Chapter 5
The Rhizosphere: Molecular Interactions Between Microorganisms and Roots

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5.1 Introduction

Roots constitute important plant organs for water and nutrient uptake. They, however, also release a wide range of carbon compounds of low molecular weight. This release can amount up to 30% of total net fixed carbon (Smith and Read 2008; Rovira 1991) and forms the basis for an environment inhabited by a highly diverse and active microbial community, the rhizosphere (Hiltner 1904; Hartmann et al. 2008), which is defined as soil compartment influenced by living roots.

The release of assimilates by plant roots results in a greater microbial density and activity in the rhizosphere than in the bulk soil. The specific conditions lead to the selection of distinct microbial communities, where fungi play an important role (Frey-Klett et al. 2005). Especially, symbiotic fungi (ecto/arbuscular mycorrhiza) release a substantial amount of plant-derived carbon to the soil, creating another sphere, the mycorrhizosphere. These organic carbon enriched spheres are highly attractive for other microorganisms. For example, the rhizosphere/bulk soil ratio for Gram-negative bacteria reaches from 2 to 20, for actinomycetes from 5 to 10, and for fungi from 10 to 20 (Morgan et al. 2005). The diversity and structure of bacterial communities are plant specific and vary over time (Smalla et al. 2001; Barriuso et al. 2005; Berg and Smalla 2009; Hartmann et al. 2009). Bacteria can have a negative, neutral, or beneficial effect to plant fitness. Detrimental effects are caused by

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R. Matyssek et al. (eds.), *Growth and Defence in Plants*, Ecological Studies 220,
DOI 10.1007/978-3-642-30645-7_5,

bacterial pathogens and parasites, and bacteria that produce phytotoxic substances. The occurrence of pathogenic bacteria is, however, low in healthy plant populations. This is due to plant defence systems, which are selective and could cause the enrichment of plant beneficial microbes within the rhizosphere (Frey-Klett et al. 2005). The plant beneficial bacteria include saprotrophs that degrade the organic litter, antagonists of plant root pathogens, and plant growth promoting rhizobacteria (PGPR) (Barea et al. 2005).

5.2 Bacteria of the Rhizosphere and Effective Metabolites

5.2.1 Plant Growth Promoting Rhizobacteria

Plant growth promoting rhizobacteria (PGPR) are usually in contact with the root surface as well as the hyphal cell walls of symbiotic fungi (Bonfante and Anca 2009), and increase plant growth (Weller 1988; Lucy et al. 2004; Haas and Défago 2005). PGPR must be able to colonise the root and have to be present in sufficient numbers to exert their functions. *Pseudomonas* spp. and *Bacillus* spp. are the most commonly investigated PGPR, and are often the dominating bacterial groups in the rhizosphere of herbs and grasses (Marilley et al. 1999; Morgan et al. 2005). Diverse PGPR strains have been used successfully for crop inoculations, including members of *Azospirillum*, *Azotobacter*, *Bacillus*, *Enterobacter*, *Pseudomonas*, *Serratia*, and *Xanthomonas* (see Lucy et al. 2004 for a comprehensive list). In addition there is a considerable diversity of endophytic microbes (bacteria and fungi) which are commonly colonising plants systemically without harming the plant, but rather exert plant growth promoting activity in some cases (Rosenblueth and Martinez-Romero 2006; de Almeida et al. 2009). For example, diazotrophic plant growth promoting bacteria belonging to the species *Herbaspirillum seropedica*, *Burkholderia tropica*, *Gluconacetobacter diazotrophicus*, *Azoarcus* sp., or *Azospirillum brasilense* occur as endophytic symbiotic bacteria in Gramineae and other plants (Rothballer et al. 2009), although their exact mechanism of symbiotic interaction is not well understood.

Two groups of PGPR exist: those that are involved in nutrient cycling and plant growth stimulation (biofertilizers), and those that are involved in the biological control of plant pathogens (biopesticides). Bacteria may support plant growth by the mobilisation of inorganic nutrients, by nitrogen fixation, by the production of phytohormones including auxins, cytokinins as well as gibberellins (Dobbelaere et al. 2001). Most interestingly, a widely distributed activity and gene cluster for the degradation of indole acetic acid was discovered recently in diverse bacteria (Leveau and Gerards 2008), which could be another means to interfere with plant growth. Volatile substances, such as acetoine or 2,3-butanediol, were also shown to stimulate plant growth substantially (Ryu et al. 2004; Barea et al. 2005). Some root-associated bacteria are able to stimulate plant growth by reducing inhibitory levels

of ethylene in the rhizosphere through the hydrolysis of the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) (Blaha et al. 2006; Glick et al. 1998; Grichko and Glick 2001; Prigent-Combaret et al. 2008) and rhizobitoxin (Sugarawa et al. 2006). Root-associated and endophytic bacteria with ACC-deaminase activity have a good potential for practical applications (Mayak et al. 2004). In soils with low phosphate (P), P-solubilising bacteria release phosphate ions from low-soluble inorganic P-containing minerals and from organic phosphate sources. Although many P-solubilising bacteria have been characterised, their relative importance in the PGPR effect is uncertain. However, if the phosphate ions are released in an area rich in mycorrhizal fungal hyphae, the hyphae may transport the P to the plants and the PGPR effect is detectable (Artursson et al. 2005; Barea et al. 2005).

The inoculation of roots with *Azospirillum* spp. often promotes plant growth, probably not primarily through biological nitrogen fixation, but mostly due to the ability of the bacteria to produce phytohormones and thus stimulate root development, increase the volume of explored soil space, and finally nutrient uptake efficiency (Steenhoudt and Vanderleyden 2000; Dobbelaere et al. 2001). PGPR often enhance plant growth through the production of plant growth regulators (Lucy et al. 2004). The auxin-type phytohormones produced by *Azospirillum* spp. induce root branching and thus improve plant nutrient uptake from the soil (Dobbelaere and Okon 2007), an example for a possible trade-off of altered resource allocation (GDB). Recently, a salt-tolerant *Azospirillum brasilense* strain NH was characterised, which produced the auxin indole acetic acid at 250 mM NaCl and conferred salt tolerance to wheat upon inoculation (Nabti et al. 2007). Interestingly, an efficient endophytic colonisation of the apoplastic space in the root epidermis by *A. brasilense* NH was observed (Nabti et al. 2010). The growth of the plants may also be stimulated by bacterial cytokinin production (Lucy et al. 2004).

5.2.2 Plant Disease Suppressing Rhizobacteria

The second major PGPR mechanism is to reduce the incidence of plant disease. Infectious diseases are often caused by soil-borne organisms including both bacteria and fungi. The soils, where soil-borne diseases are infrequent, are called suppressive soils, and it has been shown that the disease suppression is often caused by specific bacterial and fungal populations (Weller et al. 2002). Recent studies highlight three major mechanisms for the disease suppression: antagonism, direct pathogen–agonist (plant growth promoting microorganism) interactions, and induced systemic resistance in the plant (Compant et al. 2005).

Antagonists are naturally occurring organisms that express traits which enable them to interfere with pathogen growth, survival, and infection. Bacteria, antagonistic to plant pathogens, represent an important part of the rhizosphere communities, and antagonistic strains amount up to 35% of the culturable bacteria (Opelt and Berg 2004). The Gram-negative rods of *Stenotrophomonas maltophilia* (earlier

Xanthomonas maltophilia) are also typical rhizosphere inhabitants, and of scientific interest due to their potential for biological control (Nakayama et al. 1999).

The most thoroughly investigated group of PGPR agonists are still the fluorescent pseudomonads (Haas and Défago 2005). These bacteria produce diverse antagonistic secondary metabolites that suppress the growth of other organisms. As an example, the extracellular pigment pyoverdin is an efficient siderophore (iron carrier), and the production of pyoverdin by pseudomonads in iron-poor soils is an effective way to suppress the growth of non-producers by depriving the pathogens from iron (Kloepper et al. 1980). Pseudomonads also produce metal-chelating agents with proposed properties other than iron scavenging. Pyochelin, e.g., binds effectively copper and zinc, and possesses strong antimicrobial activity (Cornelis and Matthijs 2002). However, the antimicrobial effect of pyochelin, and of some other siderophores, can be explained by their effective metal-chelating activity (Haas and Défago 2005). Gram-positive PGPR antagonists, like *Bacillus subtilis* GB03 (Kloepper et al. 2004) and *B. amyloliquefaciens* FZB42 (Koumoutsi et al. 2004; Chen et al. 2009a) are also very efficient PGPR strains with biocontrol activity, also having effects on systemic resistance in plants. Since their spores withstand adverse conditions, they have wide acceptance for practical applications, because of easier handling and excellent stability of inoculant preparations.

Direct antibiosis is used by several PGPR as a mechanism for biocontrol. *Antibiosis* by PGPR pseudomonads is often caused by the production of several antimicrobial substances. These chemicals not only suppress fungi, but are often also toxic against bacteria (Compant et al. 2005). From antimicrobial compounds produced by pseudomonads, the mode of action has been partly determined for six classes of substances. These include the electron transport inhibitors phenazines, phloroglucinols (causing membrane damage in *Pythium* spp. and being phytotoxic at higher concentrations), pyrrolnitrin (acting as a fungicide), cyclic lipopeptides (surfactant properties against fungi and plants, chelation of cations), and HCN (potent inhibitor of metalloenzymes). A comprehensive list of the antibiotics, producer strains, target organisms, and effects on the host plants has been covered in a review by Raaijmakers et al. (2002). Production of siderophores, lipopeptides, and antibiotics production has been observed in other PGPR isolates as well, including *Bacillus amyloliquefaciens* (Chen et al. 2009b), *Stenotrophomonas* spp. (Compant et al. 2005), and *Streptomyces* spp.

Another group of antagonistic compounds are lytic enzymes, such as cell wall hydrolases that attack pathogens. The ability to degrade fungal cell walls by chitinases is shared by many biocontrol PGPR including *Pseudomonas*, *Serratia*, and *Streptomyces* spp. (Whipps 2001). In addition to chitinases, some bacterial strains produce β-glucanases and proteases (Dunne et al. 2000). Synergism between the action of cell wall degrading agents and antibiotics was observed by Woo et al. (2002). The authors showed that the pre-treatment of plant pathogenic fungi with cell wall degrading enzymes rendered them more susceptible to the antifungal substance, syringomycin.

Inoculation of plants with some PGPR elicits a phenomenon known as induced *systemic resistance* (ISR; Bakker et al. 2007; see Fig. 5.1). ISR allows the plants to

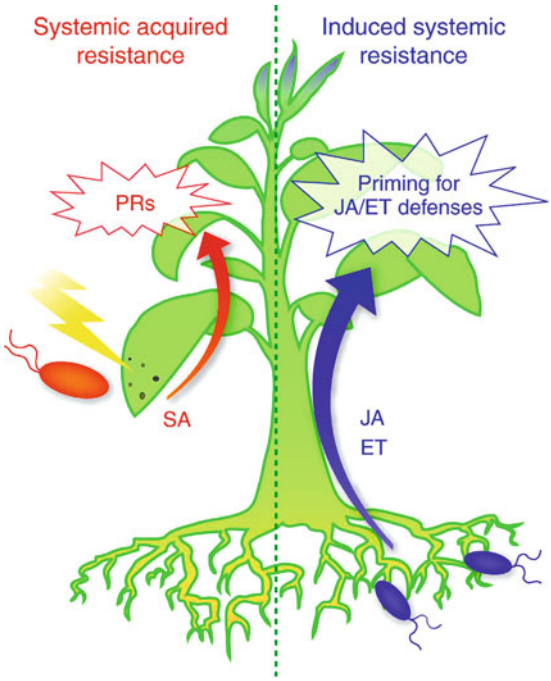

Fig. 5.1 Schematic representation of systemically induced immune responses. Systemic acquired resistance starts with a local infection and can induce resistance in yet not affected distant tissues. Transport of salicylic acid (SA) is essential for this response. Induced systemic resistance can result from root colonisation by non-pathogenic microorganisms and, by long-distance signalling, induces resistance in the shoot. Ethylene (ET) and jasmonic acid (JA) are involved in the regulation of the respective pathways. Depending on the pathogen, JA/ET can also be involved in SAR. They induce pathogenesis-related genes different from those induced by SA (taken from Pieterse et al. 2009, with permission)

endure pathogen attacks that, without bacterial pre-inoculation, could be lethal. The effect is systemic, e.g., root inoculation with the biocontrol PGPR yields the whole plant non-susceptible (Haas and Défago 2005). Thus far, *Pseudomonas*, *Burkholderia*, and *Bacillus* spp. have been shown to elicit ISR (e.g., Ryu et al. 2004), and the search for effective substances is in progress (see also Sect. 3.4.2). Root treatment of *Phaseolus vulgaris* with *Pseudomonas putida* BTP1 leads to a significant reduction of the disease caused by the pathogen *Botrytis cinerea* on leaves. Ongena et al. (2005) isolated the molecular determinant of *P. putida* mainly responsible for the induced systemic resistance and identified it as a polyalkylated benzylamine structure. Exposure to butanediol, the volatile that induces the growth of *Arabidopsis* seedlings (Ryu et al. 2003), decreased disease severity by the bacterial pathogen *Erwinia carotovora* in the same plant (Ryu et al. 2004). Transgenic lines of *Bacillus subtilis*, that emitted reduced levels of 2,3-butanediol, decreased *Arabidopsis thaliana* protection against pathogen infection compared with seedlings exposed to volatiles from wild-type bacterial lines. Furthermore,

bacterial signalling molecules of the *N*-acyl homoserine lactone type which are quorum sensing compounds of Gram-negative bacteria (Eberl 1999) were found to exert systemic functions in plants as well (see below).

The multiple mechanisms as to how plant-beneficial bacteria promote plant fitness are only beginning to be resolved. It is obvious that the use of *Pseudomonas* and *Bacillus* spp. has yielded a mass of relevant results, but much remains to be learned from the bacteria in other taxa.

5.2.3 The Special Role of Actinomycetales

Ample evidence indicates that actinomycetes are quantitatively and qualitatively important in the rhizospheres of plants, where they may influence plant growth and protect plant roots against invasion by root pathogens (for a review see Tarkka and Hampp 2008). Within the order of the Streptomycetales, members of the genus *Streptomyces* are traditionally considered as soil dwelling organisms (Janssen 2006), and have been reported to be the most prolific producers of a variety of antibiotics (Berdy 2005). There is now abundant evidence that some *Streptomyces* species colonise the root surface and even plant tissues (Sardi et al. 1992; Coombs and Franco 2003), and it has been suggested that antibiotic production by the streptomycete may protect the host plants against phytopathogens (Challis and Hopwood 2003; Tarkka and Hampp 2008). Streptomycetes causing plant disease were covered recently in Loria et al. (2006).

Streptomycetes can inhibit diverse plant pathogens, including Gram-positive and Gram-negative bacteria, fungi, and nematodes (Crawford et al. 1993; Chamberlain and Crawford 1999; El-Abyad et al. 1993; Samac and Kinkel 2001). Biocontrol strains from *Streptomyces* spp. have often been isolated from suppressive soils (Weller et al. 2002). For example, the common scab disease of potato, caused by *Streptomyces scabies* (Loria et al. 1997), is effectively suppressed by streptomycete isolates from *S. scabies* suppressive soils. The disease suppression effect can be long lasting. Liu et al. (1995) added streptomycete strains to a soil that was non-suppressive against potato scab. Even by the fourth year after the inoculation, the disease reduction due to these strains was at 63–73%. The biocontrol activity of these *Streptomyces* spp. can often be explained by direct inhibition of the growth of the pathogen (Eckwall and Schottel 1997), but it has been observed that stronger biocontrol agents also have a capacity for resource competition (Neeno-Eckwall et al. 2001; Schottel et al. 2001). Disease suppression by streptomycetes owes partially to their exudation of various antimicrobials, helminticides, and enzymes degrading fungal cell walls and insect exoskeletons (Weller et al. 2002).

To date, approximately 17% of biologically active secondary metabolites (7,600 out of 43,000; Berdy 2005) have been characterised from the filamentous streptomycetes. These commonly produce two to three dominant secondary metabolites, along with approximately ten minor compounds. For such mixtures to become effective, the bacteria often produce synergistically acting compounds,

and the cost of their production is greatly decreased due to the so-called combinatory biosynthesis. Acquisition and retention of the substance diversity are enabled due to horizontal gene transfer between streptomycete isolates, together with strong microbial competition (Firn and Jones 2000; Challis and Hopwood 2003; Davelos et al. 2004; Weissman and Leadlay 2005).

When applied as a single substance, most of the chemicals produced by streptomycetes do not possess any activity against specific target microbes unless tested at high concentrations (Firn and Jones 2000). Streptomycetes, however, simultaneously produce several bioactive secondary metabolites, which, in combination, possess a strong biological activity (Challis and Hopwood 2003). Such mixtures, often consisting of antibiotics, metal chelators, and growth regulators, may act in a synergistic way (Cocito et al. 1997; Liras 1999). Other sorts of streptomycete chemicals may also act together. In their search for secondary metabolites from streptomycetes, Fiedler et al. (2001) detected an uncommon iron-chelating substance from the culture extracts of two streptomycete strains. This was identified as enterobactin, a characteristic siderophore of *Enterobacteriaceae* spp. The production of enterobactin in addition to the common siderophores, desferri-ferrioxamine B and E, could be an important fitness factor in an iron-poor soil substrate (Challis and Hopwood 2003).

As the frequency of microbial encounters increases with increased population density, the extent to which streptomycetes release chemicals to the soil is also strongly affected. For example, populations of high density have a stronger relative benefit from antibiotics production than low-density populations (Wiener 2000). Davelos et al. (2004) showed that frequency and intensity of antibiotics production by streptomycetes are related to the location of the streptomycetes in the soil. Where high population densities were achieved, the isolates produced more frequently antibiotic substances (Davelos et al. 2004). In the rhizosphere, the microbial population densities are extremely high, and the data from Davelos et al. (2004) suggest that the rhizosphere could be a hotspot for antibiosis. Indeed, Frey-Klett et al. (2005) were able to show that the rhizosphere effect leads to the enrichment of bacteria that suppress plant pathogenic fungi.

In addition, the presence of biocontrol activity determinants other than direct antagonism has been suggested by several studies. There is evidence that bacterial influence on plant growth is also an important determinant in biocontrol. Eight antagonistic *Streptomyces* isolates were tested for their ability to control *Phytophthora* root rots on alfalfa and soybean (Xiao et al. 2002). The strongest indicator of disease suppression in alfalfa by the antagonist was an increase in alfalfa biomass following inoculation with the bacterial isolate. In this case, direct enhancement of alfalfa growth by *Streptomyces* may be one of the key mechanisms by which *Streptomyces* antagonists enhance plant health. In our own experiments, we have observed a similar dependence between plant growth promotion and increased disease resistance, while screening for streptomycete strains that are able to suppress *Brassica* dark leaf spot development in *A. thaliana* (Herold M, Schrey S, Hampp R, Tarkka M, unpublished).

Upon infection by certain rhizobacteria, plants acquire an increased resistance to pathogen attack. This phenomenon has been classified as priming (Conrath et al. 2002). Rhizosphere and endophytic streptomycetes have been recently identified as such disease resistance inducing species (Lehr et al. 2008; Conn et al. 2008). We have studied the mechanisms of disease suppression by the streptomycete GB 4-2 against *Heterobasidion* root and butt rot in Norway spruce seedlings. Unexpectedly, GB 4-2 promoted the physiology of the pathogenic fungus: mycelial growth, germination rate of fungal spores, extension of fungal germ tubes, and even early colonisation of outer cortical layer of the plant root were all enhanced in the presence of the bacteria. However, later, disease development was blocked by the bacterium, since the port of fungal entry into the vascular tissue, the root cortex, was blocked by the formation of cell wall thickenings in co-inoculated plants. In addition, the vascular tissue was rendered inaccessible due to increased xylem formation and strong lignification (Lehr et al. 2008). Together, these data indicate that the inoculation with GB 4-2 sensitised the plant to enhance its responsiveness to the root pathogen. This is another example for a trade-off of increased carbon allocation to the root system (see GDB).

An important finding was associated with this latter result. The infection of needles by *Botrytis cinerea* was also reduced in Norway spruce due to pre-treatment seedlings with *Streptomyces* GB 4-2, suggesting increased systemic defence reactions. To further analyse the underlying mechanism, the accumulation of defence-related transcripts in *A. thaliana* during its interaction with GB 4-2 in the roots and/or with the phytopathogenic fungus *Alternaria brassicicola* in the leaves was investigated (Schrey, unpublished). The aim of these gene expression analyses is to unravel if GB 4-2 provokes a plant immune response similar to ISR or systemic acquired resistance (SAR). In both ISR and SAR, prior treatment results in an enhanced defence response against a subsequent challenge by a pathogen (reviewed by Conrath 2006). In general, ISR is commonly induced upon the challenge of plants by non-pathogenic root-colonising bacteria, while SAR is mediated by phytopathogens. But leaves also can be the site of ISR (Fig. 5.1). Using a set of *A. thaliana* genes related to plant immunity (von Rad et al. 2005), we have observed that the response of *A. thaliana* to GB 4-2 involves changes in the expression of genes associated not only with SAR but also with ISR. Interestingly, ISR-related changes occur in the absence of a challenge by the pathogenic organism, indicating a novel, possibly GB 4-2 specific response pattern in *A. thaliana* (Schrey, unpublished).

Conn et al. (2008) investigated the impact of plant protecting actinomycetes on disease resistance related gene expression in *A. thaliana*. The bacterial inoculation promoted *A. thaliana* growth and endophytic colonisation in the plant tissues. Suppression of *Erwinia carotovora* soft rot as well as of *Fusarium oxysporum* wilt disease was also observed. Gene expression responses to streptomycetes were specific to the bacterial isolate, e.g., inoculation of *A. thaliana* seeds with *Streptomyces* sp. EN27 resulted in a 19-fold induction of the *PR-1* transcript, whereas the closely related strain, *Streptomyces* sp. EN28, was able to induce the defence gene *PDF1.2* by 23-fold. In dual inoculations, the bacteria were able to prime both the SAR and the ISR pathways of *A. thaliana*, upregulating genes in either pathway

depending on the infecting pathogen. The use of defence-compromised mutants of *Arabidopsis* showed that *Streptomyces* sp. EN27 induced resistance to *E. carotovora* by a NPR1 (nonexpression of PR proteins)-independent and to *F. oxysporum* by a NPR1-dependent pathway. In conclusion, the gene expression responses to streptomycetes indicate novel patterns of priming by these bacteria, sharing features of both previously characterised pathways, ISR and SAR.

Plant defence responses can be suppressed by specialised organisms. These organisms can produce physiologically active levels of metabolites such as enzymes that act on plant toxins, or exude their own toxins *in planta* that interfere with plant metabolism in ways that benefit the attacker (Bruce and Pickett 2007). They can also produce signals that disrupt the plant's own defence signalling pathways (Cui et al. 2005). There is evidence for the attenuation of plant by the streptomycete strain *Streptomyces* AcH 505.

AcH 505 is a so-called mycorrhization helper bacterium, i.e., a bacterium that facilitates the formation of ectomycorrhizal symbiosis (Frey-Klett et al. 2007). Both water soluble as well as volatile bacterial substances are involved in AcH 505–fungus–plant interactions. First of all, in ectomycorrhiza fungal mycelial growth is promoted by auxofuran, an auxin-related compound produced by AcH 505. In addition, AcH 505 produces an inhibitor of mycelial growth, the antibiotic WS-5995 B (Riedlinger et al. 2006). As the influence of WS-5995 B dominates over that of auxofuran, only the WS-5995 B tolerant fungal strains are able to colonise the host plant.

In addition, the growth of a WS-5995 B sensitive isolate of an important plant pathogen, *Heterobasidion* sp., was inhibited by AcH 505, and we envisaged a potential application for AcH 505: simultaneous growth promotion of mycorrhizal as well as growth suppression of pathogenic fungi (Maier et al. 2004; Schrey et al. 2005). To determine if AcH 505 could serve as a biocontrol agent against *Heterobasidion* root and butt rot, the bacterial influence on mycelial growth of *Heterobasidion* sp. isolates, cultured on wood discs and roots of Norway spruce, was determined. It had been previously suggested (Schottel et al. 2001) that single pathogen–antagonist strain studies may contribute only limited insights into the dynamics of antagonist–pathogen interactions. Our data agreed well with that suggestion: whereas 11 tested *Heterobasidion* strains were sensitive against the antibiotic and suppressed by the bacterium, the growth of another strain, *Heterobasidion annosum* 331, was unaffected by AcH 505. Hazardous in the light of biocontrol applications, root colonisation by *Heterobasidion annosum* 331 was promoted by the bacterium due to a decrease in defence-related gene expression in the host, Norway spruce, which is of advantage only for symbiotic fungi (Lehr et al. 2007). Using a culture system where the bacterium was separated from plant roots and fungal mycelium, we have been able to show that increased fungal colonisation is due to volatiles produced by AcH 505 (Störk M, Lehr N, Hampp R, Tarkka M, unpublished). In conclusion, metabolite production by bacteria like AcH 505 can lead to the inhibition of some and to the benefit of other microorganisms. Depending on the species spectrum in the habitat of the host plant, the development of symbiosis and/or disease may be promoted by such streptomycetes.

5.3 Quorum Sensing Within Bacterial Communities and Trans-kingdom Interactions of *N*-Acyl Homoserine Lactones with Plants

Many environmental and interactive important traits in bacteria, such as antibiotic, siderophore, or exoenzyme (like cellulose, pectinase) production, are regulated in a population density dependent manner using small signalling molecules. This phenomenon, called quorum sensing, is more widespread amongst all bacteria than originally thought. Many different bacterial species are communicating or "speaking" through various secreted molecules. The production is often sophistically regulated as an auto-inducing mechanism, such as the synthesis of *N*-acyl homoserine lactones (AHL), which occur in many variations of molecular structure in a wide variety of Gram-negative bacteria (Eberl 1999). In Gram-positive bacteria, other compounds, such as peptides, AI-2, and quinolone (PQS), regulate cellular activity and behaviour through sensing the cell density. However, it is probably not just the cell density but an integrated measure of the quality of the cellular surrounding (diffusion space, etc.) and the colonisation density, which finally provides the information about crucial habitat conditions leading to higher precision of adaptive gene expression and physiological efficiency (Hense et al. 2007), which has a direct positive impact on evolutionary selection. In addition, some of these signal molecules have also non-signalling roles for important processes, such as nutrient scavenging, ultrastructure modification, and competition between bacteria (Schertzer et al. 2009). In particular, iron siderophores, like pyochelin and quinolone (see above) which interfere with cellular iron stores, are small signal molecules with central functions in the iron homoeostasis. Most interestingly, evidence is accumulating that quorum sensing compounds produced by rhizosphere bacteria are also recognised by plants, and specific responses are induced upon specific molecular interactions. *Serratia liquefaciens*, e.g., strain MG1 is known as a producer of C4- and C6-homoserine lactones and in situ AHL production, occur in the rhizoplane (Gantner et al. 2006). When *Serratia liquefaciens* was inoculated to roots of tomato (Microtom^{R}) plants, the systemic resistance was clearly increased (Schuhegger et al. 2006). The induction of genes related to systemic pathogen response in tomato, like chitinase and PR1, by C4- and C6-homoserine lactones in axenic test systems is contrasted by the response of *A. thaliana* towards AHL compounds with short side chains, because systemic resistance responses are not elevated (von Rad et al. 2008). However, most recently, the induction of systemic resistance responses towards the leaf pathogen *Pseudomonas syringae* DS3000 by oxo-C14-homoserine lactone treatment in the rhizosphere of *A. thaliana* was demonstrated (Zuccharo and Kogel, personal communication). This indicates that there may be different cellular receptors and signalling pathways of bacterial signalling compounds in plants. In addition, AHL compounds with acyl side chain lengths smaller than C8 were found to enter the roots more readily and are transported up to the shoots (Götz et al. 2007). Additionally, it could be shown that the ^{3}H-labelled C6- and C8-AHLs are taken up into the central cylinder and that their transport within the roots is an active process, which is further accelerated by the

transpiration flow (Riedel, personal communication). AHL compounds with long side chains (e. g. larger than C10) stick to the root surface and are not transported substantially within, e.g., barley, maize, or *A. thaliana* (Götz et al. 2007; von Rad et al. 2008). In some other plants, like many legumes, the plant's AHL-hydrolyzing activities are efficiently degrading AHLs and thus prohibit a substantial uptake of the AHL compounds into the plants (Götz et al. 2007; Delalande et al. 2005).

Apart from plants, the effects of quorum sensing molecules on fungi were already found, like the induced morphological changes of *Candida albicans* under the influence of *Pseudomonas aeruginosa* (Hogan et al. 2004). Long side chain as well as short side chain *N*-acyl-homoserine lactones were found to modulate also the host immune response and inflammatory signalling pathways of invertebrates (for review see Cooley et al. 2008).

5.4 Mycorrhiza-Associated Bacteria and Their Effects on Symbiosis Development

5.4.1 Endo- and Ectomycorrhizal Fungi

There are many examples for close bacteria–mycorrhiza interactions. In the case of arbuscular mycorrhizae, a group of Gram-negative bacteria closely related to *Burkholderia*, described as *Candidatus Glomeribacter margarita*, was identified as endofungal bacteria by the group of Bonfante (Lumini et al. 2007). This bacterium could not be cultivated until now. Using fluorescent staining techniques and confocal laser scanning microscopy, these bacteria could be located mostly in spores of *Gigaspora margarita* (Bianciotto et al. 2004). There is recent evidence that these endofungal bacteria exert beneficial functions to their mycorrhizal host. When the lines of *G. margarita* containing the endosymbiotic bacteria were compared with the lines which had been cured of the bacteria, it became clear that the endosymbiotic bacteria strongly improved the presymbiotic growth of the arbuscular mycorrhiza-forming fungus, as shown by increased hyphal elongation and branching following treatment with root exudates. Therefore, these bacteria support the growth of such fungi and could thus possibly support mycorrhiza establishment under soil conditions which are unfavorable to the fungi (Frey-Klett et al. 2007). It was demonstrated by Brulé et al. (2001) that in the case of the MHB, *P. fluorescens* BBc6R8 ectomycorrhizal *Douglas-fir–Laccaria bicolor* symbiosis, a similar stimulation of presymbiotic fungal growth may occur. Furthermore, the *Paenibacillus* sp. isolate of *L. bicolor*, which was found as endofungal bacterium within hyphae of *L. bicolor* (Bertaux et al. 2003), was shown to significantly promote the growth of *L. bicolor* in vitro (Deveau et al. 2007). As suggested by Frey-Klett et al. (2007), the two mycorrhizal fungi, *G. margarita* (AM-forming) and *L. bicolor* (ectomycorrhiza (EcM)-forming) illustrate two different evolutionary processes of bacterial colonisation of fungal cells. *G. margarita* is an example of long-lasting co-evolution between the fungus and its endobacterium, *Glomeribacter gigasporarum*, through

fungal spore generations (Bianciotto et al. 2004). The small genome size of the endobacteria and the difficulties in cultivating these bacteria in vivo (Jargeat et al. 2004) support the hypothesis of co-evolution.

In contrast, *L. bicolor* harbors fluctuating endobacterial communities that appear to be environmentally acquired, because in EcM collected from forest soils, a high variety of α-proteobacteria was found to colonise hyphae of *L. bicolor* internally (Bertaux et al. 2005). It can be speculated that the intracellular colonisation of *L. bicolor* by soil bacteria would support the fungal host to adapt to changing environmental challenges, especially during the presymbiotic life in soil. This would provide examples for the hologenome theory, which states that the combined metabolic potential and activity of symbiotic associations of microorganisms with plants and animals provide a better basis for the evolution of holobionts with improved fitness in view of environmental challenges (Zilber-Rosenberg and Rosenberg 2008).

5.4.2 *Sebacinales: The Plant Growth Promoting Fungus* Piriformospora indica

More recently, endofungal bacteria were also described in fungi of the order *Sebacinales* (Basidiomycota; Sharma et al. 2008), which constitute a special group of fungi, being mostly involved in ericoid and orchid mycorrhizae (Selosse et al. 2007). *Piriformospora indica* and other members of the *Sebacinales* are wide-host range root-colonising, mostly symbiotic, fungi which allow the plants to grow better under physical and nutrient stress conditions. The big advantage in practical use over arbuscular mycorrhizae is the possibility to cultivate some of these fungi on complex and even minimal substrates on agar plates or in submerged culture. *P. indica* in particular was originally isolated from the roots of different xerophytes in the Indian Thar desert (Verma et al. 1998). Since it can be grown easily without the plant and is amenable to molecular genetic techniques, it is most interesting not only for basic research, but also for biotechnological applications (for review see Oelmüller et al. 2009). It has plant growth promoting and biofertilizer properties especially in nutrient-deficient soils and can act as a biocontrol agent against biotic and abiotic stresses including root and leaf pathogens and insect invaders (Badge et al. 2010). Furthermore, it is a bioregulator for plant growth development, early flowering, and enhanced seed production and it is a bio-agent for the hardening of tissue-culture-raised plants. The *P. indica*/*A. thaliana* as well as the *P. indica*/barley systems were successfully used in the identification of important target compounds of the fungus in the course of interaction and molecular colonisation (Oelmüller et al. 2009).

It turned out that in *P. indica* as well as in several *Sebacina vermifera* isolates, endofungal bacteria could be demonstrated by FISH analysis and confocal laser scanning microscopy or at least by 16S PCR analysis (Sharma et al. 2008). While *Rhizobium radiobacter* (formerly *Agrobacterium tumefaciens*) was identified as

endofungal bacterium, different isolates of *S. vermifera* had *Paenibacillus* sp., *Acinetobacter* sp., or *Rhodococcus* sp. as bacterial associate. With the exception of *R. radiobacter*, the other bacteria could not be cultivated. Since all attempts to cure the fungi from their endobacteria were unsuccessful so far, they seem to have important functions for the fungus. In the case of *R. radiobacter*, it could be shown that upon inoculation to barley, this bacterium produced a similar shoot weight increase and decrease in powdery mildew pustules formation as compared to inoculation with the *P. indica* (plus its endofungal *R. radiobacter*) (Sharma et al. 2008). Looking for properties of plant growth stimulation in the bacterium, not only the production of indole acetic acid, but also the biosynthesis of several *N*-acyl homoserine lactones was noticed (Li and Fekete, unpublished results). Interestingly, *R. radiobacter* also was found to use *p*-coumaric acid as a precursor for *N*-coumaryl homoserine lactone (Schaefer et al. 2008). The production of this variety of homoserine lactones may contribute to the plant growth promotion ability in the tripartite symbiotic system barley (or *A. thaliana*), *P. indica*, and the bacterium *R. radiobacter*.

5.5 Ectomycorrhiza-Forming Fungi

Among other types of mutualistic interactions, the formation of ectomycorrhizas (EcM), a symbiosis between fine roots of trees and certain soil fungi, is a way to overcome limitations in mineral nutrients and (easily degradable) carbohydrates, typical for many forest ecosystems. Due to the tree canopy, soil temperature rarely exceeds 15°C and degradation of organic substances often containing high amounts of phenolic substances results in relatively low pH values (below 5). As a consequence, bacterial turnover and mineralisation of organic matter is reduced and soil fungi have a large impact on nutrient recycling and plant mineral nutrition. EcM formation is thus typical for trees in boreal and temperate forests of the northern hemisphere, and alpine regions world-wide. The exchange of fungus-derived nutrients for plant-derived carbohydrates enables the colonisation of mineral nutrient-poor environments by the trees (Smith and Read 2008).

EcM fungi can both live in association with plant roots as symbionts and, possibly also as facultative saprotrophs. EcM fungal colonies remain functionally interconnected, revealing intense nutrient and carbohydrate exchange over variable distances. Vegetative mycelia can differentiate into distinct hyphal networks with different functions, differently densely growing solitary hyphae or organised as rhizomorphs (Agerer 2001) and hyphal mantle/intercellularly growing hyphae (Hartig net). When fungal hyphae recognise an emerging fine root of a compatible plant partner, they direct their growth towards it and colonise the root surface, forming a mantle of hyphae, which encloses the root, and isolates it physically from the surrounding soil (Blasius et al. 1986). Root hairs, which are normally formed by rhizodermal cells, are suppressed by EcM formation. After or parallel to mantle formation, fungal hyphae grow inside the colonised fine root, forming highly

branched structures in the apoplast of the rhizodermis (also in the root cortex in gymnosperms). This so-called Hartig net creates a large surface area between both the partners (Kottke and Oberwinkler 1987). EcM are usually composed of two fungal networks with different functions (Harley and Smith 1983; Kottke and Oberwinkler 1987; Smith and Read 2008): the Hartig net as plant/fungus interface, adapted to the exchange of plant-derived carbohydrates for fungus-derived nutrients and the fungal mantle for intermediate storage of nutrients that are delivered by soil growing hyphae and further directed to the Hartig net, as well as carbohydrates that are taken up by hyphae of the Hartig net and are then supplied to the mycelium growing within the soil.

5.5.1 Progress with Regard to Genetic Information from Ectomycorrhiza-Forming Fungi

The *Laccaria bicolor* genome is the first of a fungal symbiont to be sequenced and its release is taking mycorrhizal genomics one large step further (Martin et al. 2008). This, together with the available genomes of saprotrophic and pathogenic fungi (~50; e.g. Galagan et al. 2005; Soanes et al. 2008), offers the opportunity to decipher key components of functions of ectomycorrhiza-forming fungi.

The nuclear genome of *L. bicolor* is estimated to contain 60 million bases, spread out over 12 chromosomes and is thus bigger than that of most previously published fungal genomes. About 19,000 predicted coding regions are found of which almost 25% still lack characterised orthologues in other systems. The larger size is partly explained by an unusually high number of transposable elements that constitute more than 20% of the genome. The even higher density of transposable elements which have been found in *T. melanosporum* and the poplar rust, *Melampsora laricipopulina* (Martin et al. unpublished), suggests a possible relationship between biotrophy and transposable elements richness. Compared with other fungal genomes, the *L. bicolor* genome contains both more and larger gene families which evolved from common ancestors found in other fungi (Lucic et al. 2008). These include several fungal multigene families not only coding for membrane, cell wall, and secreted proteins, but also up to 1,000 lineage-specific families (Martin et al. 2008).

5.5.2 The Saprotrophic Face of ECM Fungi

Through the net carbon input (sequestration of host carbohydrates within fungal networks) and loss (decomposition of soil organic matter, for reviews see (Smith and Read 2008; Talbot et al. 2008), mycorrhizal fungi significantly influence the CO_2 sink efficiency of forest ecosystems.

In order to understand the role played by the many ECM fungal partners in complex forest ecosystems, direct methods to assess a range of activities relevant to

tree nutrition and metabolic activity on single excised ectomycorrhizal root tips were introduced. To assess their physiological activity, Jany et al. (2003) compared the [^{14}C] glucose respiration of two ECM species and found a high influence of the respective soil conditions (e.g. drought) and species-specific differences. Using sensitive microplate assays for the detection of phosphatase, chitinase, dehydrogenases, glucosidases, and other hydrolytic enzymes, impacts of season, temperature, and soil moisture effects on enzyme activities were found (Pritsch et al. 2004; Buee et al. 2005; Courty et al. 2005). It can thus be concluded that the ability of degrading litter polymers and assimilating absorbed nutrients widely varies between ECM species, that is enhanced upon carbon and nitrogen starvation, and that it fluctuates seasonally (Buee et al. 2005, 2007; Courty et al. 2007).

Laccaria bicolor possesses expanded glycosyl hydrolase families and a large set of secreted proteases, chitinases, and glucanases associated presumably with the hydrolysis of organic matter from dead organisms (Martin and Tunlid 2009; Nuutinen and Timonen 2008; Martin and Nehls 2009). The thereby indicated strong saprotrophic capability of the fungus enables a symbiosis-driven access of trees to nutrients held up in complex molecules in the soil that are outside of the symbiosis barely available to the plant partner. However, even when capable of catabolising chitin-, glucan-, and protein-complexes from decaying litter, *L. bicolor* is not able to degrade plant cell wall polysaccharides (cellulose, pectins, and pectates; Martin et al. 2008) due to massive gene loss. This prevents ECM fungi from degrading their host cells and, as a consequence, triggering plant defence reactions and could be a prerequisite of adaptation to symbiotic lifestyle. This result has to be taken, however, with care as it may reflect the evolution of one specific clade of mycorrhizal taxon and not the lifestyle of all ectomycorrhizal species. The by-products such as amino acids or monosaccharides resulting from degradation of organic matter are efficiently taken up (Fajardo Lopez et al. 2008; Lucic et al. 2008; Morel et al. 2008), and metabolised (Fajardo López et al. 2007; Deveau et al. 2008; Nuutinen and Timonen 2008; see below). Due to its importance for plant and fungal nutrition, research was mainly focused on nitrogen and only to a lesser extent on phosphate (Tatry et al. 2008), sulphur (Mansouri-Bauly et al. 2006), and potassium (Corratge et al. 2007).

The whole range of inorganic and organic nitrogen sources found in forest soils can be used by *L. bicolor*. Genes encoding putative importers for nitrate, ammonium, urea, amino acids, peptides, nucleotides, allantoin, and polyamines were found in the genome (Lucic et al. 2008). While a proof of function is currently under progress for selected *L. bicolor* gene families, individual nitrogen import proteins of other ECM fungi were previously characterised, e.g. (Willmann et al. 2007; Morel et al. 2008). Like in other organisms with the capability to exude hydrolases, the expression of genes encoding high-affinity importers is frequently induced/enhanced by nitrogen starvation while the transcript levels of low-affinity importers are only marginally affected (for reviews, see Tarkka et al. 2005; Müller et al. 2007). However, as mobilisation capability of a given nitrogen source differs between ECM fungi (Nygren et al. 2008), additional models have to be investigated in future research to take into account fungal adaptation to various forest ecosystems.

In contrast to high levels of inorganic phosphate (Pi) within living systems (in the mM range), free concentrations of Pi in soil are very low, ranging from 1 to 10 μM (Bieleski 1973; Vance et al. 2003). The low availability of Pi in soil is due to its negative charges, resulting in rapid sequestration by cations (Vance et al. 2003) and renders Pi highly immobile (Hinsinger 2001). Uptake of phosphate by plant roots quickly generates a depletion zone, making this element mostly limiting for plant growth. In the association between *Hebeloma cylindrosporum* and its natural host plant *Pinus pinaster*, accumulation of Pi in whole plants was significantly correlated with soil exploration by external hyphae, suggesting that plant Pi mainly originates from fungal Pi uptake (Aquino and Plassard 2004). Genes, revealing significant similarities to phosphate transporters, were identified for *Amanita muscaria* (Barbosa 2004) and *H. cylindrosporum* (*HcPT1* and *HcPT2*; van Aarle et al. 2007; Tatry et al. 2008). Complementation of a yeast mutant (Δpho84), defective in phosphate transport, confirmed both corresponding *H. cylindrosporum* proteins (HcPT1 and HcPT2) to mediate $Pi:H^+$ symport, exhibiting K_M values of 55 and 4 μM, respectively. Fluorescent in situ RT-PCR (van Aarle et al. 2007) and real-time RT-PCR (Tatry et al. 2008) showed that Pi starvation increased *HcPT1* expression, while transcript levels of *HcPT2* were less dependent on Pi availability, rendering both genes good candidates for a role in fungal Pi uptake from the soil solution when the host plant is grown in soil with low Pi availability.

The macronutrient sulphur is assimilated in the inorganic form by plants, fungi, and most bacteria. Starvation results in an increased uptake by plants (Lappartient et al. 1999), bacteria (Kredich 1993), and fungi (Ono et al. 1999; Van de Kamp et al. 2000), while it is strongly repressed in the presence of reduced sulphur compounds (e.g. glutathione, cysteine, H_2S; Herschbach and Rennenberg 1994; Lappartient et al. 1999; Van de Kamp et al. 2000; Westerman et al. 2001). This indicates a tight regulation by sulphur homeostasis. Contrasting the enhanced sulfate uptake by host plants (clover and maize) with arbuscular mycorrhiza (Gray and Gerdemann 1972; Banerjee et al. 1999), the ectomycorrhizal association had no impact on the rate of sulfate uptake but influenced sulfate loading into the tree xylem (Seegmüller et al. 1996; Kreuzwieser and Rennenberg 1998). Ectomycorrhizal beech roots did not reveal a de-repression of sulfate import by sulphur deficiency as non-mycorrhizal roots do, indicating a significant contribution of the fungal partner in sulfate uptake (Kreuzwieser et al. 1996; Kreuzwieser and Rennenberg 1998). As in other organisms, sulfate uptake was increased by sulphur starvation in the ECM fungus, *L. bicolor* (Mansouri-Bauly et al. 2006). In contrast to bacteria, yeast, and plants, sulfate uptake in *L. bicolor* was not inhibited by glutathione. However, the regulation of sulfate assimilation in *L. bicolor* (indicated by the reduction in activity of 3′-phosphoadenosine 5′-phosphosulfate reductase after treatment with glutathione) is again similar to that of other organisms. Together these data clearly indicate a regulatory uncoupling of sulfate uptake and its reduction in the presence of reduced sulphur compounds in *L. bicolor*, a response which has not yet been reported for other organisms. A potential explanation is that *L. bicolor* improves sulfate uptake and export towards the plant in symbiosis, and receives reduced sulphur back from the plant partner (Mansouri-Bauly et al. 2006).

5.5.3 The Symbiotic Interface of ECM Fungi

In contrast to the uptake by growing soil hyphae, nutrient export at the plant/fungus interface is still poorly understood. Two major potential nitrogen sources, ammonium and amino acids, are thought to be released at the symbiotic interface (for a review see Chalot et al. 2006), and homologs of yeast proteins involved in their excretion are found in different ECM fungi (Selle et al. 2005; Chalot et al. 2006; Lucic et al. 2008). Regarding the plant host, no data on the impact of symbiosis on amino acid import are available yet. However, high-affinity ammonium importers are clearly upregulated upon ectomycorrhiza formation (Selle et al. 2005; Couturier et al. 2007). A prerequisite for an efficient export of any nitrogen source is that the corresponding fungal import is repressed at the plant/fungus interface. In agreement with this, transcript levels of a high-affinity ammonium importer (Willmann et al. 2007), but not that of an amino acid importer (Nehls, unpublished; amino acids are organically bound nitrogen resource preferentially taken up by EcM fungi) were strongly reduced in *Amanita muscaria* ECM. In contrast, transcript levels of putative high-affinity plant ammonium importers were induced upon EcM formation in *L. bicolor* (Lucic et al. 2008). Furthermore, and similar to *A. muscaria* (Nehls, unpublished), urease transcript levels increased in *L. bicolor* during symbiosis. It has been suggested that the release of ammonium through urease activity may be involved in fungal nitrogen export in mycorrhizal symbiosis towards the host plant (Morel et al. 2005; Cruz et al. 2007; Lucic et al. 2008). As a consequence, two scenarios for nitrogen export into the plant tissue at the plant/fungus interface can be suggested: (1) Cytoplasmic ammonium is assimilated into amino acids which are transferred to the host, and re-import of leaked ammonium is enhanced to avoid unattended loss. (2) Ammonium is released by the hyphae at the symbiotic interface (here re-import is repressed by a combination of transcriptional and post-transcriptional control), while the fungal sheath may be active in ammonium re-import to avoid its loss into the soil due to leakage. To get a better understanding of the fungal nitrogen export mechanism, microdissection (distinction of metabolite content and protein activity within hyphal networks) together with the use of fungal mutants (Kemppainen et al. 2008), defective in nitrogen export, will be necessary.

5.5.4 Carbohydrate Allocation

Essential for mycorrhizal symbiosis is a continuous fungal carbohydrate nutrition by the host plant (Saravesi et al. 2008). As a consequence, ECM reveal an elevated sink strength compared to non-mycorrhizal fine roots (for a review, see Nehls 2008). The *L. bicolor* genome (Martin et al. 2008) enables for the first time the exploration of ECM fungal capacity to take up carbohydrates. Due to the lack of fungal invertase and sucrose importer genes (Fajardo Lopez et al. 2008; Martin et al. 2008), apoplastic sucrose (the supposed major carbon source) can be hydrolyzed by host-derived enzymes only, making *L. bicolor* also locally dependent on its host.

Fifteen genes encoding putative hexose importers were found, of which five are functional hexose importers as shown by heterologous expression in yeast (Fajardo Lopez et al. 2008). Transcript profiling showed an enhanced transcript level for half of these genes, indicating a strong increase in hexose uptake capacity at the plant/fungus interface, similar to what was observed for *Amanita muscaria*. However, the comparison of the two ECM fungi revealed large differences in their control of gene expression. While the expression of *A. muscaria* genes was regulated by the apoplastic hexose concentration, development-dependent control was observed for *L. bicolor* (Fajardo Lopez et al. 2008). Further contrasting the situation in *A. muscaria* (but also *H. cylindrosporum*), where glucose and fructose are taken up simultaneously by fungal hyphae, *L. bicolor* does not use fructose until the external glucose concentration is below the K_M value of its high-affinity hexose importer. This behaviour might indicate a less efficient carbohydrate exploitation by *L. bicolor* in symbiosis, reflecting different capabilities of EM fungi.

To maintain a strong carbohydrate sink in symbiosis, increased carbon fluxes through glycolysis (Kowallik et al. 1998) and into storage compounds were observed in ECM of different fungi, in addition to an enhanced hexose uptake (Martin et al. 1985; Fajardo López et al. 2007; Wiemken 2007). This view was confirmed by whole genome transcript analysis in *L. bicolor* (Deveau et al. 2008). By physical separation of ectomycorrhizal networks and monitoring of transcript levels and activities of key enzymes of fungal trehalose biosynthesis, Fajardo López et al. (2007) could localise the enhanced flux into these storage carbohydrates in *Amanita muscaria* hyphae of the plant/fungus interface. However, further biochemical analysis and generation of mutants defective in biosynthesis of trehalose and other storage compounds will be necessary to confirm their function as integral components of fungal sink generation in symbiosis.

5.5.5 *Changing the Transcriptome: A First Step Towards Understanding Ectomycorrhiza Formation*

For expression profiling of ECM development and function, comprehensive microarray-based gene expression analyses of different developmental stages (pre-symbiotic, fully developed), using different ectomycorrhizal systems (*Laccaria bicolor*/*Pisolithus microcarpus*/*Tuber borchii* + *Tilia americana*, *Paxillus involutus* + *Betula pendula*, *Laccaria bicolor* + *Populus trichocarpa* or *Pseudotsuga menziesii*, *Amanita muscaria* + *Populus tremula* × *tremuloides*), have been performed (Peter et al. 2003; Johansson et al. 2004; Menotta et al. 2004; Duplessis et al. 2005; Wright et al. 2005; Küster et al. 2007; Nehls et al. 2007; Martin et al. 2008). They provide a quantitative assessment of transcript abundance and can be used to predict gene function based on the hypothesis that functionally related genes are mainly transcriptionally regulated. With the exception of those from *L. bicolor* (Martin et al. 2008; see below), these arrays represent a maximum of about 10% of the gene repertoire of a given fungus, and conclusions, that can be drawn, are thus limited.

The number of symbiosis-regulated and symbiosis-specific *L. bicolor* transcripts is very low (<3%; Martin et al. 2008). Transcript profiling of *L. bicolor* interacting with either *Populus trichocarpa* or *Pseudotsuga menziesii* roots has revealed several genes with a striking upregulation in symbiotic tissues (>100-fold; Martin et al. 2008). Most of them are coding for proteins belonging to expanding and lineage-specific gene families (Martin and Tunlid 2009). A large number of the mycorrhiza-upregulated transcripts are encoding small, putatively secreted, cysteine-rich proteins, which may play, together with RGD-motive (arginine–glycine–aspartic acid)-containing acidic proteins (Le Quere et al. 2005) and hydrophobins (Laurent et al. 1999), a role in the construction of the novel symbiotic apoplastic interface. Several cysteine proteinase inhibitors (mycocypin gene family) are amongst the most highly upregulated transcripts suggesting that *L. bicolor* may use proteinase inhibitors for counteracting plant secreted protease activities during its apoplastic growth.

5.6 Conclusions and Relevance with Respect to SFB Data

Light, carbon dioxide, water, and nutrients are the basis for plant growth, at least under controlled conditions, e.g. greenhouses, etc. In the field, a plant has to cope with many more parameters such as competing plants, herbivores, pathogens, or pollutants.

Within the rhizosphere, multi-level organismic interactions become increasingly visible. We know now that large groups of plant-growth promoting bacteria exist within the rhizosphere and mycorrhizosphere, which release products of their secondary metabolism. These can selectively interact with plant symbiotic and plant pathogenic bacteria and fungi, thereby establishing new balances between such organisms and thus affecting plant viability. In addition, by systemic signal propagation, compounds released can also render plants more resistant to shoot pathogens. Such support is not for free, and the plant has to invest a considerable amount of its photo assimilates into the support of the rhizosphere community. This is done by allocation of carbohydrates to the rhizo-/mycorrhizosphere which affects shoot growth, delivering a good example for the GDB theory. Air pollutants such as ozone have been shown to interfere with carbon allocation, and can thus severely affect such fine-tuned organismic interactions (comp. Chap. 10). Altered fungal communities, together with less carbon allocation will also have consequences with respect to the attraction of bacterial communities. For these, twice-ambient ozone caused a shift from Gram-negative to Gram-positive bacteria. The latter include streptomycetes, which are important producers of antibiotics. These can affect pathogenic microorganisms (see Sect. 5.2.2). Surprisingly, elevated ozone resulted in both a higher microbial biomass and abundance of microbes utilising plant C (comp. Chap. 4). This could be due to the starch-enriched litter as carbon allocation into fungal symbionts of the soil is reduced. In summary, the findings show that environmental changes can considerably interact with the microbial rhizosphere communities. This should have an impact on plant performance, and could counteract

pollutant-related forms of damage (see marginal effects of elevated ozone on the investigated beech and spruce trees). Rhizospheric interactions certainly will have an impact on allocation of carbon to either growth or protection against pathogens or herbivores, and will make discussion about possible trade-offs not easier. This becomes clear from Chap. 3.

Acknowledgements As far as own research has been addressed, financial support by the Deutsche Forschungsgemeinschaft is gratefully acknowledged. We are indebted to Nina Lehr, Silvia Schrey, and Mika Tarkka for their input, and to Reinhard Agerer for critical reading and many helpful suggestions. GB 4-2 was isolated by K. Poralla (University of Tübingen).

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