljungdahl, P. O. (2005). Divergence of Stp1 and Stp2 transcription factors in *Candida albicans* places virulence factors required for proper nutrient acquisition under amino acid control. *Molecular and Cellular Biology, 25*, 9435–9446.

Martins, M., Henriques, M., Azeredo, J., Rocha, S. M., Coimbra, M. A., & Oliveira, R. (2007). Morphogenesis control in *Candida albicans* and *Candida dubliniensis* through signaling molecules produced by planktonic and biofilm cells. *Eukaryotic Cell, 6*, 2429–2436.

Martins, M., Henriques, M., Lopez-Ribot, J. L., & Oliveira, R. (2012). Addition of DNase improves the in vitro activity of antifungal drugs against *Candida albicans* biofilms. *Mycoses, 55*, 80–85.

Martins, M., Uppuluri, P., Thomas, D. P., Cleary, I. A., Henriques, M., Lopez-Ribot, J. L., et al. (2010). Presence of extracellular DNA in the *Candida albicans* biofilm matrix and its contribution to biofilms. *Mycopathologia, 169*, 323–331.

Mathe, L., & Van Dijck, P. (2013). Recent insights into *Candida albicans* biofilm resistance mechanisms. *Current Genetics, 59*, 251–264.

Maubon, D., Garnaud, C., Calandra, T., Sanglard, D., & Cornet, M. (2014). Resistance of *Candida* spp. to antifungal drugs in the ICU: where are we now? *Intensive Care Medicine, 40*(9), 1241–1255. http://dx.doi.org/10.1007/s00134-014-3404-7.

Mayer, F. L., Wilson, D., & Hube, B. (2013a). *Candida albicans* pathogenicity mechanisms. *Virulence, 4*, 119–128.

Mayer, F. L., Wilson, D., & Hube, B. (2013b). Hsp21 potentiates antifungal drug tolerance in *Candida albicans*. *PLoS One, 8*, e60417.

Mayer, F. L., Wilson, D., Jacobsen, I. D., Miramon, P., Grosse, K., & Hube, B. (2012). The novel *Candida albicans* transporter Dur31 Is a multi-stage pathogenicity factor. *PLoS Pathogens, 8*, e1002592.

Mayer, F. L., Wilson, D., Jacobsen, I. D., Miramon, P., Slesiona, S., Bohovych, I. M., et al. (2012). Small but crucial: the novel small heat shock protein Hsp21 mediates stress adaptation and virulence in *Candida albicans*. *PLoS One, 7*, e38584.

McAlester, G., O'Gara, F., & Morrissey, J. P. (2008). Signal-mediated interactions between *Pseudomonas aeruginosa* and *Candida albicans*. *Journal of Medical Microbiology, 57*, 563–569.

McKenzie, C. G., Koser, U., Lewis, L. E., Bain, J. M., Mora-Montes, H. M., Barker, R. N., et al. (2010). Contribution of *Candida albicans* cell wall components to recognition by and escape from murine macrophages. *Infection and Immunity, 78*, 1650–1658.

Meiller, T. F., Hube, B., Schild, L., Shirtliff, M. E., Scheper, M. A., Winkler, R., et al. (2009). A novel immune evasion strategy of *Candida albicans*: proteolytic cleavage of a salivary antimicrobial peptide. *PLoS One, 4*, e5039.

Meri, T., Hartmann, A., Lenk, D., Eck, R., Wurzner, R., Hellwage, J., et al. (2002). The yeast *Candida albicans* binds complement regulators factor H and FHL-1. *Infection and Immunity, 70*, 5185–5192.

Metwalli, K. H., Khan, S. A., Krom, B. P., & Jabra-Rizk, M. A. (2013). *Streptococcus mutans, Candida albicans*, and the human mouth: a sticky situation. *PLoS Pathogens, 9*, e1003616.

Miller, M. B., & Bassler, B. L. (2001). Quorum sensing in bacteria. *Annual Review of Microbiology, 55*, 165–199.

Miller, M. G., & Johnson, A. D. (2002). White-opaque switching in *Candida albicans* is controlled by mating-type locus homeodomain proteins and allows efficient mating. *Cell, 110*, 293–302.

Miramon, P., Dunker, C., Windecker, H., Bohovych, I. M., Brown, A. J., Kurzai, O., et al. (2012). Cellular responses of *Candida albicans* to phagocytosis and the extracellular activities of neutrophils are critical to counteract carbohydrate starvation, oxidative and nitrosative stress. *PLoS One, 7*, e52850.

Miramon, P., Kasper, L., & Hube, B. (2013). Thriving within the host: *Candida* spp. interactions with phagocytic cells. *Medical Microbiology and Immunology, 202*, 183–195.

Miranda, I., Silva-Dias, A., Rocha, R., Teixeira-Santos, R., Coelho, C., Goncalves, T., et al. (2013). *Candida albicans* CUG mistranslation is a mechanism to create cell surface variation. *MBio, 4*.

Mitchell, A., Romano, G. H., Groisman, B., Yona, A., Dekel, E., Kupiec, M., et al. (2009). Adaptive prediction of environmental changes by microorganisms. *Nature, 460*, 220–224.

Mitchell, K. F., Taff, H. T., Cuevas, M. A., Reinicke, E. L., Sanchez, H., & Andes, D. R. (2013). Role of matrix beta-1,3 glucan in antifungal resistance of non-albicans *Candida* biofilms. *Antimicrobial Agents and Chemotherapy, 57*, 1918–1920.

Molloy, S. (2010). Quorum sensing: setting the threshold. *Nature Reviews Microbiology, 8*, 388–389.

Monge, R. A., Roman, E., Nombela, C., & Pla, J. (2006). The MAP kinase signal transduction network in *Candida albicans*. *Microbiology, 152*, 905–912.

Morales, D. K., & Hogan, D. A. (2010). *Candida albicans* interactions with bacteria in the context of human health and disease. *PLoS Pathogens, 6*, e1000886.

Moran, G., Coleman, D., & Sullivan, D. (2012). *An introduction to the medically important* Candida *species*. ASM Press.

Morio, F., Loge, C., Besse, B., Hennequin, C., & Le Pape, P. (2010). Screening for amino acid substitutions in the *Candida albicans* Erg11 protein of azole-susceptible and azole-resistant clinical isolates: new substitutions and a review of the literature. *Diagnostic Microbiology and Infectious Disease, 66*, 373–384.

Morschhauser, J. (2010). Regulation of white-opaque switching in *Candida albicans*. *Medical Microbiology and Immunology, 199*, 165–172.

Morschhauser, J., Barker, K. S., Liu, T. T., Bla, B. W. J., Homayouni, R., & Rogers, P. D. (2007). The transcription factor Mrr1p controls expression of the *MDR1* efflux pump and mediates multidrug resistance in *Candida albicans*. *PLoS Pathogens, 3*, e164.

Mosel, D. D., Dumitru, R., Hornby, J. M., Atkin, A. L., & Nickerson, K. W. (2005). Farnesol concentrations required to block germ tube formation in *Candida albicans* in the presence and absence of serum. *Applied and Environmental Microbiology, 71*, 4938–4940.

Moyes, D. L., Murciano, C., Runglall, M., Islam, A., Thavaraj, S., & Naglik, J. R. (2011). *Candida albicans* yeast and hyphae are discriminated by MAPK signaling in vaginal epithelial cells. *PLoS One, 6*, e26580.

Moyes, D. L., Murciano, C., Runglall, M., Kohli, A., Islam, A., & Naglik, J. R. (2012). Activation of MAPK/c-Fos induced responses in oral epithelial cells is specific to *Candida albicans* and *Candida dubliniensis* hyphae. *Medical Microbiology and Immunology, 201*, 93–101.

Moyes, D. L., & Naglik, J. R. (2011). Mucosal immunity and *Candida albicans* infection. *Clinical and Developmental Immunology, 2011*, 346307.

Moyes, D. L., Runglall, M., Murciano, C., Shen, C., Nayar, D., Thavaraj, S., et al. (2010). A biphasic innate immune MAPK response discriminates between the yeast and hyphal forms of *Candida albicans* in epithelial cells. *Cell Host & Microbe, 8*, 225–235.

Moyes, D. L., Shen, C., Murciano, C., Runglall, M., Richardson, J. P., Arno, M., et al. (2014). Protection against epithelial damage during *Candida albicans* infection is mediated by PI3K/Akt and mammalian target of rapamycin signaling. *Journal of Infectious Diseases, 209*, 1816–1826.

Muhlschlegel, F. A., & Fonzi, W. A. (1997). *PHR2* of *Candida albicans* encodes a functional homolog of the pH-regulated gene *PHR1* with an inverted pattern of pH-dependent expression. *Molecular and Cellular Biology, 17*, 5960–5967.

Mukherjee, P. K., Chandra, J., Kuhn, D. M., & Ghannoum, M. A. (2003). Mechanism of fluconazole resistance in *Candida albicans* biofilms: phase-specific role of efflux pumps and membrane sterols. *Infection and Immunity, 71*, 4333–4340.

Mulhern, S. M., Logue, M. E., & Butler, G. (2006). *Candida albicans* transcription factor Ace2 regulates metabolism and is required for filamentation in hypoxic conditions. *Eukaryotic Cell, 5*, 2001–2013.

Mullick, A., Elias, M., Picard, S., Bourget, L., Jovcevski, O., Gauthier, S., et al. (2004). Dysregulated inflammatory response to *Candida albicans* in a C5-deficient mouse strain. *Infection and Immunity, 72*, 5868–5876.

Murad, A. M., Leng, P., Straffon, M., Wishart, J., Macaskill, S., MacCallum, D., et al. (2001). *NRG1* represses yeast-hypha morphogenesis and hypha-specific gene expression in *Candida albicans*. *The EMBO Journal, 20*, 4742–4752.

Murillo, L. A., Newport, G., Lan, C. Y., Habelitz, S., Dungan, J., & Agabian, N. M. (2005). Genome-wide transcription profiling of the early phase of biofilm formation by *Candida albicans*. *Eukaryotic Cell, 4*, 1562–1573.

Murzyn, A., Krasowska, A., Stefanowicz, P., Dziadkowiec, D., & Lukaszewicz, M. (2010). Capric acid secreted by *S. boulardii* inhibits *C. albicans* filamentous growth, adhesion and biofilm formation. *PLoS One, 5*, e12050.

Nace, H. L., Horn, D., & Neofytos, D. (2009). Epidemiology and outcome of multiple-species candidemia at a tertiary care center between 2004 and 2007. *Diagnostic Microbiology and Infectious Disease, 64*, 289–294.

Nadell, C. D., Xavier, J. B., Levin, S. A., & Foster, K. R. (2008). The evolution of quorum sensing in bacterial biofilms. *PLoS Biology, 6*, e14.

Naglik, J., Albrecht, A., Bader, O., & Hube, B. (2004). *Candida albicans* proteinases and host/pathogen interactions. *Cellular Microbiology, 6*, 915–926.

Naglik, J. R., Challacombe, S. J., & Hube, B. (2003). *Candida albicans* secreted aspartyl proteinases in virulence and pathogenesis. *Microbiology and Molecular Biology Reviews, 67*, 400–428 (table of contents).

Naglik, J. R., Moyes, D., Makwana, J., Kanzaria, P., Tsichlaki, E., Weindl, G., et al. (2008). Quantitative expression of the *Candida albicans* secreted aspartyl proteinase gene family in human oral and vaginal candidiasis. *Microbiology, 154*, 3266–3280.

Naglik, J. R., Moyes, D. L., Wachtler, B., & Hube, B. (2011). *Candida albicans* interactions with epithelial cells and mucosal immunity. *Microbes and Infection, 13*, 963–976.

Nailis, H., Vandenbosch, D., Deforce, D., Nelis, H. J., & Coenye, T. (2010). Transcriptional response to fluconazole and amphotericin B in *Candida albicans* biofilms. *Research in Microbiology, 161*, 284–292.

Nakagawa, Y., Kanbe, T., & Mizuguchi, I. (2003). Disruption of the human pathogenic yeast *Candida albicans* catalase gene decreases survival in mouse-model infection and elevates susceptibility to higher temperature and to detergents. *Microbiology and Immunology, 47*, 395–403.

Nash, E. E., Peters, B. M., Palmer, G. E., Fidel, P. L., & Noverr, M. C. (2014). Morphogenesis is not required for *Candida albicans-Staphylococcus aureus* intra-abdominal infection-mediated dissemination and lethal sepsis. *Infection and Immunity, 82*, 3426–3435.

Nasution, O., Srinivasa, K., Kim, M., Kim, Y. J., Kim, W., Jeong, W., et al. (2008). Hydrogen peroxide induces hyphal differentiation in *Candida albicans*. *Eukaryotic Cell, 7*, 2008–2011.

Navarathna, D. H., Hornby, J. M., Krishnan, N., Parkhurst, A., Duhamel, G. E., & Nickerson, K. W. (2007). Effect of farnesol on a mouse model of systemic candidiasis, determined by use of a *DPP3* knockout mutant of *Candida albicans*. *Infection and Immunity, 75*, 1609–1618.

Navarathna, D. H., Nickerson, K. W., Duhamel, G. E., Jerrels, T. R., & Petro, T. M. (2007). Exogenous farnesol interferes with the normal progression of cytokine expression during candidiasis in a mouse model. *Infection and Immunity, 75*, 4006–4011.

Netea, M. G., Brown, G. D., Kullberg, B. J., & Gow, N. A. (2008). An integrated model of the recognition of *Candida albicans* by the innate immune system. *Nature Reviews Microbiology, 6*, 67–78.

Netea, M. G., Gijzen, K., Coolen, N., Verschueren, I., Figdor, C., Van der Meer, J. W., et al. (2004). Human dendritic cells are less potent at killing *Candida albicans* than both monocytes and macrophages. *Microbes and Infection, 6*, 985–989.

Netea, M. G., Gow, N. A., Munro, C. A., Bates, S., Collins, C., Ferwerda, G., et al. (2006). Immune sensing of *Candida albicans* requires cooperative recognition of mannans and glucans by lectin and Toll-like receptors. *Journal of Clinical Investigation, 116*, 1642–1650.

Netea, M. G., & Kullberg, B. J. (2010). Epithelial sensing of fungal invasion. *Cell Host & Microbe, 8*, 219–220.

Netea, M. G., van Tits, L. J., Curfs, J. H., Amiot, F., Meis, J. F., van der Meer, J. W., et al. (1999). Increased susceptibility of TNF-α lymphotoxin-alpha double knockout mice to systemic candidiasis through impaired recruitment of neutrophils and phagocytosis of *Candida albicans*. *Journal of Immunology, 163*, 1498–1505.

Nett, J., Lincoln, L., Marchillo, K., Massey, R., Holoyda, K., Hoff, B., et al. (2007). Putative role of beta-1,3 glucans in *Candida albicans* biofilm resistance. *Antimicrobial Agents and Chemotherapy, 51*, 510–520.

Nett, J. E., Crawford, K., Marchillo, K., & Andes, D. R. (2010). Role of Fks1p and matrix glucan in *Candida albicans* biofilm resistance to an echinocandin, pyrimidine, and polyene. *Antimicrobial Agents and Chemotherapy, 54*, 3505–3508.

Nett, J. E., Lepak, A. J., Marchillo, K., & Andes, D. R. (2009). Time course global gene expression analysis of an in vivo *Candida* biofilm. *Journal of Infectious Diseases, 200*, 307–313.

Nett, J. E., Sanchez, H., Cain, M. T., & Andes, D. R. (2010). Genetic basis of *Candida* biofilm resistance due to drug-sequestering matrix glucan. *Journal of Infectious Diseases, 202*, 171–175.

Nett, J. E., Sanchez, H., Cain, M. T., Ross, K. M., & Andes, D. R. (2011). Interface of *Candida albicans* biofilm matrix-associated drug resistance and cell wall integrity regulation. *Eukaryotic Cell, 10*, 1660–1669.

Nicholls, S., Leach, M. D., Priest, C. L., & Brown, A. J. (2009). Role of the heat shock transcription factor, Hsf1, in a major fungal pathogen that is obligately associated with warm-blooded animals. *Molecular Microbiology, 74*, 844–861.

Nickerson, K. W., Atkin, A. L., & Hornby, J. M. (2006). Quorum sensing in dimorphic fungi: farnesol and beyond. *Applied and Environmental Microbiology, 72*, 3805–3813.

Ning, Y., Hu, X., Ling, J., Du, Y., Liu, J., Liu, H., et al. (2013). *Candida albicans* survival and biofilm formation under starvation conditions. *International Endodontic Journal, 46*, 62–70.

Nisini, R., Torosantucci, A., Romagnoli, G., Chiani, P., Donati, S., Gagliardi, M. C., et al. (2007). beta-Glucan of *Candida albicans* cell wall causes the subversion of human monocyte differentiation into dendritic cells. *Journal of Leukocyte Biology, 82*, 1136–1142.

Nobbs, A. H., Vickerman, M. M., & Jenkinson, H. F. (2010). Heterologous expression of *Candida albicans* cell wall-associated adhesins in *Saccharomyces cerevisiae* reveals differential specificities in adherence and biofilm formation and in binding oral *Streptococcus gordonii*. *Eukaryotic Cell, 9*, 1622–1634.

Nobile, C. J., Fox, E. P., Hartooni, N., Mitchell, K. F., Hnisz, D., Andes, D. R., et al. (2014). A histone deacetylase complex mediates biofilm dispersal and drug resistance in *Candida albicans*. *MBio, 5*.

Nobile, C. J., Fox, E. P., Nett, J. E., Sorrells, T. R., Mitrovich, Q. M., Hernday, A. D., et al. (2012). A recently evolved transcriptional network controls biofilm development in *Candida albicans*. *Cell, 148*, 126–138.

Nobile, C. J., & Mitchell, A. P. (2005). Regulation of cell-surface genes and biofilm formation by the *C. albicans* transcription factor Bcr1p. *Current Biology, 15*, 1150–1155.

Nobile, C. J., Nett, J. E., Andes, D. R., & Mitchell, A. P. (2006). Function of *Candida albicans* adhesin Hwp1 in biofilm formation. *Eukaryotic Cell, 5*, 1604–1610.

Nobile, C. J., Schneider, H. A., Nett, J. E., Sheppard, D. C., Filler, S. G., Andes, D. R., et al. (2008). Complementary adhesin function in *C. albicans* biofilm formation. *Current Biology, 18*, 1017–1024.

Nobile, C. J., Solis, N., Myers, C. L., Fay, A. J., Deneault, J. S., Nantel, A., et al. (2008). *Candida albicans* transcription factor Rim101 mediates pathogenic interactions through cell wall functions. *Cellular Microbiology, 10*, 2180–2196.

Noble, S. M., French, S., Kohn, L. A., Chen, V., & Johnson, A. D. (2010). Systematic screens of a *Candida albicans* homozygous deletion library decouple morphogenetic switching and pathogenicity. *Nature Genetics, 42*, 590–598.

Noverr, M. C., & Huffnagle, G. B. (2004). Regulation of *Candida albicans* morphogenesis by fatty acid metabolites. *Infection and Immunity, 72*, 6206–6210.

Nucci, M., & Anaissie, E. (2001). Revisiting the source of candidemia: skin or gut? *Clinical Infectious Diseases, 33*(12), 1959–1967.

O'Hara, A. M., & Shanahan, F. (2006). The gut flora as a forgotten organ. *EMBO Reports, 7*, 688–693.

O'Sullivan, J. M., Jenkinson, H. F., & Cannon, R. D. (2000). Adhesion of *Candida albicans* to oral streptococci is promoted by selective adsorption of salivary proteins to the streptococcal cell surface. *Microbiology, 146*(Pt 1), 41–48.

Odds, F. C. (1987). *Candida* infections: an overview. *Critical Reviews in Microbiology, 15*, 1–5.

Odds, F. C. (1988). Candida *and Candidosis*. Elsevier Science Health Science Division.

Odds, F. C., Brown, A. J., & Gow, N. A. (2004). *Candida albicans* genome sequence: a platform for genomics in the absence of genetics. *Genome Biology, 5*, 230.

Odds, F. C., Davidson, A. D., Jacobsen, M. D., Tavanti, A., Whyte, J. A., Kibbler, C. C., et al. (2006). *Candida albicans* strain maintenance, replacement, and microvariation demonstrated by multilocus sequence typing. *Journal of Clinical Microbiology, 44*, 3647–3658.

Odds, F. C., & Jacobsen, M. D. (2008). Multilocus sequence typing of pathogenic *Candida* species. *Eukaryotic Cell, 7*, 1075–1084.

Oh, K. B., Miyazawa, H., Naito, T., & Matsuoka, H. (2001). Purification and characterization of an autoregulatory substance capable of regulating the morphological transition in *Candida albicans*. *Proceedings of the National Academy of Sciences of the United States of America, 98*, 4664–4668.

d'Ostiani, C. F., Del Sero, G., Bacci, A., Montagnoli, C., Spreca, A., Mencacci, A., et al. (2000). Dendritic cells discriminate between yeasts and hyphae of the fungus *Candida albicans*. Implications for initiation of T helper cell immunity in vitro and in vivo. *Journal of Experimental Medicine, 191*, 1661–1674.

Ostrosky-Zeichner, L., Rex, J. H., Pappas, P. G., Hamill, R. J., Larsen, R. A., Horowitz, H. W., et al. (2003). Antifungal susceptibility survey of 2,000 bloodstream *Candida* isolates in the United States. *Antimicrobial Agents and Chemotherapy, 47*, 3149–3154.

Otoo, H. N., Lee, K. G., Qiu, W., & Lipke, P. N. (2008). *Candida albicans* Als adhesins have conserved amyloid-forming sequences. *Eukaryotic Cell, 7*, 776–782.

Owen, D. H., & Katz, D. F. (1999). A vaginal fluid simulant. *Contraception, 59*, 91–95.

Pan, Y. (2011). Mitochondria, reactive oxygen species, and chronological aging: a message from yeast. *Experimental Gerontology, 46*, 847–852.

Pande, K., Chen, C., & Noble, S. M. (2013). Passage through the mammalian gut triggers a phenotypic switch that promotes *Candida albicans* commensalism. *Nature Genetics, 45*, 1088–1091.

Panwar, S. L., Legrand, M., Dignard, D., Whiteway, M., & Magee, P. T. (2003). MFalpha1, the gene encoding the alpha mating pheromone of *Candida albicans*. *Eukaryotic Cell, 2*, 1350–1360.

Pappas, P. G. (2006). Invasive candidiasis. *Infectious Disease Clinics of North America, 20*, 485–506.

Park, S. J., Han, K. H., Park, J. Y., Choi, S. J., & Lee, K. H. (2014). Influence of bacterial presence on biofilm formation of *Candida albicans*. *Yonsei Medical Journal, 55*, 449–458.

Peleg, A. Y., Hogan, D. A., & Mylonakis, E. (2010). Medically important bacterial-fungal interactions. *Nature Reviews Microbiology, 8*, 340–349.

Peleg, A. Y., Tampakakis, E., Fuchs, B. B., Eliopoulos, G. M., Moellering, R. C., Jr., & Mylonakis, E. (2008). Prokaryote-eukaryote interactions identified by using *Caenorhabditis elegans*. *Proceedings of the National Academy of Sciences of the United States of America, 105*, 14585–14590.

Peltz, G., Zaas, A. K., Zheng, M., Solis, N. V., Zhang, M. X., Liu, H. H., et al. (2011). Next-generation computational genetic analysis: multiple complement alleles control survival after *Candida albicans* infection. *Infection and Immunity, 79*, 4472–4479.

Perepnikhatka, V., Fischer, F. J., Niimi, M., Baker, R. A., Cannon, R. D., Wang, Y. K., et al. (1999). Specific chromosome alterations in fluconazole-resistant mutants of *Candida albicans*. *Journal of Bacteriology, 181*, 4041–4049.

Perez, A., Pedros, B., Murgui, A., Casanova, M., Lopez-Ribot, J. L., & Martinez, J. P. (2006). Biofilm formation by *Candida albicans* mutants for genes coding fungal proteins exhibiting the eight-cysteine-containing CFEM domain. *FEMS Yeast Research, 6*, 1074–1084.

Perez, A., Ramage, G., Blanes, R., Murgui, A., Casanova, M., & Martinez, J. P. (2011). Some biological features of *Candida albicans* mutants for genes coding fungal proteins containing the CFEM domain. *FEMS Yeast Research, 11*, 273–284.

Perez, J. C., & Johnson, A. D. (2013). Regulatory circuits that enable proliferation of the fungus *Candida albicans* in a mammalian host. *PLoS Pathogens, 9*, e1003780.

Perez, J. C., Kumamoto, C. A., & Johnson, A. D. (2013). *Candida albicans* commensalism and pathogenicity are intertwined traits directed by a tightly knit transcriptional regulatory circuit. *PLoS Biology, 11*, e1001510.

Perlroth, J., Choi, B., & Spellberg, B. (2007). Nosocomial fungal infections: epidemiology, diagnosis, and treatment. *Medical Mycology, 45*, 321–346.

Peschel, A., & Sahl, H. G. (2006). The co-evolution of host cationic antimicrobial peptides and microbial resistance. *Nature Reviews Microbiology, 4*, 529–536.

Pfaller, M. A., & Diekema, D. J. (2007). Epidemiology of invasive candidiasis: a persistent public health problem. *Clinical Microbiology Reviews, 20*, 133–163.

Pfaller, M. A., Messer, S. A., Boyken, L., Tendolkar, S., Hollis, R. J., & Diekema, D. J. (2004). Geographic variation in the susceptibilities of invasive isolates of *Candida glabrata* to seven systemically active antifungal agents: a global assessment from the ARTEMIS Antifungal Surveillance Program conducted in 2001 and 2002. *Journal of Clinical Microbiology, 42*, 3142–3146.

Phan, Q. T., Myers, C. L., Fu, Y., Sheppard, D. C., Yeaman, M. R., Welch, W. H., et al. (2007). Als3 is a *Candida albicans* invasin that binds to cadherins and induces endocytosis by host cells. *PLoS Biology, 5*, e64.

Pickeral, O. K., Makalowski, W., Boguski, M. S., & Boeke, J. D. (2000). Frequent human genomic DNA transduction driven by LINE-1 retrotransposition. *Genome Research, 10*, 411–415.

Pierce, G. E. (2005). *Pseudomonas aeruginosa*, *Candida albicans*, and device-related nosocomial infections: implications, trends, and potential approaches for control. *Journal of Industrial Microbiology and Biotechnology, 32*, 309–318.

Pierce, J. V., Dignard, D., Whiteway, M., & Kumamoto, C. A. (2013). Normal adaptation of *Candida albicans* to the murine gastrointestinal tract requires Efg1p-dependent regulation of metabolic and host defense genes. *Eukaryotic Cell, 12*, 37–49.

Pierce, J. V., & Kumamoto, C. A. (2012). Variation in *Candida albicans EFG1* expression enables host-dependent changes in colonizing fungal populations. *MBio, 3*, e00117–12.

Piispanen, A. E., Grahl, N., Hollomon, J. M., & Hogan, D. A. (2013). Regulated proteolysis of *Candida albicans* Ras1 is involved in morphogenesis and quorum sensing regulation. *Molecular Microbiology, 89*, 166–178.

Pires, D. P., Silva, S., Almeida, C., Henriques, M., Anderson, E. M., Lam, J. S., et al. (2013). Evaluation of the ability of *C. albicans* to form biofilm in the presence of phage-resistant phenotypes of *P. aeruginosa*. *Biofouling, 29*, 1169–1180.

Poltermann, S., Kunert, A., von der Heide, M., Eck, R., Hartmann, A., & Zipfel, P. F. (2007). Gpm1p is a factor H-, FHL-1-, and plasminogen-binding surface protein of *Candida albicans*. *Journal of Biological Chemistry, 282*, 37537–37544.

Prieto, D., Roman, E., Correia, I., & Pla, J. (2014). The *HOG* pathway is critical for the colonization of the mouse gastrointestinal tract by *Candida albicans*. *PLoS One, 9*, e87128.

Pulimood, S., Ganesan, L., Alangaden, G., & Chandrasekar, P. (2002). Polymicrobial candidemia. *Diagnostic Microbiology and Infectious Disease, 44*, 353–357.

Puri, S., Kumar, R., Chadha, S., Tati, S., Conti, H. R., Hube, B., et al. (2012). Secreted aspartic protease cleavage of *Candida albicans* Msb2 activates Cek1 MAPK signaling affecting biofilm formation and oropharyngeal candidiasis. *PLoS One,* 7, e46020.

Puri, S., Lai, W. K., Rizzo, J. M., Buck, M. J., & Edgerton, M. (2014). Iron-responsive chromatin remodelling and MAPK signalling enhance adhesion in *Candida albicans*. *Molecular Microbiology, 93*(2), 291–305. http://dx.doi.org/10.1111/mmi.12659.

Qian, Q., Jutila, M. A., Van Rooijen, N., & Cutler, J. E. (1994). Elimination of mouse splenic macrophages correlates with increased susceptibility to experimental disseminated candidiasis. *Journal of Immunology, 152*, 5000–5008.

Quintin, J., Voigt, J., van der Voort, R., Jacobsen, I. D., Verschueren, I., Hube, B., et al. (2014). Differential role of NK cells against *Candida albicans* infection in immunocompetent or immunocompromised mice. *European Journal of Immunology, 44*, 2405–2414.

Radisky, D., & Kaplan, J. (1999). Regulation of transition metal transport across the yeast plasma membrane. *Journal of Biological Chemistry, 274*, 4481–4484.

Ramachandra, S., Linde, J., Brock, M., Guthke, R., Hube, B., & Brunke, S. (2014). Regulatory networks controlling nitrogen sensing and uptake in *Candida albicans*. *PLoS One, 9*, e92734.

Ramage, G., Mowat, E., Jones, B., Williams, C., & Lopez-Ribot, J. (2009). Our current understanding of fungal biofilms. *Critical Reviews in Microbiology, 35*, 340–355.

Ramage, G., Saville, S. P., Wickes, B. L., & Lopez-Ribot, J. L. (2002). Inhibition of *Candida albicans* biofilm formation by farnesol, a quorum-sensing molecule. *Applied and Environmental Microbiology, 68*, 5459–5463.

Ramage, G., VandeWalle, K., Bachmann, S. P., Wickes, B. L., & Lopez-Ribot, J. L. (2002). In vitro pharmacodynamic properties of three antifungal agents against preformed *Candida albicans* biofilms determined by time-kill studies. *Antimicrobial Agents and Chemotherapy, 46*, 3634–3636.

Ramage, G., VandeWalle, K., Lopez-Ribot, J. L., & Wickes, B. L. (2002). The filamentation pathway controlled by the Efg1 regulator protein is required for normal biofilm formation and development in *Candida albicans*. *FEMS Microbiology Letters, 214*, 95–100.

Rambach, G., & Speth, C. (2009). Complement in *Candida albicans* infections. *Frontiers in Bioscience (Elite Edition), 1*, 1–12.

Ramirez-Ortiz, Z. G., & Means, T. K. (2012). The role of dendritic cells in the innate recognition of pathogenic fungi (*A. fumigatus*, *C. neoformans* and *C. albicans*). *Virulence, 3*, 635–646.

Ramsey, M. M., Rumbaugh, K. P., & Whiteley, M. (2011). Metabolite cross-feeding enhances virulence in a model polymicrobial infection. *PLoS Pathogens,* 7, e1002012.

Ramsook, C. B., Tan, C., Garcia, M. C., Fung, R., Soybelman, G., Henry, R., et al. (2010). Yeast cell adhesion molecules have functional amyloid-forming sequences. *Eukaryotic Cell, 9*, 393–404.

Ray, T. L., & Payne, C. D. (1988). Scanning electron microscopy of epidermal adherence and cavitation in murine candidiasis: a role for *Candida* acid proteinase. *Infection and Immunity, 56*, 1942–1949.

Rhie, G. E., Hwang, C. S., Brady, M. J., Kim, S. T., Kim, Y. R., Huh, W. K., et al. (1999). Manganese-containing superoxide dismutase and its gene from *Candida albicans*. *Biochimica et Biophysica Acta, 1426*, 409–419.

Ricker, A., Vickerman, M., & Dongari-Bagtzoglou, A. (2014). *Streptococcus gordonii* glucosyltransferase promotes biofilm interactions with *Candida albicans*. *Journal of Oral Microbiology, 6*.

Ristow, M., & Schmeisser, S. (2011). Extending life span by increasing oxidative stress. *Free Radical Biology and Medicine, 51*, 327–336.

Robbins, N., Uppuluri, P., Nett, J., Rajendran, R., Ramage, G., Lopez-Ribot, J. L., et al. (2011). Hsp90 governs dispersion and drug resistance of fungal biofilms. *PLoS Pathogens,* 7, e1002257.

Rodaki, A., Bohovych, I. M., Enjalbert, B., Young, T., Odds, F. C., Gow, N. A., et al. (2009). Glucose promotes stress resistance in the fungal pathogen *Candida albicans*. *Molecular Biology of the Cell, 20*, 4845–4855.

Rogers, H., Williams, D. W., Feng, G. J., Lewis, M. A., & Wei, X. Q. (2013). Role of bacterial lipopolysaccharide in enhancing host immune response to *Candida albicans*. *Clinical and Developmental Immunology, 2013*, 320168.

Roman, E., Alonso-Monge, R., Gong, Q., Li, D., Calderone, R., & Pla, J. (2009). The Cek1 MAPK is a short-lived protein regulated by quorum sensing in the fungal pathogen *Candida albicans*. *FEMS Yeast Research, 9*, 942–955.

Roman, E., Nombela, C., & Pla, J. (2005). The Sho1 adaptor protein links oxidative stress to morphogenesis and cell wall biosynthesis in the fungal pathogen *Candida albicans*. *Molecular and Cellular Biology, 25*, 10611–10627.

Romani, L., Bistoni, F., & Puccetti, P. (2002). Fungi, dendritic cells and receptors: a host perspective of fungal virulence. *Trends in Microbiology, 10*, 508–514.

Romani, L., Bistoni, F., & Puccetti, P. (2003). Adaptation of *Candida albicans* to the host environment: the role of morphogenesis in virulence and survival in mammalian hosts. *Current Opinion in Microbiology, 6*, 338–343.

Romani, L., Mencacci, A., Cenci, E., Spaccapelo, R., Toniatti, C., Puccetti, P., et al. (1996). Impaired neutrophil response and $CD4^+$ T helper cell 1 development in interleukin 6-deficient mice infected with *Candida albicans*. *Journal of Experimental Medicine, 183*, 1345–1355.

Romani, L., Montagnoli, C., Bozza, S., Perruccio, K., Spreca, A., Allavena, P., et al. (2004). The exploitation of distinct recognition receptors in dendritic cells determines the full range of host immune relationships with *Candida albicans*. *International Immunology, 16*, 149–161.

Rosenbach, A., Dignard, D., Pierce, J. V., Whiteway, M., & Kumamoto, C. A. (2010). Adaptations of *Candida albicans* for growth in the mammalian intestinal tract. *Eukaryotic Cell, 9*, 1075–1086.

Rothfels, K., Tanny, J. C., Molnar, E., Friesen, H., Commisso, C., & Segall, J. (2005). Components of the ESCRT pathway, *DFG16*, and YGR122w are required for Rim101 to act as a corepressor with Nrg1 at the negative regulatory element of the *DIT1* gene of *Saccharomyces cerevisiae*. *Molecular and Cellular Biology, 25*, 6772–6788.

Roux, D., Gaudry, S., Dreyfuss, D., El-Benna, J., de Prost, N., Denamur, E., et al. (2009). *Candida albicans* impairs macrophage function and facilitates Pseudomonas aeruginosa pneumonia in rat. *Critical Care Medicine, 37*, 1062–1067.

Roy, R. M., & Klein, B. S. (2012). Dendritic cells in antifungal immunity and vaccine design. *Cell Host & Microbe, 11*, 436–446.

Rozpedowska, E., Galafassi, S., Johansson, L., Hagman, A., Piskur, J., & Compagno, C. (2011). *Candida albicans*—a pre-whole genome duplication yeast—is predominantly aerobic and a poor ethanol producer. *FEMS Yeast Research, 11*, 285–291.

Rubin-Bejerano, I., Fraser, I., Grisafi, P., & Fink, G. R. (2003). Phagocytosis by neutrophils induces an amino acid deprivation response in *Saccharomyces cerevisiae* and *Candida albicans*. *Proceedings of the National Academy of Sciences of the United States of America, 100*, 11007–11012.

Rustad, T. R., Stevens, D. A., Pfaller, M. A., & White, T. C. (2002). Homozygosity at the *Candida albicans* MTL locus associated with azole resistance. *Microbiology, 148*, 1061–1072.

Rustchenko-Bulgac, E. P. (1991). Variations of *Candida albicans* electrophoretic karyotypes. *Journal of Bacteriology, 173*, 6586–6596.

Rustchenko, E. (2007). Chromosome instability in *Candida albicans*. *FEMS Yeast Research, 7*, 2–11.

Rustchenko, E. P., Howard, D. H., & Sherman, F. (1997). Variation in assimilating functions occurs in spontaneous *Candida albicans* mutants having chromosomal alterations. *Microbiology, 143*(Pt 5), 1765–1778.

Sabina, J., & Brown, V. (2009). Glucose sensing network in *Candida albicans*: a sweet spot for fungal morphogenesis. *Eukaryotic Cell, 8*, 1314–1320.

Saidi, S., Luitaud, C., & Rouabhia, M. (2006). In vitro synergistic effect of farnesol and human gingival cells against *Candida albicans*. *Yeast, 23*, 673–687.

Samaranayake, L. P., & MacFarlane, T. W. (1982). The effect of dietary carbohydrates on the in-vitro adhesion of *Candida albicans* to epithelial cells. *Journal of Medical Microbiology, 15*, 511–517.

Samonis, G., Gikas, A., Toloudis, P., Maraki, S., Vrentzos, G., Tselentis, Y., et al. (1994). Prospective study of the impact of broad-spectrum antibiotics on the yeast flora of the human gut. *European Journal of Clinical Microbiology, 13*, 665–667.

Sampaio, P., Gusmao, L., Correia, A., Alves, C., Rodrigues, A. G., Pina-Vaz, C., et al. (2005). New microsatellite multiplex PCR for *Candida albicans* strain typing reveals microevolutionary changes. *Journal of Clinical Microbiology, 43*, 3869–3876.

Sanglard, D. (2002). Resistance of human fungal pathogens to antifungal drugs. *Current Opinion in Microbiology, 5*, 379–385.

Santos, M. A., Gomes, A. C., Santos, M. C., Carreto, L. C., & Moura, G. R. (2011). The genetic code of the fungal CTG clade. *Comptes Rendus Biologies, 334*, 607–611.

Sapaar, B., Nur, A., Hirota, K., Yumoto, H., Murakami, K., Amoh, T., et al. (2014). Effects of extracellular DNA from *Candida albicans* and pneumonia-related pathogens on *Candida* biofilm formation and hyphal transformation. *Journal of Applied Microbiology, 116*, 1531–1542.

Sasse, C., Hasenberg, M., Weyler, M., Gunzer, M., & Morschhauser, J. (2013). White-opaque switching of *Candida albicans* allows immune evasion in an environment-dependent fashion. *Eukaryotic Cell, 12*, 50–58.

Sato, T., Watanabe, T., Mikami, T., & Matsumoto, T. (2004). Farnesol, a morphogenetic autoregulatory substance in the dimorphic fungus *Candida albicans*, inhibits hyphae growth through suppression of a mitogen-activated protein kinase cascade. *Biological & Pharmaceutical Bulletin, 27*, 751–752.

Saville, S. P., Lazzell, A. L., Chaturvedi, A. K., Monteagudo, C., & Lopez-Ribot, J. L. (2008). Use of a genetically engineered strain to evaluate the pathogenic potential of yeast cell and filamentous forms during *Candida albicans* systemic infection in immunodeficient mice. *Infection and Immunity, 76*, 97–102.

Saville, S. P., Lazzell, A. L., Monteagudo, C., & Lopez-Ribot, J. L. (2003). Engineered control of cell morphology in vivo reveals distinct roles for yeast and filamentous forms of *Candida albicans* during infection. *Eukaryotic Cell, 2*, 1053–1060.

Schroppel, K., Rotman, M., Galask, R., Mac, K., & Soll, D. R. (1994). Evolution and replacement of *Candida albicans* strains during recurrent vaginitis demonstrated by DNA fingerprinting. *Journal of Clinical Microbiology, 32*, 2646–2654.

Schweizer, A., Rupp, S., Taylor, B. N., Rollinghoff, M., & Schroppel, K. (2000). The TEA/ATTS transcription factor CaTec1p regulates hyphal development and virulence in *Candida albicans*. *Molecular Microbiology, 38*, 435–445.

Sellam, A., van het Hoog, M., Tebbji, F., Beaurepaire, C., Whiteway, M., & Nantel, A. (2014). Modeling the transcriptional regulatory network that controls the early hypoxic response in *Candida albicans*. *Eukaryotic Cell, 13*, 675–690.

Selmecki, A. M., Dulmage, K., Cowen, L. E., Anderson, J. B., & Berman, J. (2009). Acquisition of aneuploidy provides increased fitness during the evolution of antifungal drug resistance. *PLoS Genetics, 5*, e1000705.

Selmecki, A., Forche, A., & Berman, J. (2006). Aneuploidy and isochromosome formation in drug-resistant *Candida albicans*. *Science, 313*, 367–370.

Selmecki, A., Forche, A., & Berman, J. (2010). Genomic plasticity of the human fungal pathogen *Candida albicans*. *Eukaryotic Cell, 9*, 991–1008.

Setiadi, E. R., Doedt, T., Cottier, F., Noffz, C., & Ernst, J. F. (2006). Transcriptional response of *Candida albicans* to hypoxia: linkage of oxygen sensing and Efg1p-regulatory networks. *Journal of Molecular Biology, 361*, 399–411.

Shapiro, R. S., & Cowen, L. E. (2012). Uncovering cellular circuitry controlling temperature-dependent fungal morphogenesis. *Virulence, 3*, 400–404.

Shapiro, R. S., Robbins, N., & Cowen, L. E. (2011). Regulatory circuitry governing fungal development, drug resistance, and disease. *Microbiology and Molecular Biology Reviews, 75*, 213–267.

Shapiro, R. S., Uppuluri, P., Zaas, A. K., Collins, C., Senn, H., Perfect, J. R., et al. (2009). Hsp90 orchestrates temperature-dependent *Candida albicans* morphogenesis via Ras1-PKA signaling. *Current Biology, 19*, 621–629.

Shareck, J., & Belhumeur, P. (2011). Modulation of morphogenesis in *Candida albicans* by various small molecules. *Eukaryotic Cell, 10*, 1004–1012.

Sherry, L., Rajendran, R., Lappin, D. F., Borghi, E., Perdoni, F., Falleni, M., et al. (2014). Biofilms formed by *Candida albicans* bloodstream isolates display phenotypic and transcriptional heterogeneity that are associated with resistance and pathogenicity. *BMC Microbiology, 14*, 182.

Shirtliff, M. E., Krom, B. P., Meijering, R. A., Peters, B. M., Zhu, J., Scheper, M. A., et al. (2009). Farnesol-induced apoptosis in *Candida albicans*. *Antimicrobial Agents and Chemotherapy, 53*, 2392–2401.

Shirtliff, M. E., Peters, B. M., & Jabra-Rizk, M. A. (2009). Cross-kingdom interactions: *Candida albicans* and bacteria. *FEMS Microbiology Letters, 299*, 1–8.

Si, H., Hernday, A. D., Hirakawa, M. P., Johnson, A. D., & Bennett, R. J. (2013). *Candida albicans* white and opaque cells undergo distinct programs of filamentous growth. *PLoS Pathogens, 9*, e1003210.

Silva, S., Henriques, M., Oliveira, R., Williams, D., & Azeredo, J. (2010). In vitro biofilm activity of non-*Candida albicans Candida* species. *Current Microbiology, 61*, 534–540.

da Silva Dantas, A., Patterson, M. J., Smith, D. A., Maccallum, D. M., Erwig, L. P., Morgan, B. A., et al. (2010). Thioredoxin regulates multiple hydrogen peroxide-induced signaling pathways in *Candida albicans*. *Molecular and Cellular Biology, 30*, 4550–4563.

Silverman, R. J., Nobbs, A. H., Vickerman, M. M., Barbour, M. E., & Jenkinson, H. F. (2010). Interaction of *Candida albicans* cell wall Als3 protein with *Streptococcus gordonii* SspB adhesin promotes development of mixed-species communities. *Infection and Immunity, 78*, 4644–4652.

Singh, S. D., Robbins, N., Zaas, A. K., Schell, W. A., Perfect, J. R., & Cowen, L. E. (2009). Hsp90 governs echinocandin resistance in the pathogenic yeast *Candida albicans* via calcineurin. *PLoS Pathogens, 5*, e1000532.

Slutsky, B., Staebell, M., Anderson, J., Risen, L., Pfaller, M., & Soll, D. R. (1987). "White-opaque transition": a second high-frequency switching system in *Candida albicans*. *Journal of Bacteriology, 169*, 189–197.

Smith, D. A., Morgan, B. A., & Quinn, J. (2010). Stress signalling to fungal stress-activated protein kinase pathways. *FEMS Microbiology Letters, 306*, 1–8.

Smith, D. A., Nicholls, S., Morgan, B. A., Brown, A. J., & Quinn, J. (2004). A conserved stress-activated protein kinase regulates a core stress response in the human pathogen *Candida albicans*. *Molecular Biology of the Cell, 15*, 4179–4190.

Sobel, J. D. (2007). Vulvovaginal candidosis. *Lancet, 369*, 1961–1971.

Soll, D. R. (1992). High-frequency switching in *Candida albicans*. *Clinical Microbiology Reviews, 5*, 183–203.

Soll, D. R. (2002). *Candida* commensalism and virulence: the evolution of phenotypic plasticity. *Acta Tropica, 81*, 101–110.

Soll, D. R. (2004). Mating-type locus homozygosis, phenotypic switching and mating: a unique sequence of dependencies in *Candida albicans*. *Bioessays, 26*, 10–20.

Soll, D. R. (2014). The role of phenotypic switching in the basic biology and pathogenesis of *Candida albicans*. *Journal of Oral Microbiology, 6*.

Sorger, P. K., & Pelham, H. R. (1987). Purification and characterization of a heat-shock element binding protein from yeast. *The EMBO Journal, 6*, 3035–3041.

Sorger, P. K., & Pelham, H. R. (1988). Yeast heat shock factor is an essential DNA-binding protein that exhibits temperature-dependent phosphorylation. *Cell, 54*, 855–864.

Sosinska, G. J., de Koning, L. J., de Groot, P. W., Manders, E. M., Dekker, H. L., Hellingwerf, K. J., et al. (2011). Mass spectrometric quantification of the adaptations in the wall proteome of *Candida albicans* in response to ambient pH. *Microbiology, 157*, 136–146.

Spellberg, B., Kontoyiannis, D. P., Fredricks, D., Morris, M. I., Perfect, J. R., Chin-Hong, P. V., et al. (2012). Risk factors for mortality in patients with mucormycosis. *Medical Mycology, 50*, 611–618.

Spiering, M. J., Moran, G. P., Chauvel, M., Maccallum, D. M., Higgins, J., Hokamp, K., et al. (2010). Comparative transcript profiling of *Candida albicans* and *Candida dubliniensis* identifies *SFL2*, a *C. albicans* gene required for virulence in a reconstituted epithelial infection model. *Eukaryotic Cell, 9*, 251–265.

Srikantha, T., Borneman, A. R., Daniels, K. J., Pujol, C., Wu, W., Seringhaus, M. R., et al. (2006). *TOS9* regulates white-opaque switching in *Candida albicans*. *Eukaryotic Cell, 5*, 1674–1687.

Srinivasa, K., Kim, J., Yee, S., Kim, W., & Choi, W. (2012). A MAP kinase pathway is implicated in the pseudohyphal induction by hydrogen peroxide in *Candica albicans*. *Molecules and Cells, 33*, 183–193.

Staab, J. F., Bradway, S. D., Fidel, P. L., & Sundstrom, P. (1999). Adhesive and mammalian transglutaminase substrate properties of *Candida albicans* Hwp1. *Science, 283*, 1535–1538.

Staab, J. F., Datta, K., & Rhee, P. (2013). Niche-specific requirement for hyphal wall protein 1 in virulence of *Candida albicans*. *PLoS One, 8*, e80842.

Steinberg, G. (2007). Hyphal growth: a tale of motors, lipids, and the Spitzenkorper. *Eukaryotic Cell, 6*, 351–360.

Stewart, P. S., & Franklin, M. J. (2008). Physiological heterogeneity in biofilms. *Nature Reviews Microbiology, 6*, 199–210.

Stichternoth, C., & Ernst, J. F. (2009). Hypoxic adaptation by Efg1 regulates biofilm formation by *Candida albicans*. *Applied and Environmental Microbiology, 75*, 3663–3672.

Stoldt, V. R., Sonneborn, A., Leuker, C. E., & Ernst, J. F. (1997). Efg1p, an essential regulator of morphogenesis of the human pathogen *Candida albicans*, is a member of a conserved class of bHLH proteins regulating morphogenetic processes in fungi. *The EMBO Journal, 16*, 1982–1991.

Strus, M., Kucharska, A., Kukla, G., Brzychczy-Wloch, M., Maresz, K., & Heczko, P. B. (2005). The in vitro activity of vaginal *Lactobacillus* with probiotic properties against *Candida*. *Infectious Diseases in Obstetrics and Gynecology, 13*, 69–75.

Su, C., Lu, Y., & Liu, H. (2013). Reduced TOR signaling sustains hyphal development in *Candida albicans* by lowering Hog1 basal activity. *Molecular Biology of the Cell, 24*, 385–397.

Sudbery, P., Gow, N., & Berman, J. (2004). The distinct morphogenic states of *Candida albicans*. *Trends in Microbiology, 12*, 317–324.

Sudbery, P. E. (2011). Growth of *Candida albicans* hyphae. *Nature Reviews Microbiology, 9*, 737–748.

Sun, F. J., Zhang, X. B., Fang, Y., Chen, J., Xing, H., Shi, H., et al. (2012). Spectrum and drug resistance of pathogens from patients with burns. *Burns, 38*, 1124–1130.

Sun, J. N., Solis, N. V., Phan, Q. T., Bajwa, J. S., Kashleva, H., Thompson, A., et al. (2010). Host cell invasion and virulence mediated by *Candida albicans* Ssa1. *PLoS Pathogens, 6*, e1001181.

Sundstrom, P., Balish, E., & Allen, C. M. (2002). Essential role of the *Candida albicans* transglutaminase substrate, hyphal wall protein 1, in lethal oroesophageal candidiasis in immunodeficient mice. *Journal of Infectious Diseases, 185*, 521–530.

Swidergall, M., Ernst, A. M., & Ernst, J. F. (2013). *Candida albicans* mucin Msb2 is a broad-range protectant against antimicrobial peptides. *Antimicrobial Agents and Chemotherapy, 57*, 3917–3922.

Swidergall, M., & Ernst, J. F. (2014). Interplay between *Candida albicans* and the antimicrobial peptide armory. *Eukaryotic Cell, 13*, 950–957.

Szafranski-Schneider, E., Swidergall, M., Cottier, F., Tielker, D., Roman, E., Pla, J., et al. (2012). Msb2 shedding protects *Candida albicans* against antimicrobial peptides. *PLoS Pathogens, 8*, e1002501.

Taff, H. T., Mitchell, K. F., Edward, J. A., & Andes, D. R. (2013). Mechanisms of *Candida* biofilm drug resistance. *Future Microbiology, 8*, 1325–1337.

Taff, H. T., Nett, J. E., Zarnowski, R., Ross, K. M., Sanchez, H., Cain, M. T., et al. (2012). A *Candida* biofilm-induced pathway for matrix glucan delivery: implications for drug resistance. *PLoS Pathogens, 8*, e1002848.

Tampakakis, E., Peleg, A. Y., & Mylonakis, E. (2009). Interaction of *Candida albicans* with an intestinal pathogen, *Salmonella enterica* serovar Typhimurium. *Eukaryotic Cell, 8*, 732–737.

Tao, L., Du, H., Guan, G., Dai, Y., Nobile, C. J., Liang, W., et al. (2014). Discovery of a "white-gray-opaque" tristable phenotypic switching system in *Candida albicans*: roles of non-genetic diversity in host adaptation. *PLoS Biology, 12*, e1001830.

Thewes, S., Kretschmar, M., Park, H., Schaller, M., Filler, S. G., & Hube, B. (2007). In vivo and ex vivo comparative transcriptional profiling of invasive and non-invasive *Candida albicans* isolates identifies genes associated with tissue invasion. *Molecular Microbiology, 63*, 1606–1628.

Torosantucci, A., Romagnoli, G., Chiani, P., Stringaro, A., Crateri, P., Mariotti, S., et al. (2004). *Candida albicans* yeast and germ tube forms interfere differently with human monocyte differentiation into dendritic cells: a novel dimorphism-dependent mechanism to escape the host's immune response. *Infection and Immunity, 72*, 833–843.

Tortorano, A. M., Kibbler, C., Peman, J., Bernhardt, H., Klingspor, L., & Grillot, R. (2006). Candidaemia in Europe: epidemiology and resistance. *International Journal of Antimicrobial Agents, 27*, 359–366.

Trejo-Hernandez, A., Andrade-Dominguez, A., Hernandez, M., & Encarnacion, S. (2014). Interspecies competition triggers virulence and mutability in *Candida albicans-Pseudomonas aeruginosa* mixed biofilms. *ISME Journal, 8*(10), 1974–1988. http://dx.doi.org/10.1038/ismej.2014.53.

Trofa, D., Gacser, A., & Nosanchuk, J. D. (2008). *Candida parapsilosis*, an emerging fungal pathogen. *Clinical Microbiology Reviews, 21*, 606–625.

Tsoni, S. V., Kerrigan, A. M., Marakalala, M. J., Srinivasan, N., Duffield, M., Taylor, P. R., et al. (2009). Complement C3 plays an essential role in the control of opportunistic fungal infections. *Infection and Immunity, 77*, 3679–3685.

Tuch, B. B., Mitrovich, Q. M., Homann, O. R., Hernday, A. D., Monighetti, C. K., De La Vega, F. M., et al. (2010). The transcriptomes of two heritable cell types illuminate the circuit governing their differentiation. *PLoS Genetics, 6*, e1001070.

Tumbarello, M., Posteraro, B., Trecarichi, E. M., Fiori, B., Rossi, M., Porta, R., et al. (2007). Biofilm production by *Candida* species and inadequate antifungal therapy as predictors of mortality for patients with candidemia. *Journal of Clinical Microbiology, 45*, 1843–1850.

Ullmann, B. D., Myers, H., Chiranand, W., Lazzell, A. L., Zhao, Q., Vega, L. A., et al. (2004). Inducible defense mechanism against nitric oxide in *Candida albicans*. *Eukaryotic Cell, 3*, 715–723.

Umezu, K., Hiraoka, M., Mori, M., & Maki, H. (2002). Structural analysis of aberrant chromosomes that occur spontaneously in diploid *Saccharomyces cerevisiae*: retrotransposon Ty1 plays a crucial role in chromosomal rearrangements. *Genetics, 160*, 97–110.

Uppuluri, P., Chaturvedi, A. K., Srinivasan, A., Banerjee, M., Ramasubramaniam, A. K., Kohler, J. R., et al. (2010). Dispersion as an important step in the *Candida albicans* biofilm developmental cycle. *PLoS Pathogens, 6*, e1000828.

Uppuluri, P., Mekala, S., & Chaffin, W. L. (2007). Farnesol-mediated inhibition of *Candida albicans* yeast growth and rescue by a diacylglycerol analogue. *Yeast, 24*, 681–693.

Uppuluri, P., Pierce, C. G., Thomas, D. P., Bubeck, S. S., Saville, S. P., & Lopez-Ribot, J. L. (2010). The transcriptional regulator Nrg1p controls *Candida albicans* biofilm formation and dispersion. *Eukaryotic Cell, 9*, 1531–1537.

Urban, C., Xiong, X., Sohn, K., Schroppel, K., Brunner, H., & Rupp, S. (2005). The moonlighting protein Tsa1p is implicated in oxidative stress response and in cell wall biogenesis in *Candida albicans*. *Molecular Microbiology, 57*, 1318–1341.

Uwamahoro, N., Verma-Gaur, J., Shen, H. H., Qu, Y., Lewis, R., Lu, J., et al. (2014). The pathogen *Candida albicans* hijacks pyroptosis for escape from macrophages. *MBio, 5*, e00003–14.

Vandenbosch, D., Braeckmans, K., Nelis, H. J., & Coenye, T. (2010). Fungicidal activity of miconazole against *Candida* spp. biofilms. *Journal of Antimicrobial Chemotherapy, 65*, 694–700.

Vandeputte, P., Tronchin, G., Larcher, G., Ernoult, E., Berges, T., Chabasse, D., et al. (2008). A nonsense mutation in the *ERG6* gene leads to reduced susceptibility to polyenes in a clinical isolate of *Candida glabrata*. *Antimicrobial Agents and Chemotherapy, 52*, 3701–3709.

de Vasconcellos, A. A., Goncalves, L. M., Del Bel Cury, A. A., & da Silva, W. J. (2014). Environmental pH influences *Candida albicans* biofilms regarding its structure, virulence and susceptibility to fluconazole. *Microbial Pathogenesis, 69-70*, 39–44.

Vediyappan, G., Rossignol, T., & d'Enfert, C. (2010). Interaction of *Candida albicans* biofilms with antifungals: transcriptional response and binding of antifungals to beta-glucans. *Antimicrobial Agents and Chemotherapy, 54*, 2096–2111.

Vilchez, R., Lemme, A., Ballhausen, B., Thiel, V., Schulz, S., Jansen, R., et al. (2010). *Streptococcus mutans* inhibits *Candida albicans* hyphal formation by the fatty acid signaling molecule trans-2-decenoic acid (SDSF). *Chembiochem, 11*, 1552–1562.

Vinces, M. D., Haas, C., & Kumamoto, C. A. (2006). Expression of the *Candida albicans* morphogenesis regulator gene *CZF1* and its regulation by Efg1p and Czf1p. *Eukaryotic Cell, 5*, 825–835.

Virag, A., & Harris, S. D. (2006). The Spitzenkorper: a molecular perspective. *Mycological Research, 110*, 4–13.

Voigt, J., Hunniger, K., Bouzani, M., Jacobsen, I. D., Barz, D., Hube, B., et al. (2014). Human natural killer cells acting as phagocytes against *Candida albicans* and mounting an inflammatory response that modulates neutrophil antifungal activity. *Journal of Infectious Diseases, 209*, 616–626.

Vylkova, S., Carman, A. J., Danhof, H. A., Collette, J. R., Zhou, H., & Lorenz, M. C. (2011). The fungal pathogen *Candida albicans* autoinduces hyphal morphogenesis by raising extracellular pH. *MBio, 2*, e00055–11.

Vylkova, S., & Lorenz, M. C. (2014). Modulation of phagosomal pH by *Candida albicans* promotes hyphal morphogenesis and requires Stp2p, a regulator of amino acid transport. *PLoS Pathogens, 10*, e1003995.

Vylkova, S., Nayyar, N., Li, W., & Edgerton, M. (2007). Human beta-defensins kill *Candida albicans* in an energy-dependent and salt-sensitive manner without causing membrane disruption. *Antimicrobial Agents and Chemotherapy, 51*, 154–161.

Wachtler, B., Citiulo, F., Jablonowski, N., Forster, S., Dalle, F., Schaller, M., et al. (2012). *Candida albicans*-epithelial interactions: dissecting the roles of active penetration, induced endocytosis and host factors on the infection process. *PLoS One, 7*, e36952.

Wachtler, B., Wilson, D., Haedicke, K., Dalle, F., & Hube, B. (2011). From attachment to damage: defined genes of *Candida albicans* mediate adhesion, invasion and damage during interaction with oral epithelial cells. *PLoS One, 6*, e17046.

Wachtler, B., Wilson, D., & Hube, B. (2011). *Candida albicans* adhesion to and invasion and damage of vaginal epithelial cells: stage-specific inhibition by clotrimazole and bifonazole. *Antimicrobial Agents and Chemotherapy, 55*, 4436–4439.

Wagener, J., Malireddi, R. K., Lenardon, M. D., Koberle, M., Vautier, S., MacCallum, D. M., et al. (2014). Fungal chitin dampens inflammation through IL-10 induction mediated by NOD2 and TLR9 activation. *PLoS Pathogens, 10*, e1004050.

Wagener, J., Schneider, J. J., Baxmann, S., Kalbacher, H., Borelli, C., Nuding, S., et al. (2013). A peptide derived from the highly conserved protein GAPDH is involved in tissue protection by different antifungal strategies and epithelial immunomodulation. *Journal of Investigative Dermatology, 133*, 144–153.

Walker, L. A., Maccallum, D. M., Bertram, G., Gow, N. A., Odds, F. C., & Brown, A. J. (2009). Genome-wide analysis of *Candida albicans* gene expression patterns during infection of the mammalian kidney. *Fungal Genetics and Biology, 46*, 210–219.

Wang, L. H., He, Y., Gao, Y., Wu, J. E., Dong, Y. H., He, C., et al. (2004). A bacterial cell-cell communication signal with cross-kingdom structural analogues. *Molecular Microbiology, 51*, 903–912.

Wang, Y. (2013). Fungal adenylyl cyclase acts as a signal sensor and integrator and plays a central role in interaction with bacteria. *PLoS Pathogens, 9*, e1003612.

Wargo, M. J., & Hogan, D. A. (2006). Fungal–bacterial interactions: a mixed bag of mingling microbes. *Current Opinion in Microbiology, 9*, 359–364.

Watanabe, T., Takano, M., Murakami, M., Tanaka, H., Matsuhisa, A., Nakao, N., et al. (1999). Characterization of a haemolytic factor from *Candida albicans*. *Microbiology, 145*(Pt 3), 689–694.

Weber, K., Schulz, B., & Ruhnke, M. (2010). The quorum-sensing molecule E,E-farnesol—its variable secretion and its impact on the growth and metabolism of *Candida* species. *Yeast, 27*, 727–739.

Weber, K., Sohr, R., Schulz, B., Fleischhacker, M., & Ruhnke, M. (2008). Secretion of E,E-farnesol and biofilm formation in eight different *Candida* species. *Antimicrobial Agents and Chemotherapy, 52*, 1859–1861.

Weindl, G., Naglik, J. R., Kaesler, S., Biedermann, T., Hube, B., Korting, H. C., et al. (2007). Human epithelial cells establish direct antifungal defense through TLR4-mediated signaling. *Journal of Clinical Investigation, 117*, 3664–3672.

Weindl, G., Wagener, J., & Schaller, M. (2010). Epithelial cells and innate antifungal defense. *Journal of Dental Research, 89*, 666–675.

Weindl, G., Wagener, J., & Schaller, M. (2011). Interaction of the mucosal barrier with accessory immune cells during fungal infection. *International Journal of Medical Microbiology, 301*, 431–435.

Weissman, Z., & Kornitzer, D. (2004). A family of *Candida* cell surface haem-binding proteins involved in haemin and haemoglobin-iron utilization. *Molecular Microbiology, 53*, 1209–1220.

Weissman, Z., Shemer, R., Conibear, E., & Kornitzer, D. (2008). An endocytic mechanism for haemoglobin-iron acquisition in *Candida albicans*. *Molecular Microbiology, 69*, 201–217.

Wellington, M., Koselny, K., Sutterwala, F. S., & Krysan, D. J. (2014). *Candida albicans* triggers NLRP3-mediated pyroptosis in macrophages. *Eukaryotic Cell, 13*, 329–340.

Wenzel, R. P. (1995). Nosocomial candidemia: risk factors and attributable mortality. *Clinical Infectious Diseases, 20*, 1531–1534.

Westwater, C., Balish, E., & Schofield, D. A. (2005). *Candida albicans*-conditioned medium protects yeast cells from oxidative stress: a possible link between quorum sensing and oxidative stress resistance. *Eukaryotic Cell, 4*, 1654–1661.

Wheeler, R. T., & Fink, G. R. (2006). A drug-sensitive genetic network masks fungi from the immune system. *PLoS Pathogens, 2*, e35.

Wheeler, R. T., Kombe, D., Agarwala, S. D., & Fink, G. R. (2008). Dynamic, morphotype-specific *Candida albicans* beta-glucan exposure during infection and drug treatment. *PLoS Pathogens, 4*, e1000227.

White, S. J., Rosenbach, A., Lephart, P., Nguyen, D., Benjamin, A., Tzipori, S., et al. (2007). Self-regulation of *Candida albicans* population size during GI colonization. *PLoS Pathogens, 3*, e184.

White, T. C. (1997). Increased mRNA levels of *ERG16, CDR*, and *MDR1* correlate with increases in azole resistance in *Candida albicans* isolates from a patient infected with human immunodeficiency virus. *Antimicrobial Agents and Chemotherapy, 41*, 1482–1487.

Whiteway, M., & Oberholzer, U. (2004). *Candida* morphogenesis and host-pathogen interactions. *Current Opinion in Microbiology, 7*, 350–357.

Whitmore, S. E., & Lamont, R. J. (2011). The pathogenic persona of community-associated oral streptococci. *Molecular Microbiology, 81*, 305–314.

Whittington, A., Gow, N. R., & Hube, B. (2014). From commensal to pathogen: *Candida albicans*. In O. Kurzai (Ed.), *Human Fungal Pathogens* (Vol. 12, pp. 3–18). Springer Berlin Heidelberg.

Williams, P. (2007). Quorum sensing, communication and cross-kingdom signalling in the bacterial world. *Microbiology, 153*, 3923–3938.

Williams, P., & Camara, M. (2009). Quorum sensing and environmental adaptation in *Pseudomonas aeruginosa*: a tale of regulatory networks and multifunctional signal molecules. *Current Opinion in Microbiology, 12*, 182–191.

Wilson, D., Citiulo, F., & Hube, B. (2012). Zinc exploitation by pathogenic fungi. *PLoS Pathogens, 8*, e1003034.

Wilson, D., Hebecker, B., Moyes, D. L., Miramon, P., Jablonowski, N., Wisgott, S., et al. (2013). Clotrimazole dampens vaginal inflammation and neutrophil infiltration in response to *Candida albicans* infection. *Antimicrobial Agents and Chemotherapy, 57*, 5178–5180.

Wilson, D., Thewes, S., Zakikhany, K., Fradin, C., Albrecht, A., Almeida, R., et al. (2009). Identifying infection-associated genes of *Candida albicans* in the postgenomic era. *FEMS Yeast Research, 9*, 688–700.

van 't Wout, J. W., Linde, I., Leijh, P. C., & van Furth, R. (1988). Contribution of granulocytes and monocytes to resistance against experimental disseminated *Candida albicans* infection. *European Journal of Clinical Microbiology, 7*, 736–741.

Wright, C. J., Burns, L. H., Jack, A. A., Back, C. R., Dutton, L. C., Nobbs, A. H., et al. (2013). Microbial interactions in building of communities. *Molecular Oral Microbiology, 28*, 83–101.

Wu, W., Lockhart, S. R., Pujol, C., Srikantha, T., & Soll, D. R. (2007). Heterozygosity of genes on the sex chromosome regulates *Candida albicans* virulence. *Molecular Microbiology, 64*, 1587–1604.

Wu, W., Pujol, C., Lockhart, S. R., & Soll, D. R. (2005). Chromosome loss followed by duplication is the major mechanism of spontaneous mating-type locus homozygosis in *Candida albicans*. *Genetics, 169*, 1311–1327.

Wysong, D. R., Christin, L., Sugar, A. M., Robbins, P. W., & Diamond, R. D. (1998). Cloning and sequencing of a *Candida albicans* catalase gene and effects of disruption of this gene. *Infection and Immunity, 66*, 1953–1961.

Xie, Z., Thompson, A., Sobue, T., Kashleva, H., Xu, H., Vasilakos, J., et al. (2012). *Candida albicans* biofilms do not trigger reactive oxygen species and evade neutrophil killing. *Journal of Infectious Diseases, 206*, 1936–1945.

Xu, H., Jenkinson, H. F., & Dongari-Bagtzoglou, A. (2014). Innocent until proven guilty: mechanisms and roles of *Streptococcus-Candida* interactions in oral health and disease. *Molecular Oral Microbiology, 29*, 99–116.

Xu, H., Nobile, C. J., & Dongari-Bagtzoglou, A. (2013). Glucanase induces filamentation of the fungal pathogen *Candida albicans*. *PLoS One, 8*, e63736.

Xu, H., Sobue, T., Thompson, A., Xie, Z., Poon, K., Ricker, A., et al. (2014). Streptococcal co-infection augments *Candida* pathogenicity by amplifying the mucosal inflammatory response. *Cellular Microbiology, 16*, 214–231.

Xu, X. L., Lee, R. T., Fang, H. M., Wang, Y. M., Li, R., Zou, H., et al. (2008). Bacterial peptidoglycan triggers *Candida albicans* hyphal growth by directly activating the adenylyl cyclase Cyr1p. *Cell Host & Microbe, 4*, 28–39.

Yan, L., Yang, C., & Tang, J. (2013). Disruption of the intestinal mucosal barrier in *Candida albicans* infections. *Microbiological Research, 168*, 389–395.

Yang, F., Kravets, A., Bethlendy, G., Welle, S., & Rustchenko, E. (2013). Chromosome 5 monosomy of *Candida albicans* controls susceptibility to various toxic agents, including major antifungals. *Antimicrobial Agents and Chemotherapy, 57*, 5026–5036.

Yang, M., Brand, A., Srikantha, T., Daniels, K. J., Soll, D. R., & Gow, N. A. (2011). Fig1 facilitates calcium influx and localizes to membranes destined to undergo fusion during mating in *Candida albicans*. *Eukaryotic Cell, 10*, 435–444.

Yapar, N. (2014). Epidemiology and risk factors for invasive candidiasis. *Therapeutics and clinical risk management, 10*, 95–105.

Yi, S., Sahni, N., Daniels, K. J., Lu, K. L., Srikantha, T., Huang, G., et al. (2011). Alternative mating type configurations (a/alpha versus a/a or alpha/alpha) of *Candida albicans* result in alternative biofilms regulated by different pathways. *PLoS Biology, 9*, e1001117.

You, T., Ingram, P., Jacobsen, M. D., Cook, E., McDonagh, A., Thorne, T., et al. (2012). A systems biology analysis of long and short-term memories of osmotic stress adaptation in fungi. *BMC Research Notes, 5*, 258.

Yu, Q., Wang, H., Cheng, X., Xu, N., Ding, X., Xing, L., et al. (2012). Roles of Cch1 and Mid1 in morphogenesis, oxidative stress response and virulence in *Candida albicans*. *Mycopathologia, 174*, 359–369.

Zacchi, L. F., Gomez-Raja, J., & Davis, D. A. (2010). Mds3 regulates morphogenesis in *Candida albicans* through the TOR pathway. *Molecular and Cellular Biology, 30*, 3695–3710.

Zakikhany, K., Naglik, J. R., Schmidt-Westhausen, A., Holland, G., Schaller, M., & Hube, B. (2007). In vivo transcript profiling of *Candida albicans* identifies a gene essential for interepithelial dissemination. *Cellular Microbiology, 9*, 2938–2954.

Zarnowski, R., Westler, W. M., Lacmbouh, G. A., Marita, J. M., Bothe, J. R., Bernhardt, J., et al. (2014). Novel entries in a fungal biofilm matrix encyclopedia. *MBio, 5*, e01333–14.

Zeidler, U., Lettner, T., Lassnig, C., Muller, M., Lajko, R., Hintner, H., et al. (2009). *UME6* is a crucial downstream target of other transcriptional regulators of true hyphal development in *Candida albicans*. *FEMS Yeast Research, 9*, 126–142.

Zelante, T., Iannitti, R. G., De Luca, A., Arroyo, J., Blanco, N., Servillo, G., et al. (2012). Sensing of mammalian IL-17A regulates fungal adaptation and virulence. *Nature Communications, 3*, 683.

Zhang, L., Yan, L., Jiang, J., Wang, Y., Jiang, Y., Yan, T., et al. (2014). The structure and retrotransposition mechanism of LTR-retrotransposons in the asexual yeast *Candida albicans*. *Virulence, 5*, 655–664.

Zhao, X., Oh, S. H., Cheng, G., Green, C. B., Nuessen, J. A., Yeater, K., et al. (2004). *ALS3* and *ALS8* represent a single locus that encodes a *Candida albicans* adhesin; functional comparisons between Als3p and Als1p. *Microbiology, 150*, 2415–2428.

Zheng, X., Wang, Y., & Wang, Y. (2004). Hgc1, a novel hypha-specific G1 cyclin-related protein regulates *Candida albicans* hyphal morphogenesis. *The EMBO Journal, 23*, 1845–1856.

Zhu, C. X., Yan, L., Wang, X. J., Miao, Q., Li, X. X., Yang, F., et al. (2014). Transposition of the Zorro2 retrotransposon is activated by miconazole in *Candida albicans*. *Biological & Pharmaceutical Bulletin, 37*, 37–43.

Zhu, W., & Filler, S. G. (2010). Interactions of *Candida albicans* with epithelial cells. *Cellular Microbiology, 12*, 273–282.

Zhu, W., Phan, Q. T., Boontheung, P., Solis, N. V., Loo, J. A., & Filler, S. G. (2012). *EGFR* and *HER2* receptor kinase signaling mediate epithelial cell invasion by *Candida albicans* during oropharyngeal infection. *Proceedings of the National Academy of Sciences of the United States of America, 109*, 14194–14199.

Zordan, R. E., Galgoczy, D. J., & Johnson, A. D. (2006). Epigenetic properties of white-opaque switching in *Candida albicans* are based on a self-sustaining transcriptional feedback loop. *Proceedings of the National Academy of Sciences of the United States of America, 103*, 12807–12812.

Zordan, R. E., Miller, M. G., Galgoczy, D. J., Tuch, B. B., & Johnson, A. D. (2007). Interlocking transcriptional feedback loops control white-opaque switching in *Candida albicans*. *PLoS Biology, 5*, e256.

Zucchi, P. C., Davis, T. R., & Kumamoto, C. A. (2010). A *Candida albicans* cell wall-linked protein promotes invasive filamentation into semi-solid medium. *Molecular Microbiology, 76*, 733—748.

CHAPTER FOUR

Tailoring Specialized Metabolite Production in *Streptomyces*

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Contents

Abstract

Streptomycetes are prolific producers of a plethora of medically useful metabolites. These compounds are made by complex secondary (specialized) metabolic pathways, which utilize primary metabolic intermediates as building blocks. In this review we discuss the evolution of specialized metabolites and how expansion of gene families in primary metabolism has lead to the evolution of diversity in these specialized metabolic pathways and how developing a better understanding of expanded primary metabolic pathways can help enhance synthetic biology approaches to industrial pathway engineering.

You can know the name of a bird in all the languages of the world, but when you're finished, you'll know absolutely nothing whatever about the bird… So let's look at the bird and see what it's doing—that's what counts.

Richard P. Feynman

1. INTRODUCTION

The widespread use of antibiotics is a relatively new addition to human health care that emerged from the discovery of penicillin in 1928 by Alexander Fleming and its subsequent development to industrial scale production by

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Howard Florey and coworkers. This discovery of bioactive metabolites produced by microorganisms and useful in human health, prompted the so-called golden era of antibiotics. Between the 1950s and 1970s the search for new metabolites resulted in the delivery of many chemical classes of antibiotic, antifungal, antihelminthics, anticancer agents, and immunosuppressive drugs to market (Davies & Davies, 2010). During this intensive period of research the actinobacteria came to the fore as prolific producers of bioactive metabolites, particularly antibiotics from the genera *Streptomyces* and *Micromonospora* (Davies & Davies, 2010; Hoskisson, Hobbs, & Sharples, 2000). This initiated the widespread public perception that antibiotics were "wonder-drugs" that signaled the end of life-threatening bacterial infections, yet this was premature. Rapidly after the introduction of these drugs to the clinic, antibiotic resistance was observed (Abraham & Chain, 1940; Davies & Davies, 2010). This began a race to discover and bring to the clinic new antibiotics to help combat the emergence of resistance. Yet with the introduction of each new drug, rapid resistance was observed. This inevitable resistance, coupled to the rising costs of development, tightening of regulatory rules, diminishing discovery rates (and rediscovery of known compounds), and the lack of financial returns due to short-treatment durations resulted in the withdrawal of large pharmaceutical companies from large-scale antibiotic discovery in the 1990s (Projan, 2003). These problems coupled with profligate use of antibiotics in medicine and agriculture lead to the emergence of extensive antibiotic resistance on a global scale. In 2009, the World Health Organization raised concerns that the rise of antibiotic resistance was becoming one of the major threats to human health and that researchers, clinicians, industry, and policy makers needed to work together to address this multifactorial problem. These concerns lead to the release of a global action plan on antimicrobial resistance (http://www.who.int/drugresistance/en/).

Synthetic biology is a new methodology that can enable development of novel, clinically useful antibiotics. Recent advances in the technology of DNA synthesis, the ability to assemble longer tracts of DNA and at much reduced cost, provide the opportunity to design and improve biosynthetic gene clusters. Moreover this synthetic biology revolution has great potential to transform the more traditional disciplines of metabolic engineering, through creation of novel heterologous hosts with engineered precursor supply or deletion of native biosynthetic clusters to reduce wastage of precursors (Gomez-Escribano & Bibb, 2011, 2012) that may help overcome metabolic limitations.

In this review we will focus on primary metabolism in streptomycetes and how a genome-level understanding of the evolution of metabolic nodes

can aid the rational development of metabolic engineering targets for improved metabolite production.

2. INTERPLAY OF PRIMARY AND SECONDARY METABOLISM IN STREPTOMYCETES

The distinction of primary and secondary metabolism probably arose through the studies of Albrecht Kossel, who proposed that plants show two different metabolisms: "primary" and "secondary." Primary metabolism is common among all organisms and is composed of all essential reactions, whereas secondary metabolism is thought to be specialized, distinct, and comprised of species-specific pathways (Firn & Jones, 2009; Hartmann, 2008). This view has been widely adopted and adapted through many fields of biology and while the designations imply secondary metabolism is less important than primary metabolism, this view has been modified over the years, and it is implicit that secondary metabolism is dependent on supply of precursors from primary metabolism.

Disconnecting primary and secondary metabolism was challenged by Firn and Jones (2009) as misleading. This accords with the term "specialized metabolites" which may be a more useful term to replace the bias implied by the term "secondary"—indicating that this aspect has less importance (Davies, 2013; van Keulen & Dyson, 2014).

3. STREPTOMYCETES AS SPECIALIZED METABOLITE PRODUCERS

The actinobacterial phylum represents a large lineage of physiologically and morphologically diverse bacteria that includes the industrially, agriculturally, and medically important genera *Bifidobacterium*, *Corynebacterium*, *Mycobacterium*, *Nocardia*, *Leifsonia*, *Frankia*, and *Streptomyces* (Ventura et al., 2007). The most speciate genus is *Streptomyces* which are sporulating soil bacteria with a filamentous growth habit, characterized by large (>7 Mbp) high G + C-content, linear genomes. The streptomycete life cycle begins when a unigenomic spore germinates and grows through apical extension to form a mat of vegetative mycelia that, in response to nutrient-limitation produce aerial hyphae, which subsequently form septa and eventually form mature spores (Flärdh & Buttner, 2009). Specialized metabolites are produced at the onset of the developmental process and molecular studies have identified common regulators of both processes (Chandra & Chater, 2013). The

plethora of specialized metabolites produced by this group of organisms have a high degree of chemical diversity including classes such as polyketides, terpenes, lactams, aminoglycosides, and nonribosomal peptides with many species able to produce multiple examples of the same class of specialized metabolite (Bérdy, 2005). To emphasize this diversity, readers are directed to the StreptomeDB database (Lucas et al., 2013; http://www.pharmaceutical-bioinformatics.de/streptomedb/), where the structures of more than 2400 compounds from more than 1900 species are held. These estimates are increasing and will continue to increase exponentially along with the expansion of whole genome sequencing projects and specialized metabolite predictions software such as antiSMASH (Medema et al., 2011) and EvoMining (Cruz-Morales & Barona-Gómez Personal Communication). In addition, the sequences of about 7758 actinobacteria genomes are currently available in the Genomes Online Database (GOLD; Reddy et al., 2015) with the number continually increasing. This represents a vast resource for identification of biotechnologically useful genes and gene clusters. One particularly revealing actinobacterial genome feature that the next-generation sequencing revolution has opened our eyes to is the vast array of antibiotic (and other specialized metabolite) biosynthetic gene clusters present in the genomes of actinobacterial strains. This was first observed in well-studied species such as *Streptomyces coelicolor* for which, prior to the availability of the whole genome sequences, we knew of only four bioactive metabolites produced by the strain, yet the whole genome sequence revealed more than 20 specialized metabolite gene clusters in its repertoire, that are often referred to as cryptic or silent biosynthetic clusters (Bentley et al., 2002). This trend has continued with the release of each streptomycete genome. Significantly, the biosynthetic clusters that are cryptic or poorly expressed in their natural hosts offer great potential for the discovery of novel, clinically useful compounds. Moreover these gene clusters represent a significant resource of genes for synthetic biology to create novel metabolites through synthetic biology or semisynthesis, where existing compounds may be biosynthesized as a chemical backbone and then modified further through synthetic chemistry. Great advances have been made in this area in recent years (Wu, 2000). However semisynthetic derivatives of natural products are often limited by availability of starting material. Semisynthetic derivatives of erythromycin such as azithromycin require increasing amounts of the starter molecule yet the natural producing strains have been difficult to engineer to high production levels when compared to related species (Wu et al., 2011). Given that specialized metabolites, such as

antibiotics, are derived from primary metabolic starter units, a deeper understanding of how these precursors are synthesized and channeled into the biosynthesis of these specialized metabolites offers great potential in metabolic engineering and manipulation through synthetic biology approaches for the development of novel compounds. Many specialized metabolites have acetyl-CoA, malonyl-CoA (Olano, Méndez, & Salas, 2010), or amino acids as direct precursors (Stirrett, Denoya, & Westpheling, 2009). Competition for the same precursors between central metabolism and specialized metabolism may represent key conflicts in supply of precursors such as in fatty acid metabolism and polyketide synthesis (Rodriguez, Navone, Casati, & Gramajo, 2012). Given these issues, a better understanding of precursor supply may enable increased production of the poorly understood cryptic/silent biosynthetic clusters.

Therefore there are several challenges facing researchers in this field, such as how to awaken or enhance production of the array of cryptic gene clusters emerging from whole genome sequencing projects and how we can understand better the links between primary and secondary metabolism so that we can increase metabolite flow to maximize industrial yield of new and existing medically useful compounds.

4. EVOLUTION OF PRIMARY AND SPECIALIZED METABOLISM

Primary metabolism refers to the core pathways of central metabolism that provides building blocks for all the cellular macromolecules including DNA, RNA, proteins, lipids/fatty acids, etc., and also provides the precursors for specialized metabolites. Surprisingly many primary metabolic genes are nonessential for survival due to genetic redundancy providing isoenzymes or alternative reactions that allow the cell to adapt to changing environmental conditions providing an adaptive robustness to metabolism (Kim & Copley, 2007). The use of the term "redundancy" may be misleading as it suggests nonessentially. However, there may be multiple routes to a metabolic intermediate under a given set of environmental conditions. Therefore "contingency," "metabolic flexibility," or "enzyme expansion" may be more suitable terms to reflect this phenomenon (Challis & Hopwood, 2003; Noda-García & Barona-Gómez, 2013; Treangen & Rocha, 2011). Such terms would also account for the so-called "moonlighting" enzyme functions and catalytic promiscuity where the main catalytic function is supplemented by the catalysis of additional reactions (Copley, 2003, 2012,

2014, 2015). The main reason for these promiscuous activities is that evolution of the "perfect" catalytic site is difficult and natural selection acts upon those that provide a "good enough" functionality (Copley, 2015). This indicates that such accidental catalysis may shift fitness effects to other cellular functions and can aid in the generation of new pathways and chemistry within cells, such as specialized metabolism.

An interesting feature of streptomycete genomes is that multiple genes are often annotated to code for the same biochemical function in central carbon metabolism (Bentley et al., 2002; Hiltner & Hoskisson, unpublished; Figure 1). Understanding the relationship between function and evolution is a key to elucidating metabolic plasticity and exploiting these traits in biotechnology. In metabolic models these homologous functions are often combined into one flux pathway, yet this does not reflect the nuances of regulation and allostery for each gene product. However, knowledge of regulation and allostery is necessary to understand the roles of these gene expansions to fully appreciate functionality at the biochemical level. These redundant functions are hypothesized to allow cellular flexibility and adaptation in dynamic environments. Interestingly, given that streptomycetes exhibit so many of these gene expansions it may also, in part, reflect their ability to produce such a vast variety of specialized metabolites, as these pathways are usually connected to core metabolism and use common intermediates. There are well-studied examples of such gene family expansions in streptomycete developmental genes and DNA-binding protein families (Clark & Hoskisson, 2011; Girard et al., 2013). However, little attention has been paid to central metabolism. An example of gene expansion and evolution relating to specialized metabolites is that of polyketide biosynthesis for which the multimodular and iterative polyketide synthases that perform the stepwise condensation of activated carboxylic acid subunits provide the carbon backbones for the polyketides. It appears that these pathways have evolved from fatty acid synthases (Jenke-Kodama, 2005). This premise is based on the presence of highly conserved modules such as the ketoacyl synthase domains and acyl carrier proteins which through duplications, deletion, and horizontal gene transfer (HGT) have lead to the diverse chemistry array of polyketide chemistry observed today (Jenke-Kodama, 2005; Ridley, Lee, & Khosla, 2008).

The nature of these gene expansions needs to be carefully considered as it is generally assumed that these expansions arise through gene duplications. However, little consideration has been paid to the role of HGT in metabolic gene expansion (Noda-García & Barona-Gómez, 2013; Noda-García et al.,

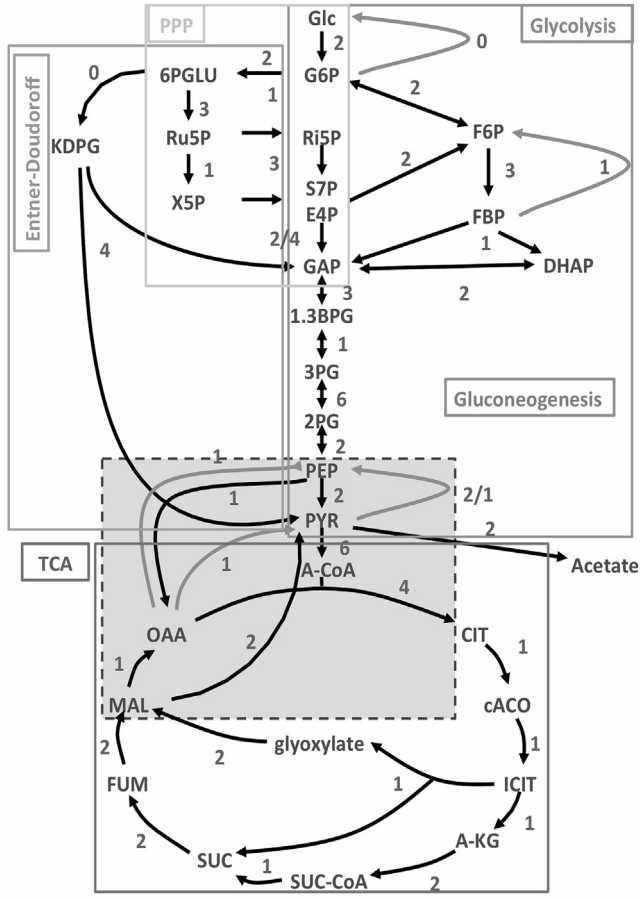

Figure 1 ***Schematic overview with main metabolites of the central carbon metabolism grouped according to pathway.*** Numbers indicate number of genes predicted in the genome of *Streptomyces coelicolor*. Glc, glucose; G6P, glucose-6-phosphate; 6-PGLU, 6-phosphogluconate; Ru5P, ribulose-5-phosphate; X5P, xylose-5-phosphate; KDPG, 2-keto-3-deoxy-6-phosphogluconate; F6P, fructose-6-phosphate; FBP, fructose 1,6-bisphosphate; DHAP, dihydroxyacetone phosphate; Ri5P, ribose-5-phosphate; S7P, seduheptulose-7-phosphate; E4P, erythrose-4-phosphate; GAP, glyceraldehyd-3-phosphate; 1.3BGP, 1.3-bisphosphoglycerate; 3 PG, 3-phosphoglycerate; 2 PG, 2-phosphoglycerate; PEP, phosphoenolpyruvate; PYR, pyruvate; ACoA, acetyl-CoA; Cit, citrate; cAco cisAconitate; ICit, isocitrate; A-KG, alpha-ketoglutarate (2-oxoglutarate); SucCoA, succinyl-CoA; Suc, succinate; Fum, fumarate; Mal, malate; OAA, oxaloacetate.

2013). This reflects the idea that gene duplication is an important source of biological innovation, where orthologous genes exhibit conserved functionality, and paralogs tend toward diverged function. However, integrating the role of HGT in this process requires sophisticated tools to identify HGT

events in metabolic genes, coupled with thorough studies of enzyme function to tease out detailed mechanisms. Recently, Noda-Garcia (Noda-García et al., 2013) showed that horizontally acquired metabolic genes could drive evolution of existing metabolic function through altered substrate specificity, and this may reflect a common, but under appreciated mechanism for enzyme expansion in prokaryotic genomes.

This is a developing area of interest that requires integration of a range of techniques (genomics, molecular genetics, biochemistry, X-ray crystallography, molecular dynamics simulations, and evolutionary modeling), but offers great potential for deep understanding of evolution of enzyme function and how this has contributed to metabolic plasticity. Ultimately studies such as these can be valuable to inform metabolic engineering for biotechnology.

5. THE PEP-PYR-OAA NODE AS TARGET FOR METABOLIC ENGINEERING

The phosphoenolpyruvate-pyruvate-oxaloacetate node is a major branch point within central carbon metabolism of all organisms that acts as a connection point for glycolysis, gluconeogenesis, and the TCA cycle (Figure 2). Many key precursors for specialized metabolites are derived from the node or pathways are limited by flux through it. Yet it is surprisingly diverse among bacteria. Sauer and Eikmanns (2005) examined this node in *Escherichia coli*, *Corynebacterium glutamicum*, and *Bacillus subtilis* in their roles as major workhorses for the industrial production of bulk chemicals such as amino acids, organic acids, or proteins. These authors concluded that this node of metabolism is a key target for metabolic engineering in bacteria.

This node represents a major flux distribution point for carbon skeletons in the cell with the key metabolites being phosphoenolpyruvate (PEP), pyruvate (PYR), and oxaloacetate (OAA). The reactions that interconvert each of these substrates are listed in Table 1. Pyruvate can be metabolized further into either OAA or acetyl-CoA, the former being a precursor for amino acids such as aspartate, lysine, methionine, threonine, and isoleucine and the latter for fatty acids and or polyketides. Across a range of species the architecture of this node can be highly variable and likely reflects the ecology of individual organisms. Here we will focus mainly on *Streptomyces* and the related actinobacterium, *Corynebacterium*, which with only one copy of each gene per reaction has a much reduced gene expansion at this node when compared to *Streptomyces* (Table 2).

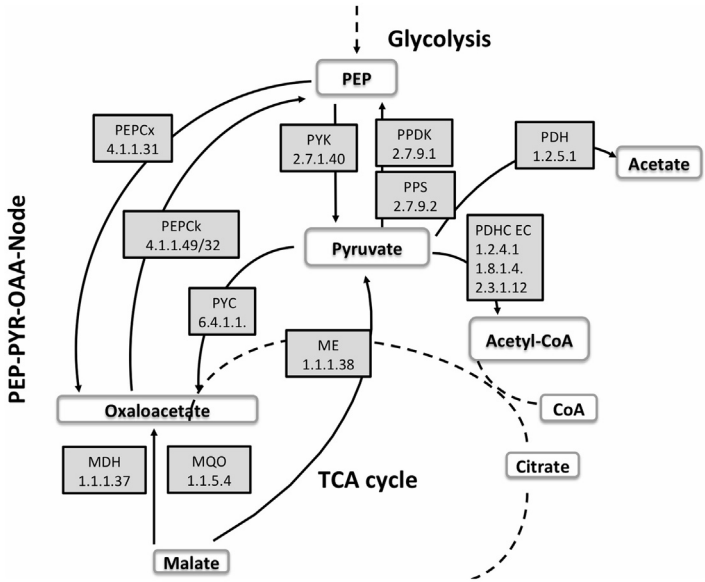

Figure 2 General overview of the reactions that form the phosphoenolpyruvate-pyruvate-oxaloacetate node of central carbon metabolism including the EC numbers of the enzyme responsible for each reaction.

It is also known that manipulating primary metabolic pathways in the PEP-PYR-OAA node can lead to higher product yields in a range of bacterial species. In *C. glutamicum*, a well-studied amino acid producing organism, it is well established that this node is crucial for amino acid production. Much can be learned from studying the PEP-PYR-OAA node in this organism in terms of how the basal node functions. PEP carboxylase (PEPC) is not essential for lysine production and has no effect on the growth rate when deleted. However, a mutant lacking both PEPC and pyruvate kinase (Pyk) has decreased growth rates as well as reduced rates of lysine production rates (Koffas & Stephanopoulos, 2005). Moreover, inducing low expression levels of *aceE* (E1 subunit of the pyruvate dehydrogenase complex (PDHC)) through promoter exchange and deletion of *pqo* (pyruvate:quinone oxidoreductase) and *ppc* (PEP carboxylase) leads to an increase in L-valine production (Buchholz et al., 2013). It was also shown that L-lysine production could be increased by reducing PDHC levels indicating that this enzyme activity levels has an important influence on the carbon flux and can increase pyruvate-derived molecules (Blombach, Arndt, Auchter, & Eikmanns, 2009; Buchholz et al., 2013; Eikmanns & Blombach, 2014). The PEPCk gene in

Table 1 Reactions of the PEP-PYR-OAA node including their name, abbreviations in the text, EC numbers, and the reactions they catalyze

Enzyme	Code	EC number	Reaction catalyzed	Part of minimal set of genes
PEP carboxylase	PEPCx	4.1.1.31	H_2O + PEP + CO_2 <-> PO_4^{3-} + OAA	No
PEP carboxykinase	PEPCk	4.1.1.32	GTP + OAA <-> GDP + PEP + CO_2	Yes
Malic enzyme	ME	1.1.1.38	(S)-MAL + NAD^+ <-> PYR + CO_2 + NADH + H^+ OAA <-> PYR + CO_2	Yes
Malate dehydrogenase (quinone)	MQO	1.1.5.4	(S)-MAL + a quinone <-> OAA + reduced quinone	No
Malate dehydrogenase	MDH	1.1.1.37	(S)-MAL + NAD^+ <-> OAA + NADH + H^+	Yes
Pyruvate carboxylase	PYC	6.4.1.1	ATP + PYR + HCO^{3-} <-> ADP + PO_4^{3-} + OAA	No
Pyruvate kinase (pyk)	PYK	2.7.1.40	ADP + PEP <-> PYR + ATP	Yes
Pyruvate phosphate dikinase (PPDK)	PPDK	2.7.9.1	ATP + PYR + P <-> AMP + PEP + diP	No
PEP synthase (PPS)	PPS	2.7.9.2	ATP + PYR + P + H_2O <-> AMP + PEP + diP	No
Pyruvate dehydrogenase	PDH	1.2.5.1	PYR + ubiquinone + H_2O <-> ACE + ubiquinol + CO_2	No
Pyruvate dehydrogenase complex	PDHC E1	1.2.4.1	PYR + ThdP <-> HeThdP + CO_2 HeThdP + lipoamide-E <-> S-acetyldihydrolipoamide-E + ThdP	Yes
Pyruvate dehydrogenase complex	PDHC E2	2.3.1.12	CoA + S-acetyldihydrolipoamide-E <-> acetyl-CoA + lipoamide-E	
Pyruvate dehydrogenase complex	PDHC E3	1.8.1.4	PYR + CoA + NAD^+ <-> acetyl-CoA + CO_2 + NADH + H^+	

ACE, acetate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; ATP, adenosine triphosphate; GDP, guanosine diphosphate; GTP, guanosine triphosphate; HeThdP, 2-(alpha-hydroxyethyl)thiamine diphosphate; MAL, malate; NAD, nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; OAA, oxaloacetate; PEP, phosphoenolpyruvate; PYR, pyruvate; ThdP, thiamin diphosphate.

Table 2 Comparison of PEP-PYR-OAA node expansion in a range of bacteria, indicating the number of homologues indicating the level of gene expansion at each point. The nucleotide cofactor is indicated where appropriate

	Streptomyces coelicolor	***Streptomyces rimosus***	***Saccharopolyspora erythraea***	***Streptomyces tsukubaensis***	***Corynebacterium glutamicum***	***Escherichia coli***	***Bacillus subtilis***
Pyk	2	2	1	2	1	2	1
PEPCx	1	1	0	1	1	1	0
PEPCk	1 GTP	1 GTP	1 GTP	1 GTP	1 GTP	1 ATP	1 ATP
Pyc	1	0	1	0	1	0	0
ME	2	1	2	2	1	1	4
mdh	1	1	1	1	1	1	1
mqo	0	0	1	0	1	1	0
PEP synthase	1	1	3	0	1	1	2
PPDK	2	2	0	1	1	0	0
PDH	2	1	2	1	1	1	0
PDHC	≥6 E1	≥3 E1	≥6 E1	≥3 E1	1	1	4

Mdh, malate dehydrogenase; ME, malic enzyme; Mqo, malate dehydrogenase (quinone); PDH, pyruvate dehydrogenase; PDHC, pyruvate dehydrogenase complex; PEPCk, PEP carboxykinase; PEPCx, PEP carboxylase; PPDK, pyruvate phosphate dikinase; Pyc, pyruvate carboxylase; Pyk, pyruvate kinase.

C. glutamicum also influences the production of glutamate and lysine; inactivation leads to an increase in production whereas overexpression decreases production (Riedel et al., 2001). This indicates that blocking certain routes within metabolism can increase flux in diverse pathways. Disrupting these processes can also be mediated through mutating the transcriptional regulators. For example, deletion of *pckR* (Cg0196), a negative repressor of PEPCk during growth on glucose results in cellular PEPCk activity even in the presence of glucose (Hyeon, Kang, Kim, You, & Han, 2012).

Overexpression of pyruvate carboxylase (*pyc*) in *C. glutamicum* results in increased PCx activity and glutamate production in an optimized lysine-producing strain. However, inactivation of *pyc* resulted in no PCx activity and lower levels of glutamate production levels (Peters-Wendisch et al., 1998). A similar effect was observed for threonine production and its precursor homoserine, pointing toward the importance of Pyc and PCx in amino acid metabolism and industrial production (Peters-Wendisch et al., 2001). In a lysine overproducing strain, deletion of pyruvate kinase (*pyk*) resulted in similar growth rates but higher rates of overflow metabolites such as dihydroxyacetone and glycerol as well as a shift from pyruvate carboxylase to phosphoenolpyruvate carboxylase flux during glucose utilization (Becker, Klopprogge, & Wittmann, 2008) which enabled a metabolic bypass via malic enzyme to account for deletion of *pyk*. This highlights the level of metabolic flexibility that this node provides in central carbon metabolism.

The Gram-negative bacterium *E. coli* has two genes encoding pyruvate kinase; *pykA* and *pykE* (Muñoz & Ponce, 2003). Disruption of a single pyruvate kinase (*pykA*) in a phenylalanine overproducing strain in combination with inactivation of a Phosphotransferase system (PTS) sugar transporter, leads to redistribution of cellular carbon flux when glucose was the substrate. Inactivation of PTS and PykA (which is allosterically regulated by AMP) resulted in decreased PCx, PEPCk, and TCA cycle activities. Inactivation of PTS and PykF (which is allosterically regulated by fructose 1,6 biphosphate) resulted in increased OAA formation from PEP and flux through the TCA cycle. Interestingly, both strains showed increased production of phenylalanine compared to the parental strain (Meza, Becker, Bolivar, Gosset, & Wittmann, 2012). The enlargement of this part of the PEP-PYR-OAA node in *E. coli* compared to *C. glutamicum* and the results reported by Meza (Meza et al., 2012) indicate that dissection of these aspects of enzyme expansion in metabolism is difficult. In the streptomycetes, this is especially true when up to six copies of some enzymes within the PEP-PYR-OAA node are present (Figure 2 and Table 2).

The PEP-PYR-OAA node is poorly understood in streptomycetes. However, some primary metabolic enzymes that have undergone gene expansion in *Streptomyces* have been studied in more detail. One such example is the presence of three copies of the glycolytic enzyme phosphofructokinase (SCO1214—*pfkA*3; SCO2119—*pfkA*1; and SCO5426—*PfkA*2) catalyzing the addition of a second phosphate to fructose-6-phosphate at the C1 position. Deletion of *pfkA*2 leads to an increase of undecylprodigiosin and actinorhodin production on certain media, whereas deletion of either *pfkA*1 or *pfkA*3 does not show the same phenotype. The *pfkA*2 mutant also had higher intracellular pools of glucose-6-phosphate and fructose-6-phosphate and radiolabeling experiments indicated increased flux through the pentose phosphate pathway (PPP) and concomitant increased levels of reduced nicotinamide adenine dinucleotide phosphate (NADPH) showing that despite having three genes encoding the same function they have different physiological roles (Borodina et al., 2008). These data also correlate with higher production of methylenomycin and increased PPP flux during slow growth (Obanye, Hobbs, Gardner, & Oliver, 1996). In *Streptomyces clavuligerus*, two glyceraldehyde-3-phosphate dehydrogenase (GAPDH) genes are present in the genome, *gap*1 and *gap*2. Disruption of *gap*1 leads to an increase in clavulanic acid production, but not when *gap*2 is deleted. Since clavulanic acid biosynthesis starts with the condensation of L-arginine and glyceraldehyde-3-phosphate (G3P), this downstream block of *gap*1 appears to redirect flux toward clavulanic acid biosynthesis rather than glycolysis. Furthermore it demonstrates the different physiological roles played by the different isoforms of GAPDH (Li & Townsend, 2006). In the model streptomycete *S. coelicolor* disruption of either of the genes encoding the two isoforms of glucose-6-phosphate dehydrogenase *zwf*1 (SCO6661) and *zwf*2 (SCO1937) leads to changes in the production of the polyketide actinorhodin. Again, two genes encoding for the same function seem to play different roles. It appears that *zwf*2 plays a more important role than *zwf*1 for directing the flux toward actinorhodin production (Butler et al., 2002; Ryu, Butler, Chater, & Lee, 2006). Similarly it has also been shown that disruption of *zwf* in *Streptomyces lividans*, which also has two copies results in higher production of actinorhodin and undecylprodigiosin production (Butler et al., 2002). Further studies in chemostat culture of *S. lividans* indicate that the flux of carbon was dependent on growth rate and the carbon source (Avignone Rossa et al., 2002). When gluconate was utilized as carbon source a higher flux through PPP was observed than on glucose

and a decline in secondary metabolite production resulted when growth rate was increased (Avignone Rossa et al., 2002).

Within the PEP-PYR-OAA node of *Streptomyces* only two genes have been studied in detail—PEPCx and the malic enzymes from *Streptomyces coelicolor*. The activity of this enzyme increased during biosynthesis of actinorhodin and overexpression of PEPCx in *Streptomyces lividans* reduced the growth rate of the strain, delaying actinorhodin biosynthesis (Bramwell, Nimmo, Hunter, & Coggins, 1993). The study of the two malic enzymes SCO2951 and SCO5261 revealed that the mutant in SCO2951 and the double mutant show decreased actinorhodin production, due to a decrease of expression of *actIIORF4* and the double mutant also has decreased triacylglycerol storage levels during exponential growth (Rodriguez et al., 2012). These data indicate the importance of this node to specialized metabolite production, especially polyketides, and highlight the potential for further investigation.

Recent global metabolomics studies in *Streptomyces* have also revealed key findings about the metabolic flexibility at the PEP-PYR-OAA node at onset of specialized metabolite production. Studies conducted during phosphate and L-glutamate depletion indicate that most changes in the global metabolite pool (metabolome) were in amino acid and organic acid levels (Wentzel, Sletta, Consortium, Ellingsen, & Bruheim, 2012). During phosphate depletion the amino acid pools of histidine, phenylalanine, tyrosine, alanine, valine, leucine, glycine, proline, isoleucine, and lysine were increased in addition to the intracellular pools of succinate and ornithine pools. Intracellular pools of glutamate and aspartate were both reduced as was pyruvate, citrate, 2-oxoglutarate, fumarate, and malate. Under glutamate depletion the pools of histidine, phenylalanine, tyrosine, alanine, valine, leucine, glycine, proline, and lysine decreased initially before recovering at around 40 h. This was also observed for citrate and succinate. Pyruvate, 2-oxoglutarate, fumarate, malate, and ornithine were all reduced in addition to the amino acids glutamate, glutamine, and aspartate (Wentzel et al., 2012). These data confirm the central role for pyruvate in balancing central carbon metabolism during growth and reinforces our understanding of key branch points in specialized metabolite production. An additional study by this group to develop cultivation media for studying metabolic shifts tested a wide range of carbon sources (arabinose, alanine, aspartate, glucose/glutamate, glucose, glutamate, proline, Tween 20, Tween 40, Tween 60, Tween 80, and xylose) and examined expression of the PDHC genes. Only one of the genes (SCO2183) showed decreased

expression on Tween and alanine. SCO2181, SCO2180, and SCO4919 had increased expression, yet under all other conditions no expression differences were observed, which may suggest that the gene products of the PDHC may act as a metabolic bottleneck during glucose growth (Wentzel et al., 2012). Furthermore, studies of carbon preferences using ^{13}C-glucose to label metabolites during culture on glucose and glutamate as a mixed carbon source, indicate that during rapid growth glycolysis and PPP are enriched for ^{13}C compounds, but upon cessation of growth ^{13}C-labeled PPP intermediates decreased. The TCA cycle is generally low in ^{13}C-labeled intermediates indicating that glutamate is a preferred carbon source, being catabolized via 2-oxoglutarate following deamination and release of ammonium ions. Interestingly 2-oxoglutarate can be decarboxylated in the TCA cycle to form malate and further decarboxylated to pyruvate which can be converted to acetyl-CoA by PDHC. Acetyl-CoA is an important precursor for fatty acids and polyketides. Clearly glutamate is the preferred carbon source providing the main cellular carbon and with glucose playing an ancillary role (Wentzel et al., 2012). Interestingly, it is known that *Streptomyces* secrete pyruvate and 2-oxoglutarate during growth under certain conditions, prior to specialized metabolite production (Hobbs et al., 1992), which may reflect the inefficiency of formation of acetyl-CoA from pyruvate under some physiological conditions. PDHC expansion in streptomycetes may be an evolutionary solution to this phenomenon. These natural examples may provide a framework for metabolic engineering strategies.

6. CONCLUDING REMARKS

Detailed insight into the evolution, regulation, and biochemistry of primary metabolism and how this feeds into specialized metabolism will help us understand better complex biological systems and will allow targeting key points in metabolism for metabolic engineering. While global "omics" studies and modeling can help with general phenomena, a full understanding of gene function in a classical reductionist manner is the only way to gain true biological insight. These approaches guide synthetic biology strategies for strain and pathway construction and increased production of medically useful metabolites. There are key points within central metabolism that represent excellent metabolic engineering targets, such as the PEP-PYR-OAA node, which are particularly well suited

for engineering metabolite production and will make it more efficient and easier to enhance industrial production processes.

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REFERENCES

Abraham, E. P., & Chain, E. (1940). An enzyme from bacteria able to destroy penicillin. *Reviews of Infectious Diseases, 10*, 677–678.

Avignone Rossa, C., White, J., Kuiper, A., Postma, P. W., Bibb, M., & Teixeira de Mattos, M. J. (2002). Carbon flux distribution in antibiotic-producing chemostat cultures of *Streptomyces lividans*. *Metabolic Engineering, 4*, 138–150.

Becker, J., Klopprogge, C., & Wittmann, C. (2008). Metabolic responses to pyruvate kinase deletion in lysine producing *Corynebacterium glutamicum*. *Microbial Cell Factories, 7*, 8.

Bentley, S. D., Chater, K. F., Cerdeno-Tarraga, A. M., Challis, G. L., Thomson, N. R., James, K. D., et al. (2002). Complete genome sequence of the model actinomycete *Streptomyces coelicolor* A3(2). *Nature, 417*, 141–147.

Bérdy, J. (2005). Bioactive Microbial Metabolites. *The Journal of Antibiotics, 58*(1), 1–26.

Blombach, B., Arndt, A., Auchter, M., & Eikmanns, B. J. (2009). L-valine production during growth of pyruvate dehydrogenase complex-deficient *Corynebacterium glutamicum* in the presence of ethanol or by inactivation of the transcriptional regulator SugR. *Applied and Environmental Microbiology, 75*, 1197–1200.

Borodina, I., Siebring, J., Zhang, J., Smith, C. P., van Keulen, G., Dijkhuizen, L., et al. (2008). Antibiotic overproduction in *Streptomyces coelicolor* A3(2) mediated by phosphofructokinase deletion. *The Journal of Biological Chemistry, 283*, 25186–25199.

Bramwell, H., Nimmo, H. G., Hunter, I. S., & Coggins, J. R. (1993). Phosphoenolpyruvate carboxylase from *Streptomyces coelicolor* A3(2): purification of the enzyme, cloning of the ppc gene and over-expression of the protein in a streptomycete. *Biochemical Journal, 293*(Pt 1), 131–136.

Buchholz, J., Schwentner, A., Brunnenkan, B., Gabris, C., Grimm, S., Gerstmeir, R., et al. (2013). Platform engineering of *Corynebacterium glutamicum* with reduced pyruvate dehydrogenase complex activity for improved production of l-lysine, l-valine, and 2-ketoisovalerate. *Applied and Environmental Microbiology, 79*, 5566–5575.

Butler, M. J., Bruheim, P., Jovetic, S., Marinelli, F., Postma, P. W., & Bibb, M. J. (2002). Engineering of primary carbon metabolism for improved antibiotic production in *Streptomyces lividans*. *Applied and Environmental Microbiology, 68*, 4731–4739.

Challis, G. L., & Hopwood, D. A. (2003). Synergy and contingency as driving forces for the evolution of multiple secondary metabolite production by *Streptomyces* species. *Proceedings of the National Academy of Sciences USA, 100*(Suppl. 2), 14555–14561.

Chandra, G., & Chater, K. F. (2013). Developmental biology of *Streptomyces* from the perspective of 100 actinobacterial genome sequences. *FEMS Microbiology Reviews, 38*. n/a–n/a.

Clark, L. C., & Hoskisson, P. A. (2011). Duplication and evolution of devA-like genes in *Streptomyces* has resulted in distinct developmental roles. *PLoS One, 6*, e25049.
Copley, S. D. (2003). Enzymes with extra talents: moonlighting functions and catalytic promiscuity. *Current Opinion in Chemical Biology, 7*, 265–272.
Copley, S. D. (2012). Moonlighting is mainstream: paradigm adjustment required. *Bioessays, 34*, 578–588.
Copley, S. D. (2014). An evolutionary perspective on protein moonlighting. *Biochemical Society Transactions, 42*, 1684–1691.
Copley, S. D. (2015). *An evolutionary biochemist's perspective on promiscuity 1*–7. Elsevier Ltd.
Davies, J. (2013). Specialized microbial metabolites: functions and origins. *The Journal of Antibiotics, 66*, 361–364.
Davies, J., & Davies, D. (2010). Origins and evolution of antibiotic resistance. *Microbiology and Molecular Biology Reviews, 74*, 417–433.
Eikmanns, B. J., & Blombach, B. (2014). The pyruvate dehydrogenase complex of *Corynebacterium glutamicum*: an attractive target for metabolic engineering. *Journal of Biotechnology, 192*(Pt B), 339–345.
Firn, R. D., & Jones, C. G. (2009). A Darwinian view of metabolism: molecular properties determine fitness. *Journal of Experimental Botany, 60*, 719–726.
Flärdh, K., & Buttner, M. J. (2009). *Streptomyces* morphogenetics: dissecting differentiation in a filamentous bacterium. *Nature Reviews Microbiology, 7*, 36–49.
Girard, G., Traag, B. A., Sangal, V., Mascini, N., Hoskisson, P. A., Goodfellow, M., et al. (2013). A novel taxonomic marker that discriminates between morphologically complex actinomycetes. *Open Biology, 3*, 130073.
Gomez-Escribano, J. P., & Bibb, M. J. (2011). Engineering *Streptomyces coelicolor* for heterologous expression of secondary metabolite gene clusters. *Microbial Biotechnology, 4*, 207–215.
Gomez-Escribano, J. P., & Bibb, M. J. (2012). *Streptomyces coelicolor* as an expression host for heterologous gene clusters. *Methods in Enzymology, 517*, 279–300.
Hartmann, T. (2008). The lost origin of chemical ecology in the late 19th century. *Proceedings of the National Academy of Sciences USA, 105*(12), 4541–4546.
Hobbs, G., Obanye, A. I., Petty, J., Mason, J. C., Barratt, E., Gardner, D. C., et al. (1992). An integrated approach to studying regulation of production of the antibiotic methylenomycin by *Streptomyces coelicolor* A3(2). *Journal of Bacteriology, 174*, 1487–1494.
Hoskisson, P. A., Hobbs, G., & Sharples, G. P. (2000). Response of *Micromonospora echinospora* (NCIMB 12744) spores to heat treatment with evidence of a heat activation phenomenon. *Letters in Applied Microbiology, 30*, 114–117.
Hyeon, J. E., Kang, D. H., Kim, Y. I., You, S. K., & Han, S. O. (2012). GntR-type transcriptional regulator PckR negatively regulates the expression of phosphoenolpyruvate carboxykinase in *Corynebacterium glutamicum*. *Journal of Bacteriology, 194*, 2181–2188.
Jenke-Kodama, H. (2005). Evolutionary implications of bacterial polyketide synthases. *Molecular Biology and Evolution, 22*, 2027–2039.
van Keulen, G., & Dyson, P. J. (2014). Production of specialized metabolites by *Streptomyces coelicolor* A3(2). *Advances in Applied Microbiology, 89*, 217–266.
Kim, J., & Copley, S. D. (2007). Why metabolic enzymes are essential or nonessential for growth of *Escherichia coli* K12 on glucose. *Biochemistry, 46*, 12501–12511.
Koffas, M., & Stephanopoulos, G. (2005). Strain improvement by metabolic engineering: lysine production as a case study for systems biology. *Current Opinion in Biotechnology, 16*, 361–366.
Li, R., & Townsend, C. A. (2006). Rational strain improvement for enhanced clavulanic acid production by genetic engineering of the glycolytic pathway in *Streptomyces clavuligerus*. *Metabolic Engineering, 8*, 240–252.

Lucas, X., Senger, C., Erxleben, A., Grüning, B. A., Döring, K., Mosch, J., et al. (2013). StreptomeDB: a resource for natural compounds isolated from *Streptomyces* species. *Nucleic Acids Research, 41*, D1130−D1136.

Medema, M. H., Blin, K., Cimermancic, P., de Jager, V., Zakrzewski, P., Fischbach, M. A., et al. (2011). antiSMASH: rapid identification, annotation and analysis of secondary metabolite biosynthesis gene clusters in bacterial and fungal genome sequences. *Nucleic Acids Research, 39*, W339−W346.

Meza, E., Becker, J., Bolivar, F., Gosset, G., & Wittmann, C. (2012). Consequences of phosphoenolpyruvate:sugar phosphotranferase system and pyruvate kinase isozymes inactivation in central carbon metabolism flux distribution in *Escherichia coli*. *Microbial Cell Factories, 11*, 127.

Muñoz, E., & Ponce, E. (2003). Pyruvate kinase: current status of regulatory and functional properties. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 135*, 197−218. Elsevier.

Noda-García, L., & Barona-Gómez, F. (2013). Enzyme evolution beyond gene duplication: a model for incorporating horizontal gene transfer. *Mobile Genetic Elements, 3*, e26439.

Noda-García, L., Camacho-Zarco, A. R., Medina-Ruíz, S., Gaytán, P., Carrillo-Tripp, M., Fülöp, V., et al. (2013). Evolution of substrate specificity in a recipient's enzyme following horizontal gene transfer. *Molecular Biology and Evolution, 30*, 2024−2034.

Obanye, A. I. C., Hobbs, G., Gardner, D. C. J., & Oliver, S. G. (1996). Correlation between carbon flux through the pentose phosphate pathway and production of the antibiotic methylenomycin in *Streptomyces coelicolor* A3(2). *Microbiology, 142*, 133−137.

Olano, C., Méndez, C., & Salas, J. A. (2010). Post-PKS tailoring steps in natural product-producing actinomycetes from the perspective of combinatorial biosynthesis. *Natural Product Reports, 27*, 571−616.

Peters-Wendisch, P. G., Kreutzer, C., Kalinowski, J., Pátek, M., Sahm, H., & Eikmanns, B. J. (1998). Pyruvate carboxylase from *Corynebacterium glutamicum*: characterization, expression and inactivation of the pyc gene. *Microbiology, 144*(Pt 4), 915−927.

Peters-Wendisch, P. G., Schiel, B., Wendisch, V. F., Katsoulidis, E., Möckel, B., Sahm, H., et al. (2001). Pyruvate carboxylase is a major bottleneck for glutamate and lysine production by *Corynebacterium glutamicum*. *Journal of Molecular Microbiology and Biotechnology, 3*, 295−300.

Projan, S. J. (2003). Why is big Pharma getting out of antibacterial drug discovery? *Current Opinion in Microbiology, 6*, 427−430.

Reddy, T. B. K., Thomas, A. D., Stamatis, D., Bertsch, J., Isbandi, M., Jansson, J., et al. (2015). The Genomes OnLine Database (GOLD) v.5: a metadata management system based on a four level (meta)genome project classification. *Nucleic Acids Research, 43*, D1099−D1106.

Ridley, C. P., Lee, H. Y., & Khosla, C. (2008). Evolution of polyketide synthases in bacteria. *Proceedings of the National Academy of Sciences USA, 105*, 4595−4600.

Riedel, C., Rittmann, D., Dangel, P., Möckel, B., Petersen, S., Sahm, H., et al. (2001). Characterization of the phosphoenolpyruvate carboxykinase gene from *Corynebacterium glutamicum* and significance of the enzyme for growth and amino acid production. *Journal of Molecular Microbiology and Biotechnology, 3*, 573−583.

Rodriguez, E., Navone, L., Casati, P., & Gramajo, H. (2012). Impact of malic enzymes on antibiotic and triacylglycerol production in *Streptomyces coelicolor*. *Applied and Environmental Microbiology, 78*, 4571−4579.

Ryu, Y. G., Butler, M. J., Chater, K. F., & Lee, K. J. (2006). Engineering of primary carbohydrate metabolism for increased production of actinorhodin in *Streptomyces coelicolor*. *Applied and Environmental Microbiology, 72*, 7132−7139.

Sauer, U., & Eikmanns, B. J. (2005). The PEP-pyruvate-oxaloacetate node as the switch point for carbon flux distribution in bacteria. *FEMS Microbiology Reviews, 29*, 765–794.

Stirrett, K., Denoya, C., & Westpheling, J. (2009). Branched-chain amino acid catabolism provides precursors for the Type II polyketide antibiotic, actinorhodin, via pathways that are nutrient dependent. *Journal of Industrial Microbiology and Biotechnology, 36*, 129–137.

Treangen, T. J., & Rocha, E. P. C. (2011). Horizontal transfer, not duplication, drives the expansion of protein families in prokaryotes. *PLoS Genetics,* 7, e1001284.

Ventura, M., Canchaya, C., Tauch, A., Chandra, G., Fitzgerald, G. F., Chater, K. F., et al. (2007). Genomics of actinobacteria: tracing the evolutionary history of an ancient phylum. *Microbiology and Molecular Biology Reviews,* 71, 495–548.

Wentzel, A., Sletta, H., Consortium, S., Ellingsen, T. E., & Bruheim, P. (2012). Intracellular metabolite pool changes in response to nutrient depletion induced metabolic switching in *Streptomyces coelicolor*. *Metabolites, 2*, 178–194.

Wu, Y. J. (2000). Highlights of semi-synthetic developments from erythromycin A. *Current Pharmaceutical Design, 6*, 181–223.

Wu, J., Zhang, Q., Deng, W., Qian, J., Zhang, S., & Liu, W. (2011). Toward improvement of erythromycin A production in an industrial *Saccharopolyspora erythraea* strain via facilitation of genetic manipulation with an artificial attB site for specific recombination. *Applied and Environmental Microbiology,* 77, 7508–7516.

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