Impact of root exudates and plant defense signaling on bacterial communities in the rhizosphere. A review

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REVIEW ARTICLE

Impact of root exudates and plant defense signaling on bacterial communities in the rhizosphere. A review

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Abstract Despite significant advances in crop protection, plant diseases cause a 20% yield loss in food and cash crops worldwide. Therefore, interactions between plants and pathogens have been studied in great detail. In contrast, the interplay between plants and non-pathogenic microorganisms has received scant attention, and differential responses of plants to pathogenic and non-pathogenic microorganisms are as yet not well understood. Plants affect their rhizosphere microbial communities that can contain beneficial, neutral and pathogenic elements. Interactions between the different elements of these communities have been studied in relation to biological control of plant pathogens. One of the mechanisms of disease control is induced systemic resistance (ISR). Studies on biological control of plant diseases have focused on ISR the last decade, because ISR is effective against a wide range of pathogens and thus offers serious potential for practical applications in crop protection. Such applications may however affect microbial communities associated with plant roots and interfere with the functioning of the root microbiota. Here, we review the possible impact of plant defense signaling on bacterial communities in the rhizosphere. To better assess implications of shifts in the rhizosphere microflora we first review effects of root exudates on soil microbial communities. Current knowledge on inducible defense signaling in plants is discussed in the context of recognition and systemic responses to pathogenic and beneficial microorganisms. Finally, the as yet limited knowledge on effects of plant defense on rhizosphere microbial communities is reviewed and we discuss future directions of research that will contribute to unravel the molecular interplay of plants and their beneficial microflora.

Keywords Biological control · Induced systemic resistance · Microbial diversity · Rhizosphere · Root colonization · Root exudates · Systemic acquired resistance

Contents

1 Introduction

Plants are under continuous attack by pathogenic microorganisms and pest insects. Despite significant advances that

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have been achieved to prevent crop losses due to diseases (Cook 2000), it is estimated that plant diseases cause a 20% yield loss in food and cash crops worldwide (Schumann and D'Arcy 2006). Disease incidence can be minimized by agricultural practices such as crop rotation, the application of chemical pesticides and breeding of resistant crop varieties. However, occurrence of pesticide-tolerant pathogens, the banning of chemical pesticides, and public concern about genetically modified crops, urge the development of biological control of plant diseases (Alabouvette et al. 2006). According to Cook and Baker (1983), "Biological control is the reduction of the amount of inoculum or disease-producing activity of a pathogen accomplished by or through one or more organisms other than man." There are multiple mechanisms by which naturally occurring beneficial bacteria and fungi can suppress disease incidence or severity, including antibiosis, competition for nutrients and space, and the production of lyctic enzymes (Weller 1988; Chet et al. 1990; Chet and Inbar 1994; Handelsman and Stabb 1996; Raaijmakers et al. 2002; Haas and Defago 2005; Van Loon 2007). Of special interest is the enhancement of plant innate defense responses against pathogens by beneficial bacteria and fungi that occur naturally on plant roots (Zehnder et al. 2001; Kloepper et al. 2004; Hoitink et al. 2006; Van Wees et al. 2008; Segarra et al. 2009). This induced resistance of plant defenses is effective against a wide variety of plant pathogens for prolonged periods. It is unknown, however, how the natural microflora on plant roots, that plays an important role in maintaining plant health, reacts to an augmented defensive state of the plant. Whereas fungi, oomycetes, and protozoa constitute important elements of the microbiota associated with plants, this review focuses on the more extensively studied bacterial communities in the rhizosphere.

2 Bacterial abundance and diversity in soil

The number of prokaryotic cells on earth is estimated to exceed 10^{30}, of which the biggest fraction occurs in the soil (Whitman et al. 1998). The soil matrix is a favorable niche for bacteria since both temperature and humidity are relatively stable (Lavelle and Spain 2001). The community structure of the indigenous bacterial microflora in soil is determined by many variables, including geographic location and soil structure (Gelsomino et al. 1999), soil particle size (Postma and Van Veen 1990; Ranjard et al. 2000; Sessitsch et al. 2001), mineral composition (Carson et al. 2009), and agricultural practices (Benizri et al. 2007; Rooney and Clipson 2009). Fierer and Jackson (2006) analyzed almost 100 soil samples from across the North and the South American continent by DNA fingerprinting methods to compare the bacterial community composition and diversity across sites. The authors showed that the diversity of soil bacterial communities differed by edaphic variables, particularly pH, whereas site temperature and latitude were of no influence. In general, pH-neutral soils showed a higher bacterial diversity, whereas acidic soils were least diverse.

Such extensive molecular studies were impossible until a relatively short time ago, when microbial abundance could be assessed mainly by cultivation-dependent techniques, of which counting of bacterial colony-forming units (cfu) on (semi-)selective media was the most popular. But also other methods, such as assessing bacterial enzymatic activities or measurement of soil respiration were of valuable use (Jenkinson and Ladd 1981). Implementation of molecular techniques have greatly contributed to our understanding of the microbial diversity in soil, since they do not depend on a culturable-dependent bias. For instance, PCR-fingerprinting techniques based on differences in the nucleotide sequence of phylogenetic markers, of which the small subunit 16S rDNA is predominantly used, are now widely employed. Nevertheless, culturable-dependent techniques remain important for the physiological and genetic characterization of specific bacterial species containing functionally important traits. Hence, culturable plating methods are still required (Nichols 2007) and further optimized (Janssen et al. 2002).

Microbial diversity is now estimated to comprise up to 10^7 species. Hence, describing bacterial community structure is still a daunting and challenging task (Hughes et al. 2001; Bent and Forney 2008; Little et al. 2008). Torsvik et al. (1990) demonstrated that the number of bacterial genomes in a deciduous forest soil exceeded the genetic diversity found by selective plating by about 200-fold, indicating that bacteria isolated by culturable-dependent techniques are only a fraction of the total soil bacterial diversity. The acceptance that bacterial cell densities revealed by culture-dependent techniques represent only 1–10% of the total bacterial microflora present in soil is now known as 'the great plate count anomaly' (Amann et al. 1995). Therefore, prokaryotic taxonomy is nowadays based on genomic data, which allow classification of non-culturable bacteria as well (Rosselló-Mora and Amann 2001; Konstantinidis and Tiedje 2005).

2.1 Root exudates influence soil microbial communities

Soil organisms have to compete for nutrients and other resources that are sparsely available in soil. Because of these limiting circumstances, bacterial proliferation in soil is slow. However, microbial activity in soil is greatly influenced by plant roots (Bais et al. 2006). The main reason for this is the loss of carbon-containing metabolites from the roots into the soil matrix as a result of rhizodeposition. Rhizodeposition includes shedding of root

cells and the exudation, secretion and leakage of, e.g., sugars, organic acids, and amino acids into the soil (Bertin et al. 2003; Bais et al. 2006). Microorganisms can use these compounds as substrates, resulting in an increased microbial biomass and activity around the roots, the so-called rhizosphere effect. The term rhizosphere, meaning the soil compartment influenced by plant roots, was first defined in 1904 by Lorentz Hiltner (Hiltner 1904; Hartmann et al. 2008), and after a century of rhizosphere research it can be concluded that many microbial interactions occur in this specific environmental niche (Whipps 2001; Lugtenberg et al. 2002).

Up to 40% of photosynthetically fixed carbon is secreted into the rhizosphere (Bais et al. 2006). Root exudation has long been regarded as a passive process. However, increasing evidence is available that ATP-binding cassette transporters in the roots are involved in the translocation of phytochemicals into the rhizosphere, indicating that plants actively secrete metabolites into the environment (Loyola-Vargas et al. 2007; Badri et al. 2008). A wide variety of plants possess specialized root cells that contain many mitochondria, Golgi stacks and Golgi-derived vesicles, indicative of active secretion of metabolites (Brigham et al. 1995, 1999; Hawes et al. 2000; Vicré et al. 2005). These cells were designated border cells; they become detached from the root and enmeshed in the mucilage surrounding the root surface (Hawes et al. 1998). Although they are common in most plant species, border cells were initially not observed in various Brassicaceae, including *Arabidopsis thaliana* (Driouich et al. 2007). However, Vicré et al. (2005) observed a different organization of border cells in *Arabidopsis*, which are therefore designated as border-like cells. Proposed functions of border cells include attraction of beneficial microorganisms, reduction of sensitivity to heavy metals such as aluminum and entrapment of pathogenic bacteria and nematodes in the mucilage surrounding the roots (Hawes 1990; Hawes et al. 2000; Miyasaka and Hawes 2001).

Among the most prevalent rhizosphere bacteria are the *Pseudomonas* spp., which areubiquitously present in soils, easily culturable in vitro, and possess a variety of traits that are relevant for the biological control of plant diseases. Traits of *Pseudomonas* spp. that enable successful rhizosphere colonization are well documented (Weller 1988; Lugtenberg et al. 2001). Among these traits is the flagellar motility towards substrates, such as the organic acids and amino acids secreted by plant roots (De Weert et al. 2002).

The composition of root exudates depends on plant species and cultivar, developmental stage, plant growth substrate, and stress factors (Uren 2000). Analysis of tomato, cucumber and sweet pepper root exudates from plants grown under gnotobiotic conditions on rock wool showed that the exudates contained higher total amounts of organic acids than of sugars. Citric, succinic, and malic acid were the major organic acids, and fructose and glucose the major sugars (Kamilova et al. 2006b). Root exudate composition is also influenced by the rhizosphere microflora itself. Application of the bacterial biocontrol strain *Pseudomonas fluorescens* WCS365 (WCS365) on tomato roots resulted in increased levels of total organic acids, whereas the amount of succinic acid decreased (Kamilova et al. 2006a). Inoculation of the tomato roots with the pathogenic fungus *Fusarium oxysporum* f. sp. *radicis-lycopersici* caused severe foot and root rot and led to decreased amounts of citric acid, while the amount of succinic acid increased compared with non-treated control plants (Kamilova et al. 2006a). When both WCS365 and the pathogen were present, disease was much less severe and the content of succinic acid in the root exudate was decreased (Kamilova et al. 2006a). Thus, availability and composition of the nutritional diet for microorganisms in the rhizosphere are highly dynamic. As root exudation also depends on as yet unpredictable interactions between microorganisms, the analysis of root exudates in gnotobiotic systems is just at the beginning of understanding conditions in the rhizosphere.

2.2 Plants affect their microbial rhizosphere community

Soil is the main reservoir of the potential bacterial rhizosphere community (Normander and Prosser 2000; De Ridder-Duine et al. 2005; Berg and Smalla 2009). Evidence is increasing that plants actively select specific elements of their bacterial rhizosphere microflora, establishing a habitat which is favorable for the plant (Latour et al. 1996; Bais et al. 2004; Garbeva et al. 2004a; Robin et al. 2007; Broeckling et al. 2008; Houlden et al. 2008; Rudrappa et al. 2008). Indeed, plant species-specific rhizosphere communities have been reported. Smalla et al. (2001) monitored the bacterial rhizosphere communities of strawberry, oilseed rape and potato for two consecutive years by the culturable-independent fingerprinting method, denaturing gradient-gel electrophoresis (DGGE). Plant species-specific rhizosphere communities were observed, and differences became more pronounced in the second year. In both years, seasonal effects on both the abundance and composition of the bacterial rhizosphere populations were also observed. Lemanceau et al. (1995) studied the effect of flax and tomato roots on the diversity of *Pseudomonas* populations. In their study, both plant species affected *Pseudomonas* populations differentially, and rhizosphere populations differed from those in bulk soil. Glandorf et al. (1993) studied the *Pseudomonas* diversity on the roots of potato, grass and wheat. Most characterized isolates from each crop were not observed on the other two crops, indicating that composition of *Pseudomonas* populations differed between these plant species. Plant genotype also affects fungal

communities in the rhizosphere, as for example demonstrated by Viebahn et al. (2005) for ascomycete communities in the rhizospheres of field-grown potato and wheat

Plant defenses have the potential to affect bacterial populations in the rhizosphere by either recruiting beneficial bacteria or actively repressing pathogen proliferation. One of the best-studied examples is the biological control of the fungus *Gaeumannomyces graminis* var. *tritici* (*Ggt*), the causal agent of take-all in wheat. When wheat is continuously grown in the same field, a build-up of the pathogen occurs. However, after several years of wheat monoculture and a severe outbreak of the disease, a decrease in take-all is observed, a phenomenon known as take-all decline (TAD). TAD is associated with the proliferation of specific strains of fluorescent *Pseudomonas* spp. in the wheat rhizosphere that produce the antibiotic 2,4-diacetylphloroglucinol (DAPG) and successfully suppress *Ggt* (Weller et al. 2002; Kwak et al. 2009).

Recently, Rudrappa et al. (2008) demonstrated in a gnotobiotic system that infection of *Arabidopsis* with the bacterial leaf pathogen *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst*) results in the recruitment of the biocontrol strain *Bacillus subtilis* FB17 to the roots. The authors demonstrated that roots of *Pst*-infected plants secrete large amounts of malic acid, which is a chemo-attractant for FB17, and it was postulated that diseased plants "signal for help" in the rhizosphere (Rudrappa et al. 2008).

Root exudates can also have direct defensive qualities. Pathogen-activated plant defenses can result in root secretion of antimicrobial compounds. Hairy root cultures of *Ocimum basilicum* challenged with *Pythium ultimum* produce rosmarinic acid, a caffeic acid derivative with antimicrobial activity against multiple soilborne microorganisms (Bais et al. 2002). In another study (Bais et al. 2005), it was shown that root-derived antimicrobial metabolites from *Arabidopsis* confer resistance to a variety of *P. syringae* pathovars. It was also predicted that transgenic plants that produce antimicrobial proteins can influence rhizosphere microbial communities (Glandorf et al. 1997)

All these results were obtained predominantly in vitro and cannot be easily extrapolated to in situ conditions. Moreover, to what extent the indigenous non-pathogenic bacterial community that is intimately associated with plants is affected by plant defenses remains mostly untouched.

2.3 Plant–microbe interactions in the rhizosphere

In view of the immense diversity of microbial life in the soil and the rhizosphere, it is not only important to assess microbial abundance and diversity, but also to relate the presence of the variety of microorganisms to ecological function (Kent and Triplett 2002; Torsvik and Øvreås 2002). In the natural environment, microbial root colonization leads to multiple types of physical and chemical interactions between microorganisms and plants. These interactions can vary from neutral to beneficial on the one side, and deleterious on the other side when plant-pathogenic microorganisms are involved (Lugtenberg et al. 2002; Singh et al. 2004; Mercado-Blanco and Bakker 2007; Raaijmakers et al. 2009). To complicate matters, microorganisms can transition between pathogenic and symbiotic states depending on environmental conditions (Newton et al. 2010a, b)

Many non-pathogenic soil bacteria have the ability to promote the growth of plants and, therefore, are often designated as plant growth-promoting rhizobacteria (PGPR) (Kloepper et al. 1980; Glick 1995; Bloemberg and Lugtenberg 2001; Persello-Cartieaux et al. 2003; Van Loon 2007). Different mechanisms are involved, of which fixation of atmospheric nitrogen to ammonia by diazotrophs has been studied most (Dobbelaere et al. 2003). Rhizobia show a highly specific symbiotic association with leguminous plants in which the rhizobia induce the plant to form root nodules, a specialized organ wherein the rhizobia reside and provide the plant with directly available nitrogen in the form of ammonia (Oldroyd and Downie 2008). Besides fixing nitrogen, the diazotroph *Azospirillum* secretes several plant hormones involved in the direct promotion of plant growth, namely auxins, cytokinins and gibberellins. Auxins, which are quantitatively the most abundantly secreted hormone by *Azospirillum*, stimulate root development, thereby promoting growth of the whole plant (Steenhoudt and Vanderleyden 2000).

Another mechanism of plant growth stimulation by PGPR is the production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Glick et al. 2007). ACC is the immediate precursor of the plant hormone ethylene (ET), which is involved in stress signaling and negatively regulates root elongation. The bacterial enzyme ACC deaminase hydrolyzes ACC to ammonia and α-ketobutyrate. Glick et al. (1998) postulate that plants release ACC into the rhizosphere, and that this ACC is hydrolyzed by the bacterial ACC deaminase, thereby reducing ET-mediated suppression of root growth. This interaction is also beneficial for the bacteria, as ammonia and α-ketobutyrate are sources of N and C, respectively. Ryu et al. (2003) demonstrated that the volatiles 2,3-butanediol and acetoin produced by two *Bacillus* spp. can also enhance growth of *Arabidopsis*, indicating that a physical interaction between the PGPR and the plant is not necessarily required (Ping and Boland 2004).

Besides promoting plant growth directly, plant growth promotion by PGPR can also be indirect. The rhizosphere microflora can benefit plants by increasing tolerance to abiotic stresses such as drought (2009), nutrient deficiency (Yang et

al. 2009), and heavy metal toxicity (Zhuang et al. 2007), as well as protection against pathogens through microbial antagonism and increasing plant defensive capacity (Bent 2006; Van Loon 2007). Thus, beneficial soil bacteria can protect plants against diseases caused by different types of pathogens. A growing understanding of the mechanisms involved has made it clear that many PGPR strains have the potential to be implemented as biological control agents against plant pathogens.

3 Biological control of pathogens by beneficial bacteria

Soil suppressiveness is the phenomenon that in spite of the presence of a virulent pathogen and a susceptible host plant, disease does not occur. General soil suppressiveness is the capacity of the total microbial biomass to suppress the growth or activity of deleterious organisms, whereas specific soil suppressiveness generally depends on a single organism with the ability to antagonize a specific pathogenic species or genus (Weller et al. 2002). This knowledge has been implemented by introducing antagonistic bacteria to plants roots to control diseases (Weller 2007). Under commercial conditions application of fluorescent Pseudomonas spp. has been demonstrated to be very effective in suppression of soil borne diseases, for example control of Fusarium wilt in radish (Fig. 1, Leeman et al. 1995a). Microbial populations can be stimulated by the addition of organic amendments such as manure or compost (Hoitink and Boehm 1999). This can make a conducive soil suppressive (Weller et al. 2002; Garbeva et al. 2004a).

Specific suppression of plant pathogens has been found for representatives of a wide variety of bacterial genera, including *Agrobacterium*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Enterobacter*, *Erwinia*, *Pseudomonas*, *Rhizobium*, *Serratia*, *Streptomyces*, and *Xanthomonas* (Weller 1988; Whipps 2001), but also for fungi, for example non-pathogenic *F. oxysporum* (Alabouvette et al. 1998). Efficient root colonization and establishment of biocontrol bacteria is of key importance for effective suppression of deleterious organisms (Weller 1988; Lugtenberg et al. 2001). Therefore, focus has been mainly on fluorescent *Pseudomonas* spp. because of their excellent root-colonizing capacity and ability to produce antimicrobial compounds (Lugtenberg et al. 2001; Haas and Keel 2003; Haas and Defago 2005; Weller 2007). Several studies have demonstrated a correlation between inoculum density and efficacy of disease suppression. For example, Raaijmakers et al. (1995) demonstrated that effective biological control of Fusarium wilt in radish by *P. fluorescens* WCS374r (WCS374r) or *Pseudomonas putida* WCS358r (WCS358r) required at least 10^5 cfu/g root. A small decline in population density below this threshold resulted in a rapid decrease of efficacy. Once biocontrol bacteria are established in the rhizosphere, a wide variety of mechanisms can result in suppression of plant pathogens.

3.1 Competition for iron

Can suppress various soilborne diseases, for example *Fusarium* wilt in carnation (Duijff et al. 1994) and radish (Raaijmakers et al. 1995; De Boer et al. 2003). Because of its extremely low solubility, iron is often a limiting element in the soil and rhizosphere. Hence, most microorganisms secrete siderophores that chelate iron which is subsequently acquired through membrane receptors (Loper and Buyer 1991; Neilands 1995). Under iron-limiting conditions, WCS358 secretes the fluorescent siderophore pseudobactin-358. Iron chelated by pseudobactin-358 is taken up by a

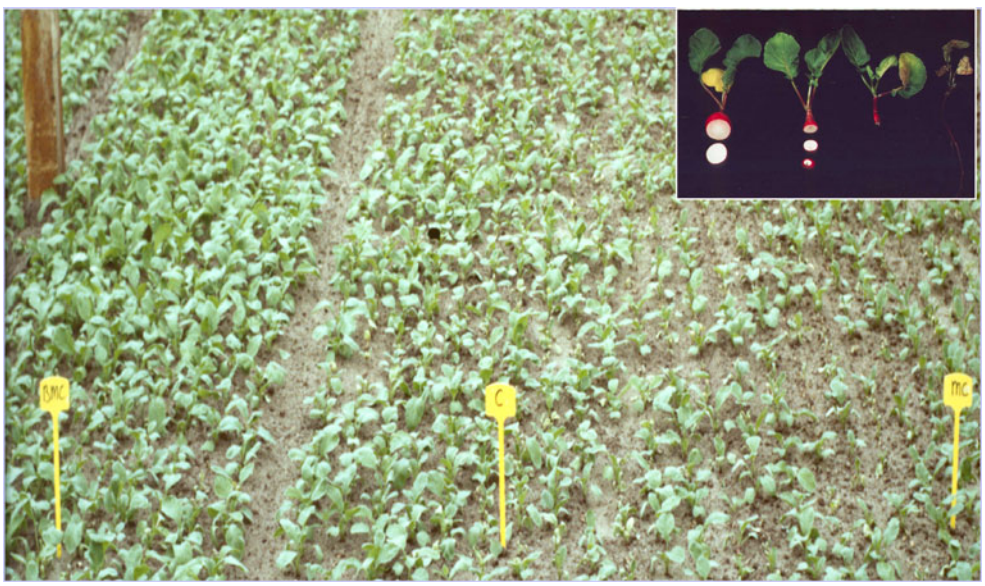

Fig. 1 Control of Fusarium wilt of radish by a seed-coating treatment with *Pseudomonas fluorescens* WCS347 in a commercial greenhouse that was naturally infested with *Fusarium oxysporum* f. sp. *raphani*. Plants on the right hand side were grown from seed not treated (*C*) or treated with just a coating (*MC*), and plants on the left from seeds coated with the pseudomonas bacteria. (Leeman et al. 1995a). The inserted picture shows details of symptoms of Fusarium wilt of radish (*left*, healthy; *right*, completely wilted and dead)

highly specific receptor, and therefore unavailable to organisms that do not possess this receptor (De Weger et al. 1988). Thus, WCS358r can monopolize the available iron in the environment, thereby effectively antagonizing deleterious organisms in their growth or activity. However, in field soils disease suppression by competition for iron is often inconsistent, since iron availability varies in time and space and can be affected by the utilization of heterologous siderophores by other organisms or degradation of the siderophore-iron complex (Loper and Henkels 1997, 1999).

Van Wuytswinkel et al. (1999) overexpressed the iron-storage protein ferritin in tobacco. As a consequence, the transgenic tobacco accumulated iron, thereby depleting the already low level of available iron in the soil. Robin et al. (2006) demonstrated that the composition of *Pseudomonas* spp. on ferritin-overexpressing tobacco roots was altered compared with wild-type tobacco. The *Pseudomonas* community on the roots of the transgenic tobacco was less susceptible to the iron-stress conditions, and moreover, individual isolates from the transgenic tobacco roots showed an increased in vitro antagonism against the plant pathogen *Pythium aphanidermatum* Op4 (Robin et al. 2007).

3.2 Antibiosis

Is the antagonistic effect of a beneficial microorganism by the production of secondary metabolites, such as antibiotics or biosurfactants. Antibiotics are low-molecular weight compounds produced by microorganisms that are deleterious to the metabolism or growth of other microorganisms. A wide variety of antibiotics exists, for example phenazines, DAPG, pyoluteorin, and pyrrolnitrin, and their involvement in biological control of plant diseases has been well studied (Raaijmakers et al. 2002; Chin-A-Woeng et al. 2003; Haas and Keel 2003; Haas and Defago 2005). Production of antibiotics is now often implicated as an important mechanism of biological control, resulting from the fact that it is a relatively easy mechanism to study and can provide a highly effective mode of action (Handelsman and Stabb 1996).

Biosurfactants are amphiphilic compounds that can damage cellular membranes, thereby causing leakage and cytolysis (Maier 2003; Raaijmakers et al. 2006). They can have antimicrobial activity against a variety of organisms, including the pathogenic oomycetes *Pythium* and *Phytophthora*, the fungus *Rhizoctonia*, as well as a number of Gram-positive and Gram-negative bacteria that are pathogenic to humans, such as *Staphylococcus aureus* and *Proteus vulgaris* (Raaijmakers et al. 2006; Das et al. 2008).

3.3 Lytic enzymes

Can degrade several components that are present in the cell walls of fungi and oomycetes (Chet and Inbar 1994). A wide variety of bacterial lytic enzymes are known, including cellulases, glucanases, proteases, and chitinases. A ß-1,3-glucanase-producing *Pseudomonas cepacia* significantly decreased the incidence of diseases caused by *Rhizoctonia solani*, *Sclerotium rolfsii*, and *P. ultimum* (Fridlender et al. 1993). Garbeva et al. (2004b) studied the effect of agricultural practices on the composition of *Pseudomonas* spp. and their antagonistic activity towards *R. solani*. They observed that disease suppressiveness against *R. solani* was higher in grassland than in arable land, and linked this to an increased number of antagonistic *Pseudomonas* spp. possessing chitinolytic activity. However, De Boer et al. (1998) demonstrated that besides the production of lytic enzymes also other mechanisms, such as the production of antibiotics, are involved in the suppression of pathogens.

3.4 Induction of systemic resistance

By beneficial rhizosphere bacteria increases the defensive capacity of the plant and thereby reduces disease incidence or severity after pathogen attack (Van Loon et al. 1998). Van Peer et al. (1991), and Wei et al. (1991) independently demonstrated that induced systemic resistance (ISR) was expressed while the bacterial inoculum and the pathogen were applied and remained spatially separated. The spatial separation excluded the possibility of a direct antagonistic effect of the biocontrol bacteria on the pathogen. Unlike direct bacterial antagonism towards soilborne pathogens, ISR is also effective in above-ground plant parts against a broad range of bacterial, fungal, and oomycetous pathogens, and even sometimes against viruses, nematodes, and herbivorous insects (Van Loon et al. 1998; Van Loon and Bakker 2003; Van Oosten et al. 2008).

ISR can be induced by many different rhizosphere bacteria (Bent 2006) in a variety of plant species (Bakker et al. 2003, 2007). However, successful elicitation is based on a specific interaction between the inducing strain and the host plant (Pieterse et al. 2002; Meziane et al. 2005; Van Loon 2007; Van Wees et al. 2008). For example, Leeman et al. (1995b; 1996) demonstrated that ISR can be elicited in radish by *P. fluorescens* WCS417r (WCS417r) and WCS374r, but not by WCS358r. Conversely, WCS358r and WCS417r are capable of inducing ISR in *Arabidopsis* accession Columbia (Col-0), whereas WCS374r does not (Van Wees et al. 1997). However, when grown at high temperature prior to inoculation (Ran et al. 2005), or when applied at a low inoculum density (Djavaheri 2007), WCS374r does elicit ISR. Variation in the ability to express ISR is observed between different *Arabidopsis* accessions. Whereas the accessions Col-0 and Landsberg *erecta* (L*er*-0) are able to express WCS417r-elicited ISR, the accessions RLD1 and Wassilewskija (WS-0) are not (Van Wees et al. 1997; Ton et al. 1999; Ton et al. 2001). Genetic studies

revealed that the inability of RLD1 and WS-0 to express ISR is mediated by one single dominant gene, *ISR1*, that is associated with sensitivity of the plant to ET (Ton et al. 1999; Ton et al. 2001).

4 Activation of plant inducible defense responses

Plants possess different strategies to recognize and counteract pathogen attack (Jones and Dangl 2006; Boller and He 2009). As a first line of defense, the plant cell surface contains pattern recognition receptors (PRRs) that recognize potential pathogens by conserved pathogen-associated molecular patterns (PAMPs), such as flagella, outer membrane lipopolysaccharides (LPS) and other cell wall or secreted components (Zipfel 2008). Non-pathogenic microorganisms are recognized in a similar way. Hence, their elicitors are designated as microbe-associated molecular patterns (MAMPs) (Bittel and Robatzek 2007). In addition to flagella (Gómez-Gómez and Boller 2002; Zipfel et al. 2004), and LPS (Newman et al. 2007), there are various bacterial compounds that can be recognized by the plant, including *N*-acyl-L-homoserine lactones (Schuhegger et al. 2006), biosurfactants (Ongena et al. 2007; Tran et al. 2007), siderophores (Höfte and Bakker 2007), and the antibiotics DAPG (Iavicoli et al. 2003) and pyocyanin (Audenaert et al. 2002).

Recognition of any of these PAMPs/MAMPs can lead to the activation of a defense signaling cascade, thereby enhancing plant immunity (Bittel and Robatzek 2007; Van Wees et al. 2008). One of the best understood PAMP/MAMP—receptor interactions is the recognition of flagellin, the main component of the bacterial flagellum, by the PRR FLAGELLIN SENSING 2 (FLS2) (Gómez-Gómez and Boller 2000; Zipfel 2008). In *Arabidopsis*, Chinchilla et al. (2006) demonstrated direct binding of flg22, a conserved 22-amino-acid peptide of bacterial flagellin, to the transmembrane leucine-rich-repeat-receptor kinase FLS2. Upon binding of flg22 by FLS2, a mitogen-activated protein kinase (MAPK) signaling cascade is triggered (Asai et al. 2002). Both MAPK3 and MAPK6 are rapidly activated, resulting in the induction of plant defenses and the biosynthesis of antimicrobial metabolites (Nühse et al. 2000; Denoux et al. 2008). The bacterial Tu elongation factor (EF-Tu), and the elf18 peptide derived from the EF-Tu N terminus, seem to be recognized by *Arabidopsis* in a similar manner by a LRR receptor kinase called EFR (Zipfel et al. 2006). Early signaling in recognition of flg22 and EF-Tu by FLS2 and EFR involves calcium-associated membrane anion channel opening (Jeworutzki et al. 2010).

Although non-pathogenic microorganisms lack virulence factors and thereby the ability to effectively exploit plants, recognition of MAMPs can also lead to the elicitation of ISR (Zipfel et al. 2004; Bittel and Robatzek 2007; Van Wees et al. 2008). Plant defenses are regulated by a complex network of signaling pathways (Koornneef and Pieterse 2008; Grant and Jones 2009), in which the plant hormones salicylic acid (SA), jasmonic acid (JA), and ET play major roles (Thomma et al. 2001; Pozo et al. 2004; Van Loon et al. 2006; Loake and Grant 2007). Also other hormones, such as abscisic acid, auxins, gibberellins, cytokinins and brassinosteroids are involved (Grant and Jones 2009; Pieterse et al. 2009). By using hormone signaling mutants, Pieterse et al. (1996, 1998) demonstrated that the signaling pathway underlying ISR in *Arabidopsis* differs from the classic form of systemically induced resistance, systemic acquired resistance (SAR), that results from limited pathogen infection (Ross 1961). Whereas SAR is associated with local and systemic accumulation of SA and the expression of pathogenesis-related (PR) genes (Métraux et al. 1990; Uknes et al. 1992; Sticher et al. 1997; Mauch-Mani and Métraux 1998; Durrant and Dong 2004), ISR requires responsiveness of the plant to JA and ET (Pieterse et al. 1996, 1998). However, ISR is not associated with an increased synthesis of these hormones, nor with an increased expression of known defense-related genes (Van Wees et al. 1999; Pieterse et al. 2000; Verhagen et al. 2004).

Both SAR and ISR enhance plant innate immunity by a mechanism designated priming, which enables the plant to react faster and more strongly to subsequent pathogen attack (Conrath et al. 2002, 2006). Primed plants do not exhibit augmented expression of defense-related genes in the absence of pathogen attack. Instead, an accelerated activation of plant defenses occurs upon pathogen recognition, providing a stronger and faster defense response. Possible mechanisms of priming in SAR and ISR involve the expression of signaling components such as transcription factors (Van der Ent et al. 2008, 2009), or the activation of protein kinases such as MAPK3 and MAPK6 (Beckers et al. 2009), which stay inactive until pathogen recognition. Another mechanism behind priming appears to be a change in chromatin structure (Bruce et al. 2007; Van den Burg and Takken 2009). DNA methylation and/or histone modification can result in a more accessible chromatin structure, allowing a quicker transcriptional response upon pathogen attack.

Expression of plant defenses is necessary for a plant to ward off attack by a pathogen. However, the expression of plant defenses in the absence of deleterious organisms involves fitness costs (Heil 2002; Heil and Baldwin 2002; Heidel et al. 2004; Van Hulten et al. 2006; Walters and Heil 2007). The *Arabidopsis* mutant *cpr1* (*constitutive expressor of* PR *genes 1*) constitutively expresses SA-dependent defenses and is more resistant to a variety of pathogens (Bowling et al. 1994). However, *cpr1* has a dwarf phenotype and produces fewer seeds compared with the

wild-type Col-0 plants (Bowling et al. 1994; Heidel et al. 2004). In contrast, the *edr1* (*enhanced disease resistance 1*) mutation in *Arabidopsis* results in a plant that is constitutively primed for SA-dependent defenses (Frye and Innes 1998). The e*dr1* mutant shows only a slightly lower fitness compared with the wild type and performed considerably better than *cpr1* in the absence of pathogens (Van Hulten et al. 2006). Moreover, upon challenge inoculation with *Pst* or *Hyaloperonospora arabidopsidis*, *edr1* displayed a comparable level of disease protection as *cpr1*, similar to that in wild-type plants in which defenses were activated by chemical elicitors. These observations indicate that priming of inducible defenses outweighs the fitness costs in an environment in which disease occurs (Van Hulten et al. 2006).

4.1 Inducible defense signaling

In general, pathogens with a necrotrophic lifestyle are resisted by JA/ET-dependent defenses, whereas SA-dependent defenses are effective against pathogens with a biotrophic lifestyle (Glazebrook 2005). This differential effectiveness of plant defenses is also displayed by ISR and SAR (Ton et al. 2002). For example, ISR is effective against the necrotrophic fungus *Alternaria brassicicola* whereas SAR is not, while SAR is effective against the biotrophic turnip crinkle virus, and ISR is not. A schematic representation of the SAR and ISR signal-transduction pathways is shown in Fig. 2.

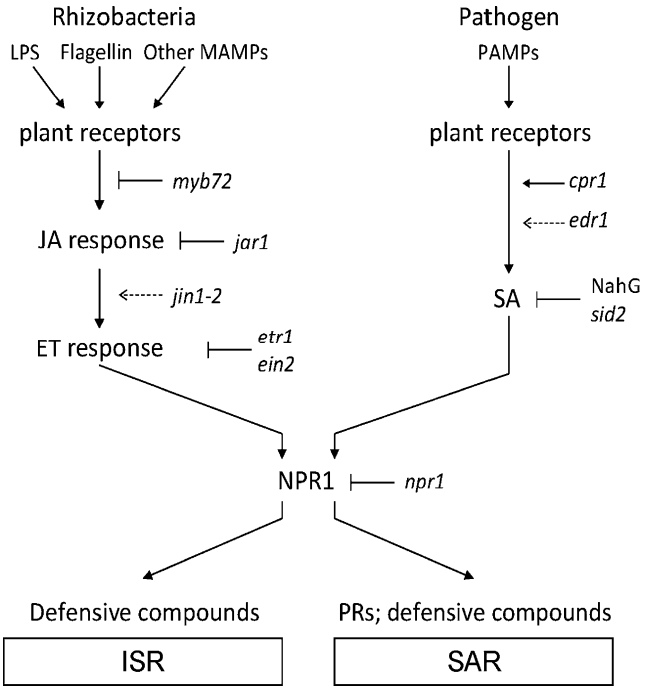

Fig. 2 Schematic representation of the signal-transduction pathways leading to rhizobacteria-mediated induced systemic resistance (*ISR*) and pathogen-induced systemic acquired resistance (*SAR*) in *Arabidopsis*. *Solid arrows* indicate stimulation; *dotted arrows* indicate priming for stimulation, *T-bars* indicate repression (see text for details). *PAMPs* pathogen-associated molecular patterns, *LPS* lipopolysaccharides, *MAMPs* microbe-associated molecular patterns, *JA* jasmonic acid, *ET* ethylene, *SA* salicylic acid, *PRs* pathogenesis-related proteins. Adapted from Pieterse et al. (1998) and Ton et al. (2006)

4.1.1 Systemic acquired resistance signaling

By transforming tobacco with the bacterial gene *NahG*, which encodes the enzyme salicylate hydroxylase that converts SA into catechol, it was demonstrated that accumulation of SA is required for the expression of PR proteins and SAR (Gaffney et al. 1993; Delaney et al. 1994; Van Loon 1997). Similarly, *Arabidopsis* genotypes that are unable to synthesize SA, such as the mutants *sid1* (*salicylic acid induction-deficient 1*), *sid2*, and *pad4* (*phytoalexin deficient 4*), are deficient in the expression of both PR proteins and SAR as well (Lawton et al. 1995; Zhou et al. 1998; Nawrath and Métraux 1999). Local synthesis of SA is necessary for the activation of SAR. However, SA is not the mobile signal required for the systemic activation of SAR (Vernooij et al. 1994). In a search for this mobile signal, it was proposed that locally produced SA is esterified to methyl salicylate (MeSA), which is transported to systemic tissues and there converted back to SA (Seskar et al. 1998; Park et al. 2007). However, Attaran et al. (2009) demonstrated that in *Arabidopsis* the synthesis of MeSA does not coincide with the expression of SAR. Earlier, Maldonado et al. (2002) suggested that a lipid-based molecule could function as the long-distance regulator of SAR in *Arabidopsis*. The mutant *dir1* (*defective in induced resistance 1*) is impaired in the synthesis of a lipid-transfer protein and in the systemic, but not the local, accumulation of SA. Moreover, a recent study by Jung et al. (2009) suggests azelaic acid to be the transported mobile signal required for the systemic activation of SAR in *Arabidopsis*. Although also JA signaling occurs in the early response of SAR, JA biosynthesis, or downstream signaling are not required for the systemic expression of SAR (Truman et al. 2007; Vlot et al. 2008; Attaran et al. 2009).

Subsequent signaling in the SAR signal-transduction pathway requires the function of NON-EXPRESSOR OF *PR* GENES 1 (NPR1), also known as NON-INDUCIBLE IMMUNITY 1, or SALICYLIC ACID-INSENSITIVE 1, which serves as a key regulator of induced resistance signaling (Cao et al. 1994; Pieterse and Van Loon 2004). Upon SA accumulation, inactive NPR1 oligomers in the cytosol are reduced to active monomers, and translocated into the nucleus (Kinkema et al. 2000; Mou et al. 2003). There, NPR1 interacts with TGA and WRKY transcription factors to regulate the expression of defense-related genes,

such as *PR-1* (Zhang et al. 1999; Pieterse and Van Loon 2004; Wang et al. 2006). Besides this regulatory function, NPR1 also controls the expression of the protein secretory machinery, which is required for the translocation of defense proteins into the apoplast (Wang et al. 2005).

4.1.2 Induced systemic resistance signaling

By using a transcriptomic approach, Verhagen et al. (2004) found only priming of defense-related genes in the leaves. However, WCS417r-treated roots showed upregulation of 97 genes. Among these, the R2R3-MYB-like transcription factor gene *MYB72* was specifically expressed in the roots upon colonization by *P. putida* WCS358r, *P. fluorescens* WCS417r, and crude cell walls of WCS417r, concomitant with the elicitation of ISR (Verhagen et al. 2004; Van der Ent et al. 2008). MYB72 binds in vitro to the ETHYLENE INSENSITIVE3 (EIN3)-LIKE transcription factor, indicating a link with the ET-response pathway. Moreover, *MYB72* was found to be essential for the activation of ISR, since *myb72* knockout mutants did not exhibit ISR after treatment with WCS417r. However, activation of *MYB72* is not sufficient for the expression of ISR, since overexpressing *35S*::*MYB72 Arabidopsis* plants did not show enhanced resistance against different pathogens tested (Van der Ent et al. 2008).

Microarray analysis further demonstrated that the promoter regions of MeJA-responsive genes that were primed by WCS417r were enriched for binding sites of the transcription factor MYC2 (Pozo et al. 2008). Moreover, *MYC2* expression was found to be upregulated in the leaves of WCS417r-induced plants. A role for *MYC2* in the ISR signal-transduction pathway was demonstrated by the observation that the *MYC2* mutant, *jasmonate-insensitive 1*, had lost the ability to express WCS417r-elicited ISR.

5 Impact of defense on the rhizosphere microflora

Plant defenses are directed against pathogenic microorganisms, but possible effects on the indigenous rhizosphere microflora have hardly been investigated. Generating such knowledge will allow more sensible implementation of beneficial rhizosphere bacteria for biological control of diseases. Whereas interactions between plants and pathogens have been studied in great detail, the interplay between plants and non-pathogenic microorganisms has received scant attention (Bisseling et al. 2009). Most investigations in which a possible impact of plant defenses has been studied use *Arabidopsis* as model plant, because a wide variety of wild-type plants, constitutive expressors, as well as mutants impaired in the expression of defenses are available.

It can be postulated that highly susceptible plants harbor a more diverse and/or more abundant microbial community compared with more resistant plants. The impact of constitutive expression of SAR on bacterial community structure in the rhizosphere of *Arabidopsis* was studied using T-RFLP (Hein et al. 2008). Although differences in bacterial diversity were observed, they could not be linked to the expression of induced resistance. Bacterial communities in the rhizosphere of *Arabidopsis* accessions RLD and WS-0 were distinct from those of Col-0 and five other accessions (Micallef et al. 2009). These data do suggest a relation between defense signaling and bacterial community structure, because compared with the other accessions, RLD and WS-0 are relative insensitive to ET and impaired in the expression of ISR (Ton et al. 1999; Ton et al. 2001). In tobacco, ET insensitivity also affects the indigenous microflora, as evidenced by studies that used ET-insensitive transgenic plants. Knoester et al. (1998) and Geraats et al. (2002, 2003, 2007) observed spontaneous infection by various soilborne fungi and oomycetes in ET-insensitive Tetr tobacco. Based on DGGE fingerprint analysis of amplified bacterial ribosomal DNA, it was concluded that also the indigenous rhizosphere bacterial community structure of tobacco was affected by ET insensitivity (Geraats 2003).

Because of the complexity of interactions that occur between microorganisms and plants, perturbations could be provoked in multiple ways. As stated earlier, plant root exudates can selectively attract microorganisms, resulting in the establishment of a rhizosphere microflora that is favorable to plant growth (Latour et al. 1996; Bais et al. 2004; Garbeva et al. 2004a; Robin et al. 2007; Broeckling et al. 2008; Houlden et al. 2008; Rudrappa et al. 2008). The composition of root exudates is a reflection of the plants physiological state which in turn can be affected by both biotic and abiotic factors. For example, the expression of inducible plant defenses requires energy. This energy demand is nicely demonstrated for *Arabidopsis* in the observation that there is a significant fitness cost for the activation of defense responses (Van Hulten et al. 2006). As indicated by local decreases of photosynthetic activity, activated plant defenses require energy in order to prioritize production of defense-related compounds (Berger et al. 2007; Bolton 2009). Increased expression of apoplastic invertases is observed under stress conditions, resulting in increased local sink strength to provide hexoses for stress alleviation (Roitsch et al. 2003; Bolton 2009). Increased transport of carbohydrates into the cells deprives apoplast-colonizing pathogens from readily available nutrients (Fotopoulos et al. 2003). In a similar way non-pathogenic microorganisms may be affected by changes in the plants energy balance.

A more direct effect on the non-pathogenic microflora can be expected from secondary metabolites with antimicrobial activity that are secreted as a results of activation of defense

responses. Roots of *Arabidopsis* treated with SA secrete numerous secondary metabolites (Walker et al. 2003). These include butanoic acid, ferulic acid, and 3-indolepropanoic acid, all of which exhibit in vitro antibacterial activity against pathogenic *Erwinia* spp., *Xanthomonas campestris,* and *P. syringae* at the concentrations detected in the exudates. Whereas growth of a non-pathogenic *P. fluorescens* was less sensitive to these exudates, effects on the microbial community were not investigated in this study. Activation of SA-dependent defenses by foliar application of SA to field-grown *Arabidopsis* resulted in a reduced diversity of bacterial endophytes in the leaves (Kniskern et al. 2007). In a similar experiment in the greenhouse, no significant effects of activated SA-dependent defenses on the bacterial rhizosphere microflora of *Arabidopsis* were observed (Doornbos et al. 2009).

Activation of SA-dependent defenses in barley by application of BION, which contains benzothiadiazole, a functional analog of SA, did not affect the composition of free-living soil biota or infection by mycorrhizal fungi (Sonnemann et al. 2002). However, increased root infections by the parasitic nematode *Pratylenchus* were evident. One of the explanations for these results is a signaling conflict or trade-off between different types of defense responses, as suggested by Heil (2001). Activation of SA-dependent defenses antagonizes the JA-dependent signaling pathway (Beckers and Spoel 2006; Koornneef and Pieterse 2008; Grant and Jones 2009). Thus, prioritizing of SA-dependent defenses over JA-dependent defenses can result in an increased susceptibility to deleterious organisms that are resisted by JA-dependent defense responses.

6 Concluding remarks

We are just at the start of understanding the complex interactions between plant roots and its highly diverse and dynamic microflora.The ability of plants to react differentially to microbial pathogens and to beneficial microorganisms is crucial for its survival. Pathogens need to be stopped quickly and efficiently, whereas the beneficials should be stimulated. Such an ideal situation seems to exist in the study by Rudrappa et al. (2008), who demonstrated that infection of *Arabidopsis* leaves by *P. syringae* pv *tomato* leads to increased rhizosphere populations of ISR eliciting bacteria. The ISR eliciting, beneficial *P. fluorescens* and *P. putida* strains were reported to have reduced root colonization on a mutant of *Arabidopsis* affected in the expression of ISR (Doornbos et al. 2009), suggesting that there is a mutual benefit of ISR for the plant, becoming more resistant to pathogens, and the bacteria, reaching higher population densities. Whereas only a limited number of studies have investigated effects of plant defense on the non-pathogenic and beneficial microflora, most of these studies suggest that effects on the indigenous microflora are negligible. Development of methodologies to study shifts in the rhizosphere microflora have been stimulated by studies that have focused on the impact of introducing genetically modified bacteria that produce broad spectrum antibiotics (Glandorf et al. 2001; Bakker et al. 2002; Blouin-Bankhead et al. 2004; Timms-Wilson et al. 2004; Viebahn et al. 2003, 2006). Most of those studies used fingerprinting techniques of group specific PCR amplified 16S (bacteria) or 18S (fungi) rDNA, enabling detection of shifts but lacking power to identify the organisms affected. The new generation PhyloChip that contains 60,000 bacterial operational taxonomic units (Hazen et al. 2010) facilitates assessment of qualitative and quantitative shifts in microbial communities. Unraveling the molecular interplay of plant–microbe interactions will not only enable us to manipulate plant defense to our benefit, but more so to stimulate development of a beneficial rhizosphere microflora.

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