The occurrence and ecological role of plasmids in bacterial mycosphere dwellers
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Chapter 2
(Introduction)

The mycosphere constitutes an arena for horizontal gene transfer with strong evolutionary implications for bacterial-fungal interactions

Miaozhi Zhang, Michele C Pereira e Silva, Maryam Chaib De Mares, Jan Dirk van Elsas

Abstract

In the microhabitat that surrounds fungal hyphae in soil, coined the mycosphere, carbonaceous compounds that are released from the hyphae stimulate the growth of heterotrophic bacteria, and thus activate organism-to-organism contacts through genetic interactions. Therefore, the mycosphere is postulated to constitute a gene transfer arena, in which a plethora of genes, including locally adaptive ones, are swapped across the resident microbial communities. Such genetic transfers may have plasmids, in particular ones with broad host ranges, as the basis. Indeed, evidence is increasing for the contention that plasmids play crucial roles as accelerators of evolution in the mycosphere, serving as a horizontal gene pool and, therefore, providing competence factors to local bacteria as well as fungi. The evidence so far points at mycosphere roles for two major plasmid classes, the IncP-1 and PromA groups. Moreover, recent data indicate that bacterium-to-fungus gene transfers are detectable and have been evolutionarily important. The large gene pool present in the mycosphere, coupled with the chances for cell-to-cell contact between mycosphere dwellers allows enhanced recombination frequencies, and as such, organisms are selected locally for enhanced fitness.
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**Microbial lifestyle and evolution in soil** - Soils have been on Earth ever since the solid crust formed, roughly some 10–12 billion years ago. Soil is constituted of solid particles of inorganic and organic compounds, which are interspersed with water and gaseous phases. Together, soils constitute a heterogeneous matrix of diverse solid, liquid and gaseous materials. Importantly, most soils are teeming with organismal life, and most of this life is microbial (bacterial and fungal) in nature. Indeed, it has been estimated that 1 g of soil contains up to 1 billion bacteria cells consisting of tens of thousands of taxa and up to 200 m fungal hyphae (Bardgett, 2005; Roesch et al., 2007). In addition, organisms such as protozoans and nematodes are also highly diverse in the living soil (Nazir et al., 2010). Thus, the soil constitutes a true playground for such natural microbial communities and their past and present activities and interactions confer true trajectories toward evolution.

In functional terms, the active soil microbiota is crucial for global ecosystem processes such as the cycling of carbon and nitrogen. Concerning the carbon cycle, microbial life plays an important role in the decomposition and mineralization of soil organic matter (Coleman et al., 2004; Wardle et al., 2004), whereas in the nitrogen cycle there are crucial steps in nitrogen transformations that are exclusively microbial. Given its importance and complexity, the soil microbiota has been coined a crucial ‘last frontier’ in science (van Elsas et al., 2007). However, in spite of the considerable levels of organic carbon that are stored in the organic matter in most soils, this carbon is often unavailable to soil bacteria. We hypothesize that reasons for this carbon unavailability are twofold: (1) spatial separation between bacterial cells and potential substrate in conjunction with general immobility of soil bacteria; and (2) the general overall recalcitrance of soil organic matter to degradation. These factors characterize the life of bacteria in soil as being harsh. Most bacteria, with the exception of autotrophic ones, will thus occur in a state of permanent starvation for most of their lifetime, potentially interspersed with short periods of active substrate uptake and activity. This modus of life has been coined a ‘feast and famine’ type of existence, in which the feast part is likely to be of short duration and the famine part is often quite extended.

**What drives activity and so accelerates the evolution of soil microorganisms?** - This is a crucial question that pertains to most of the organismal activity in soil. Overall, one can discern three types of microenvironments, collectively coined soil hot spots for enhanced activity, that is (1) the rhizosphere, the narrow layer of soil in the vicinity of plant roots that is
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directly influenced by the root (Hiltner, 1904); (2) decaying material of animal or plant origin – the latter referred to as the residuesphere (Sengeløv et al., 2000); and (3) the mycosphere, the microhabitat that surrounds the fungal hyphae in soil (Warmink and van Elsas, 2008). Traditionally, the rhizosphere is known as a hot spot in the context of carbon availability spurring bacterial activity and so adaptive processes leading to evolution (van Elsas et al., 1986). Here, we pose questions with respect to the mycosphere as a key habitat where enhanced carbon availability may similarly spur evolution and adaptation of bacterial soil inhabitants.

Heterotrophy is currently an overriding modus of microbial life in soil, both in bacteria and fungi. Let us consider the drivers of evolution of such organisms: as heterotrophs – in their adaptation to their local environment and according to the Darwinian principle – strive for optimal fitness in the face of their competitors, and so it is likely that bound carbon has been a major driver of those adaptive events that have been successful. Moreover, in a nutrient-rich environment such as the mycosphere, microbial matrices called biofilms are expected to form. Microorganisms growing in such biofilms might experience an enhanced degree of protection against inhibitory compounds and are therefore selected under specific inhibitory conditions. Therefore, protection through biofilm formation can also be considered to constitute driver of successful adaptation and evolution. In the light of their high reproduction and mutation rates, bacteria are in principle able to evolve fast and to adapt to new habitats (Thomas and Nielsen, 2005); but, to what extent have they been able to do so in the emerging mycospheres vs. the bulk soil? And how have they affected the life of their fungal counterparts? To answer these questions we have to dive into fungal lifestyles and what constitutes the mycosphere.

Fungal lifestyles and the mycosphere

Soil fungi have significant roles in microbial processes, including those affecting soil fertility, decomposition, plant health and nutrition. A daunting and largely unknown diversity of fungi inhabit our soils. In functional terms, soil fungi can be divided into three groups, namely (1) saprotrophic, (2) plant-pathogenic, and (3) plant-symbiotic (Finlay, 2007). Saprotrophy, that is the growth off soil organic compounds, is one of the most important fungal roles in soil, as it is the main driver of the recycling of carbonaceous plant materials that occur in soil, mainly including cellulose, hemicelluloses, and lignocelluloses (Nazir et al., 2010). In addition, plant pathogenicity also allows soil fungi to obtain carbon, by attacking
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their host and tinkering with host cell physiology. A third key fungal life strategy, involving association with host plants, is interactive without damage to the plant, that is by formation of mycorrhiza. The term mycorrhiza refers to a range of fungal-plant associations, including ectomycorrhizal and arbuscular mycorrhizal (AM) ones. Mycorrhizal fungi interact with plant roots in a complex way, receiving photosynthate from the plant as sources of bound carbon and donating sources of phosphorus and nitrogen to the plant. In fact, the mycelia of some fungi are capable of solubilizing nutrients or mineral particles through the production of organic acids (Duponnois et al., 2005). This distinction in three major fungal types in soil is probably a simplified version of reality, as the same (e.g. ectomycorrhizal) fungus may not always be beneficial to the host. In fact, there is a gray area in between mutualism and pathogenesis, given mycorrhizal fungi may sometimes use same pathways as pathogenic fungi to interact with their hosts (Plett and Martin, 2011). Similarly, it is highly debated whether mutualistic types can sometimes also behave as saprotrophs.

**The mycosphere** - In the mycosphere, fungal hyphae are in direct touch with the surrounding soil, yielding a colonizable hypha– soil interface. The interface is dynamic, as fungal hyphae commonly grow by extension and so constitute a moving front. One particularly important part of the mycosphere consists of the region that surrounds the dense hyphal bundle that emerges from the soil and is beneath fungal fruiting bodies (Warmink and van Elsas, 2008). Much like the overall mycosphere, this dense bundle is also thought to have a limited life span (up to several weeks), with poorly known fate after this period. However, it confers an ephemeral site in soil where one expects abundant nutrients to become available. For instance, several studies have identified glycerol, formate, acetate, mannitol (Timonen et al., 1998; Boersma et al., 2010), trehalose and fructose (Frey et al., 1997) as easily available carbon compounds emerging from living fungal hyphae. Erythritol, arabitol and amino acids such as glutamate and asparagine were also found to be secreted, in this case by the fungus *Cantharellus cibarius* (Rangel-Castro et al., 2002). Generally speaking, mycorrhizal fungi derive their bound carbon from plant photosynthates, whereas saprotrophic fungi may release compounds acquired following their feeding on soil organic matter. The compounds released by fungi constitute ecological opportunities for (heterotrophic) bacteria that are locally present and will strive for utilization of and growth on the substrates. So, what bacterial types have been shown to typically associate with soil fungi c.q. the mycosphere? Recent work revealed the occurrence of a plethora of bacterial types in the mycosphere, including *Variovorax, Paenibacillus, Bacillus and Pseudomonas spp.* (Warmink et al., 2009). Similarly, Warmink
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and van Elsas (2008) found several bacterial types, including species of Burkholderia, Dyella and Pseudomonas, which were selected in the mycosphere of Laccaria proxima. Furthermore, bacterial numbers in the L. proxima mycosphere were significantly higher than those in the corresponding bulk soil, indicating a clear activating influence of the mycosphere on bacterial abundance (Warmink and van Elsas, 2008). Until now, however, this fungal-soil interface has not been sufficiently investigated, which leads to a lack of information about the mechanisms mediating fungal-bacterial interactions in soil. In addition, due to the phenomenon known as ‘the great plate count anomaly’ (Staley and Konopka, 1985), and the fact that the majority of soil bacteria is commonly unculturable, most of our current knowledge on mycosphere bacterial communities is derived from studies on culturable bacterial communities.

Interactions in the mycosphere

The mycosphere constitutes the microhabitat where bacterial-fungal interactions occur. It is likely that a plethora of complex interactions between members of the two groups has evolved. The organisms may compete, cooperate or be neutral with each other; different interactions occur in accordance with the phenologies of both partners. In such interactions, physical contacts between the bacterial cells and the fungal hyphae are often detected (Garbaye, 1994; Toljander et al., 2006). Given the osmotrophic nature of fungi (i.e. they secrete extracellular enzymes to the environment to degrade available polymers and subsequently use membrane-localized transport proteins for nourishment; Worrall, 1999), it is expected, and in many cases has been proven, that chemical elicitors in fungal exudates are involved in the mutual recognition. Electron microscopy studies have shown that bacteria do not distribute uniformly on fungal hyphae as well as on plant roots. Cells of Pseudomonas did heavily populate cell junctions and other presumed sites of exudation (reviewed in Lugtenberg et al., 2001). However, both the matrices formed by extracellular exudates (Bianciotto et al., 2001) and specific cell receptors (Holmes et al., 1996) have been shown to mediate physical contact between the partners.

So what do bacteria gain from the presence of a fungal partner and vice versa? The hyphae of fungi, be these mycorrhizal or saprotrophic, supply carbon sources that support the growth of bacteria. Besides, mycorrhizal establishment can change the chemical composition of plant root exudates, providing nutrients for bacteria (Artursson et al., 2006). Interestingly, growing fungal hyphae in soil can develop novel microhabitats for the bacteria in their vicinity (Marschner et al., 2001). The surroundings of fungal hyphae serve as specific niches,
and therefore play a significant role in the selection of soil bacteria (Warmink and van Elsas, 2008). Next to the release of nutrients, the influences of fungi on bacteria may include pH changes, production of inhibitory or stimulatory compounds and adaptations of the soil structure (Johansson et al., 2004). Recently, new data have pinpointed several specific bacterial species as being typical for particular soil fungi. Specifically, the diversity of *Pseudomonas* communities was found to increase dramatically in the mycosphere of the ectomycorrhizal fungus *L. proxima*, when compared to bulk soil (Warmink et al., 2009).

In turn, bacteria may modulate fungal physiology in various ways. For instance, certain bacteria, coined mycorrhization helper bacteria (MHB) increase the fungal ability to colonize plants (Frey-Klett et al., 2007). Bacteria like *Streptomyces* sp. were shown to activate the expression of specific genes of the ectomycorrhizal fungus *Amanita muscaria* (Schrey et al., 2005). It is clear that a complex range of interactions occurs in the mycosphere, with the fungal partner affecting the bacterial associates and vice versa. However, there still is a paucity of knowledge about the mechanisms that allow these bacterial-fungal interactions to occur in soil. We know that the interactions may be driven by compounds released by soil fungi and that bacterial motility, secretion of enzymes, biofilm formation and action of a type three secretion system (T3SS) play important roles. This highly evolved T3SS, which is often found in bacterial pathogens of both animals and plants, functions as a molecular syringe to introduce effector proteins into the cytoplasm of these eukarya (He et al., 2004) and depends on the physical contact with the host. By PCR based on the *hrcR* gene, a highly conserved gene involved in T3SS, it was observed that the percentage of randomly picked isolates that carry the *hrcR* gene showed a significant increase, from 2.8 in the bulk to 13.4 in the *L. proxima* mycosphere soil (Warmink and van Elsas, 2008). However, we still lack a comprehensive picture of the interactive events between the organisms at the fungal surface, and to what extent horizontal transfer of genes might influence genome-level interactions between bacteria and fungi.

**Horizontal gene transfer (HGT) and plasmids in the mycosphere**

In addition to mutations and recombination within genomes, HGT is an important mechanism of microbial evolution (Arber, 2000). Out of three well-established HGT mechanisms in bacteria (i.e. transformation, transduction and conjugation), conjugation has emerged as a mechanism of major importance in soil. Conjugation involves the action of mobile genetic elements (MGEs) such as plasmids and conjugative transposons. Generally, these MGEs contain type IV secretion systems (T4SS), which allow the building of a
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cytoplasmic bridge between the cells that interconnect genetically. Furthermore, the two types of elements often carry so-called accessory genes that may become important under particular ecological conditions but are dispensable for the host most of the time. Thus, such MGE-encoded accessory genes provide the currency of HGT with incidental selective value. The fact that many such genes result in functionality outside of the cell (secreted compounds) has incited the intriguing hypothesis that HGT may also play a role in microbial sociality (Rankin et al., 2011).

The large number of bacteria present in the mycosphere is propitious to enhanced contact between the resident bacterial cells, potentially increasing genetic interactions. Therefore, the mycosphere emerges as a major arena of accelerated microbial evolution by gene transfer in soil (Fig. 1). In addition, direct evidence from triparental exogenous isolation experiments has revealed that plasmid transfer frequencies were significantly increased from communities from the mycospheres of several fungi in a forest soil as compared to those from bulk soil (Zhang et al., 2014). What evidence do we have for HGT-driven adaptive processes in the mycosphere? HGT among bacteria or across domains of life can be studied either by genome sequence comparisons, using diverse criteria as in (Philippe and Douady, 2003), or via direct experimental studies with MGE. Using both types of approaches, we will address evidence for (1) bacterial-bacterial, and (2) bacterial-fungal HGT.
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Fig. 1 The mycosphere as a HGT arena. (a) fungal hyphae are depicted by light brown strings of cells, creating the ‘arena’. Shaded areas illustrate nutrients released by fungi where heterotrophic bacteria agglomerate. There, diverse bacteria and yeast, shown as cells of different sizes, shapes and colors, are found in close association and therefore have the opportunity to interact and transfer genes more frequently than in other locations in the soil. Plasmids can also be available freely in the soil and are drawn as small, purple circles. The mechanisms of HGT underlying three different scenarios are shown in insets (gray squares). (b) Conjugation between different bacterial cells can result in either integration or replication of the plasmid. (c) Conjugation-like mechanisms between bacterial and fungal cells are proposed to occur in soils. Current evidence points at integration into the fungal genome as a result. (d) Bacteria, and possibly fungi, would be able to acquire freely available plasmids, perhaps following lysis of other microbial cells. This could result in either integration or replication of the plasmid in the bacterial recipient’s genome, or in integration into the genome of the fungal acquirer.

Bacterial-bacterial gene transfers in the mycosphere - The large number of potentially interacting partners in the mycosphere makes this microhabitat especially favorable for HGT. Do mycosphere dwellers show evidence of HGT-driven evolution or adaptation to this habitat? Our analyses of the genome of the fungal-interactive bacterium *Burkholderia terrae* BS001 revealed the presence of up to 16% of the 11.5-Mb genome, or about 1.8 Mb of sequence, to be located in so-called regions of genomic plasticity (RGPs), indicating their proneness to transfer (I.U. Haq, K. Graupner, R. Nazir, J.D. Van Elsas, unpublished data) This included an island of about 72 kb in size, which contained the whole canonical T4SS plus other plasmid-typical genes, indicating the presence of an integrated plasmid. Would association with soil fungi be conducive to enhanced prevalence of inserted ‘flexible’ DNA? This is an interesting and testable hypothesis, which draws our attention to plasmids as key vehicles for gene transfer in the mycosphere.

In spite of this coincidental finding, we still understand very little about the types, diversity and potential ecological benefits conferred to hosts by plasmids in the mycosphere. The traditional view of ecologically important traits carried by plasmids has been antibiotic/heavy metal resistance and/or biodegradative capacity (Thomas, 2000), among other easily detectable traits. On the other hand, other plasmids have been coined ‘cryptic’, which indicates that their potential roles, other than being ‘parasitic’ in a DNA-evolutionary sense, remain unknown (Tauch et al., 2002; Heuer et al., 2004). It is likely that the degree of loading with accessory genes is a reflection of past evolutionary events that involved plasmids (e.g. the number of recombination coupled to selection events). Transfer is a key facet of plasmid ‘life’, as it provides a new genetic background to a potentially selectable trait, which may thus give rise to a new ecologically successful combination of host- and plasmid-borne
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traits (Bergstrom et al., 2000). This contention about the driver of plasmid ‘life’ assumes periodic selection for novel forms (being combinations of incoming and resident fitness-enhancing traits) to be at the basis. Now, would mycospheres, that is habitats that ephemerally occur in soil and provide selective pressure for fitness in utilization of the available carbon sources, confer the periodic selection pressure that plasmids need to evolve and persist across bacterial populations? And, would they be the main drivers of HGT from bacteria into fungal hosts?

What can we learn from the structures of plasmids themselves? In a generic sense, plasmids offer opportunities to acquire and lose genes encoding a wide variety of functions. Plasmid genomes are modular in structure, meaning that their genes are arranged into operons encoding specific functions (Boyd et al., 1996; Norman et al., 2009). Such operons, via HGT, can accelerate genome innovations as functional modules rather than slowly evolving genes are acquired (Jain et al., 2003). Insertion sequence (IS) elements and transposons are generally believed to be important factors influencing the modular evolution of plasmids.

<table>
<thead>
<tr>
<th>Host</th>
<th>Habitat</th>
<th>Plasmid and encoded traits</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Variovarax paradoxus</em></td>
<td>Mycosphere of <em>Laccaria proxima</em></td>
<td>pHB44: iron uptake; biofilm formation</td>
<td>M.Zhang, J.A.Warmink, M.C. Pereira et Silva et al., unpublished data</td>
</tr>
<tr>
<td><em>Variovarax paradoxus</em></td>
<td>Mycosphere of <em>Laccaria proxima</em></td>
<td>pBS64: biofilm formation; possibly using a substrate from the fungal host</td>
<td>M.Zhang, J.A.Warmink, M.C. Pereira et Silva et al., unpublished data</td>
</tr>
<tr>
<td><em>Collimonas fungivorans</em></td>
<td>Mycophasous with <em>Fusarium</em></td>
<td>pTer331: cryptic, probably providing genetic flexibility, ie retrotransfer, transfer and mobilization</td>
<td>Mela et al., 2008</td>
</tr>
<tr>
<td><em>Pseudomonas fluorescens</em></td>
<td>Mycorrizosphere</td>
<td>plasmid-encoded <em>xy/E</em> and <em>xy/MA</em> genes: monoaromatics degradation</td>
<td>Sarand et al., 1998</td>
</tr>
</tbody>
</table>

What do we know about plasmids in bacteria that might associate with the mycosphere? In recent work, it was shown that IncP-1β plasmids are present in a major bacterial group (*Variovorax paradoxus*, 15 isolates) that typically occurs in the mycosphere of *L. proxima* (Boersma, 2010; M. Zhang, J.A.Warmink, M.C. Pereira et Silva et al., unpublished data). These plasmids were found in 2 of the 15 isolates and both stimulated the production of biofilms in their host as compared to plasmidless variants. One may argue that the increased
biofilm formation was due to the physical nature of conjugation (cell-to-cell contact through specialized conjugative pili), considering the observation that various pili-like cell appendages are involved in biofilm formation, without the involvement of ‘accessory’ genes (O’Toole and Kolter, 1998; Pratt and Kolter, 1999). However, this observation might also indicate that an important asset of successful mycosphere colonizers could be enhanced (Warmink et al., 2009). Indeed, genomic analysis of one plasmid indicated the presence of predicted coding regions for enzymes involved in biofilm formation, such as polysaccharide deacetylase and polybeta-1,6-N-acetyl-D-glucosamine (M.C. Pereira e Silva, J.K. Brons, M. Zhang, J.D. van Elsas, unpublished data). Furthermore, the aforementioned study by M. Zhang, J.A. Warmink, M.C. Pereira et Silva et al., unpublished data provided strong evidence that an iron uptake system assisted the fitness of the host endowed with one plasmid, pHB44, in the mycosphere soil, an effect that was only detectable in the case the local FeCl₃ levels were low. On the contrary, in the soil with plentiful Fe, the plasmid endowed strain revealed poorer survival than its plasmidless counterpart, indicating that the cost of the plasmid to the host cell was detrimental to its survival. This result supported the hypothesis that HGT contributes to the functioning of microbial sociality (Rankin et al., 2011).

Moreover, there is mounting evidence for the presence of cryptic plasmids, of the PromA group, in mycospheres. In this respect, plasmid pTer331 was detected in a mycosphere-isolated Collimonas fungivorans strain (Mela et al., 2008), whereas plasmid pIPO2 was obtained from a rhizosphere in which fungi possibly played a role (van Elsas et al., 1998; Tauch et al., 2002). We recently obtained evidence for the presence of PromA group plasmids in mycospheres of several ectomycorrhizal fungi in a forest soil (Zhang et al., 2014). Such cryptic plasmids might be considered a pool to create standing genetic variation within a population, allowing bacterial communities to gain robustness in the uncertainty of environmental changes (Jain et al., 2003; Hermisson and Pennings, 2005). This can be thought of as a ‘gene fishing’ strategy for their host, by their ability to retrotransfer or retromobilize plasmids (Szpirer et al., 1999; Ronchel et al., 2000). Such gene fishing makes the mobile gene pool in the habitat effectively accessible to the host. Moreover, cryptic plasmids are also good examples of selfish DNA. Their prevalence in a bacterial population might result from their high transfer efficiency, rather than the reproductive success of the plasmid-containing individuals (Lilley et al., 2000; Thomas, 2000; Velmurugan et al., 2003).

The aforementioned observations appear to support the contention that the two plasmid classes, IncP-1β and PromA, might play fundamental roles in the adaptation of
bacteria in the mycosphere (Table 1), the IncP-1 group by carrying diverse accessory genes, and the PromA group by conferring the capacity to retrotransfer to their host (van Elsas et al., 1998). Although IncP-1 plasmids are also able to retrotransfer, the efficiency is much lower than PromA.

**Bacterial-fungal gene transfers in the mycosphere** - In early work, Heinemann and Sprague (1989) already showed that conjugative plasmids of *Escherichia coli* could be transferred to the yeast *Saccharomyces cerevisiae* where, most likely, cytoplasmic bridges had been formed probably as a result of the T4SS present on the plasmid between the two interacting partners. Now, what do we know about the role of plasmids in HGT in soil, or in general, that connect bacteria and fungi? The system that is perhaps closest to answering this question is based on *Agrobacterium tumefaciens*. This organism contains a plasmid, denoted the tumor-inducing (Ti) plasmid, which can be moved from the bacterial host to plant cells, slicing off a stretch - the T-DNA - that may insert into the plant nucleus. This is a clear route of interkingdom gene transfer. Remarkably, *Agrobacterium* mediated Ti based gene transfer to yeast (Bundock et al., 1995) as well as to filamentous fungi (De Groot et al., 1998; Chen et al., 2000; Kemppainen et al., 2005) has been shown under laboratory conditions. The final fate of transferred DNA in these studies was either integration into the genome (leading to fixation), or abortion. T-DNA integrations in the fungal genomes are often erratic and may occur via nonhomologous recombination. For instance, in *S. cerevisiae* random integrations occurred in both coding and noncoding regions (54 T-DNA copies studied) (Bundock et al., 2002). Random integration of whole Ti plasmids has also been found in soil fungi such as *Fusarium oxysporum* (Islam et al., 2012) and *Rosellinia necatrix* (Kano et al., 2011). T-DNA integration in the ectomycorrhizal fungus *L. bicolor* (Kemppainen et al., 2005) occurs with a bias toward genes, but it is not known whether or not this is an artifact of the large number of genes present in this species compared with other fungi. There is evidence for different insertion biases in other fungi. Recently, successful integrations of T-DNA were reported in six white-rot fungi (Sharma and Kuhad, 2010). In this study, transfers did not require wound formation of fungal cells. Instead, transmission electron microscopy analyses revealed attachment of bacterial cells to fungal hyphae, supporting the contention that biofilm formation is an important trait during this interaction (Warmink et al., 2009; Zhang et al., 2104). We thus may be seeing glimpses of the mycosphere-determined gene transfer arena (Fig. 1), in which intimate cell-to-cell contact, such as in biofilms, spurs the genetic interconnections between the cells, of which we see experimental as well as retrospective (from genome comparisons)
evidences (see later). The possibility remains that DNA freely available in the soil as a result of cell lysis and protected against degradation by binding to particle surfaces and biofilms, is taken up not only by bacteria (i.e. transformation), but also by fungi (Fig. 1d). There is sparse evidence that such ‘competence’ exists in these eukaryotes (Bryngelsson et al., 1988; Hoffman et al., 1994).

Other cases of HGT from bacteria into fungi are mostly restricted to selfish genetic elements, for example introns, transposons and complete metabolic clusters or operons (reviewed in Rosewich and Kistler, 2000), but information on the potential transfer mechanisms involved is sparse. Taking all evidence together, HGT has been justifiably invoked as an important force in the evolution of fungi (Rosewich and Kistler, 2000; Richards et al., 2011).

Table 2. Genes from bacterial origin related to enhanced degradation capabilities transferred to fungi that are frequently encountered in soils

<table>
<thead>
<tr>
<th>Protein putative function</th>
<th>Gene Accessions</th>
<th>Fungal Recipient</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta-glucuronidase</td>
<td>AAV91787</td>
<td>Penicillium canescens</td>
<td>Wenzl et al. (2005)</td>
</tr>
<tr>
<td>Polyketide synthase</td>
<td>AAR90278</td>
<td>Cochliobolus heterostrophus</td>
<td>Krogen et al. (2003)</td>
</tr>
<tr>
<td>Catalase-peroxidase 2</td>
<td>XP_959745</td>
<td>Neurospora crassa</td>
<td>Klotz and Loewen (2003) and</td>
</tr>
<tr>
<td>Catalase</td>
<td>AAB47761;</td>
<td>Aspergillus fumigatus,</td>
<td>Zamocky et al. (2009)</td>
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<tr>
<td>Salicylate synthase</td>
<td>Afr0007119</td>
<td>Penizycomycotina</td>
<td>Johnson et al. (2002) and</td>
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<td>Glucosyltransferase</td>
<td>Fox0002676</td>
<td>Penizycomycotina</td>
<td>Marct-Houben and Gabaldon (2010)</td>
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<td>Peptidase M48</td>
<td>Kla0008461</td>
<td>Saccharomycotina</td>
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<td>Hydrolase</td>
<td>Nfi0004707</td>
<td>Pezizycomycotina</td>
<td></td>
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<td>Beta-lactamase</td>
<td>Nha0022224;</td>
<td>Pezizycomycotina</td>
<td></td>
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<td>Peptidase M24</td>
<td>Nha0011677;</td>
<td>Pezizycomycotina</td>
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<td>L-lactate transport</td>
<td>Pbl0008111</td>
<td>Zygomycomta</td>
<td></td>
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<td>Beta-galactosidase</td>
<td>Uma0002204</td>
<td>Pezizycomycotina, Basidiomycota</td>
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<td>Chitin-binding domain 3</td>
<td>Uma0005439</td>
<td>Basidiomycotia</td>
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<td>Nematicidal protein</td>
<td>Ure0005752</td>
<td>Pezizycomycotina</td>
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<td>Esterase</td>
<td>Ang0004940</td>
<td>Pezizycomycotina</td>
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<td>Secreted hydrolase-like</td>
<td>Lbi0008653</td>
<td>Pezizycomycotina, Basidiomycota</td>
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<td>Glycosyl transferase 1</td>
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<td>Ferulic acid esterase</td>
<td>Sno0015155</td>
<td>Pezizycomycotina</td>
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</tbody>
</table>
Importantly, the advent of available whole genome sequences is increasingly reporting convincing cases of HGT from bacteria to fungi. Several transferred genes actually contribute to important functional innovations, allowing fungi to thrive in novel habitats, utilize resources previously unavailable for the recipient species or enhance biosynthetic capabilities. Richards et al. (2011) recently described 323 HGT events into fungi, the majority of genes derived from bacteria. The functional annotations of these genes are dominated by putative functions in sugar, nitrogen, amino acid, nucleotide and secondary metabolism. In fact, a wide diversity of metabolic functions is covered, supporting the hypothesis that HGT from bacteria plays a significant role in expanding the metabolic and nutrient-processing capacities in many fungi. We collated published examples of genes conferring degrading capacity that were transferred horizontally between bacteria and fungi in soils (Table 2). The presented list is based on a revalidation study by Richards et al. (2011), and the added references meet the criteria proposed by the authors. We do not consider transfers among fungi, but a few have been reported to date (Richards et al., 2011). As is generally true in HGT studies, most examples provided in Table 2 cannot provide unequivocal determination of the donor lineage due to the incompleteness of genomic databases. The direction of the transfers can be proposed, though, and it is based on the assumption that the taxon of the broadest representation of the given gene family is the most likely (donor) source of the transferred gene (Koonin et al., 2001). All genes presented in Table 2 are bacterial in origin. Transfers in the opposite direction may also be possible, and there are reported cases of Eukarya-to-Prokarya transfers (Keeling and Palmer, 2008), but, to date, none within the context of the mycosphere.

Wenzl et al. (2005) identified the transfer of β-glucuronidase genes from Gram-positive bacteria to the soil fungi *Penicillium canescens* and *Scopulariopsis* sp. that may have enabled these fungi to utilize glucuronides in vertebrate urine as a carbon source. Likewise, numerous transfers of glycosidases, xylanases, esterases and acetyltransferases have been recorded into soil filamentous fungi affiliated to the *Pezizomycotina* (Marcet-Houben and Gabaldon, 2010). In addition, a recent study found HGT of carbohydrate esterases, namely
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Putative poly[(R)-3-hydroxybutyrate] (PHB) depolymerases, from a bacterial donor into ectomycorrhizal Amanita species (C. De Mares, J. Hess, D. Floudas, A. Lipzen, C. Choi, M. Kennedy, I. V. Grigoriev, A. Pringle unpublished data). The transfer may have enabled innovations in the carbon metabolism of these species by allowing them to exploit carbon storage molecules (PHB) from soil bacteria. We thus see evidence for drivers of HGT from the early soil inhabitants (bacteria) into later ones (fungi), allowing the latter organisms to expand their resource utilization capacities and hence their ecological competences in a soil setting.

Conclusions and prospects

From the foregoing and other available evidence, it appears that the mycosphere constitutes an arena in soil, in which local conditions are propitious to HGT both in terms of the presence of interacting cellular partners and in terms of cell-activating carbonaceous compounds. Habitats that provide opportunities for dense and intimate associations of microorganisms, such as the rhizosphere, have previously been proposed to contribute to and enhance HGT (van Elsas et al., 1986). Thus, the mycosphere, with its ability to generate abundant surface areas and increased nutrient availability to its associates, can be thought of an excellent provider of opportunities for HGT. Indeed, Zhang et al. (2014) have shown that transfer frequencies within bacterial communities from the mycosphere of Russula, Ampulloclitocybe and Inocybe were significantly higher than with those from the corresponding bulk soils, ranging from $10^{-8}$ to $10^{-6}$ per recipient. The cellular partners that are present, that is bacteria of different types and fungi, are probably in a continuous quest for available substrate, for which they compete, but occasionally they may also cooperate. In their quest for the nutrients, HGT and in particular the genetic flexibility offered by plasmids may constitute an evolutionary/adaptive asset for the mycosphere inhabitants. The fitness-enhancing traits, that are of key yet ephemeral value, are quite quickly swapped between partners, and, as soon as they are not needed anymore due to changed conditions, these may be discarded, for example genes or by plasmid loss from the population. This discarding of the transiently required and selected genetic information is thought to reduce the metabolic burden on the cell and thus on the local population.

In addition, the evidence for the past occurrence of HGT between bacteria and fungi is compelling, and it seems to suggest that this facet of microbial life in the soil is much more prominent than has been thought for a long time. The evidence presented here is twofold: (1)


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mechanistic, that is on the basis of data obtained from real experiments, and (2) obtained from comparative genomics. Both lines of evidence provide clear underpinnings of the contention that HGT from bacteria to fungal hosts in the mycosphere, followed by fixation by selection, is rampant.

To what extent can we predict what the implications are of any current-day gene swapping between organisms, be these just bacteria or bacteria and fungi, in the mycosphere? For instance, do bacterial genes that enhance the ecological competence of soil fungi that cause disease in plants have an effect on fungal pathogenesis? Conversely, do such transfers enhance the ecological competence of fungi that we use as biocontrol agents or ecological enhancers in the soil? Finally, are bacteria indeed genetically more flexible when they spend part of their life span in soil in the mycosphere and how does this affect their genomic build up? Do particular bacterial types with relevance for human or plant pathogenesis shape up in the mycosphere in terms of enhancing their competence in pathogenesis situations? These are relevant questions that may direct our future strategies with a focus to enhance our understanding of mycosphere-bound gene transfer processes. The mycosphere is a long-overlooked arena in soil where genetic interconnections between the inhabitants are potentially strong, affecting the adaptations of the latter, with potentially very far-reaching consequences.

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