

Chapter 2

Bacteria for Plant Growth Promotion and Disease Management

Brahim Bouizgarne

2.1 Introduction

Soil is an excellent niche of growth of many microorganisms: protozoa, fungi, viruses, and bacteria. Some microorganisms are able to colonize soil surrounding plant roots, the rhizosphere, making them come under the influence of plant roots (Hiltner 1904; Kennedy 2005). These bacteria are named rhizobacteria. Rhizobacteria are rhizosphere competent bacteria able to multiply and colonize plant roots at all stages of plant growth, in the presence of a competing microflora (Antoun and Kloepper 2001) where they are in contact with other microorganisms. This condition is widely encountered in natural, non-autoclaved soils.

Generally, interactions between plants and microorganisms can be classified as pathogenic, saprophytic, and beneficial (Lynch 1990). Beneficial interactions involve plant growth promoting rhizobacteria (PGPR), generally refers to a group of soil and rhizosphere free-living bacteria colonizing roots in a competitive environment and exerting a beneficial effect on plant growth (Kloepper and Schroth 1978; Lazarovits and Nowak 1997; Kloepper et al. 1989; Kloepper 2003; Bakker et al. 2007). However, numerous researchers tend to enlarge this restrictive definition of rhizobacteria as any root-colonizing bacteria and consider endophytic bacteria in symbiotic association: Rhizobia with legumes and the actinomycete *Frankia* associated with some phanerogams as PGPR genera. Among PGPRs are representatives of the following genera: *Acinetobacter*, *Agrobacterium*, *Arthrobacter*, *Azoarcus*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Klebsiella*, *Pseudomonas*, *Rhizobium*, *Serratia*, and *Thiobacillus*. Some of these genera such as *Azoarcus* spp., *Herbaspirillum*, and *Burkholderia* include endophytic species.

B. Bouizgarne (✉)

Laboratoire de biotechnologies et valorisation des ressources naturelles (LBVRN),

Agadir, Morocco

e-mail: b.bouizgarne@ucam.ac.ma

However, *Pseudomonas* and *Bacillus* species constitute, together with *Streptomyces* species, the most bacteria often found in the rhizosphere of many crop plants.

In recent years, interest in the use of PGPR to promote plant growth has increased. Beneficial effect of PGPR on plant growth involves abilities to act as phytostimulators; biofertilizers. PGPR could enhance crop yield through nutrient uptake and plant growth regulators. PGPR could also act as biocontrol agents by production of antibiotics, triggering induced local or systemic resistance, or preventing the deleterious effects of xenobiotics by degradation (rhizoremediators) by acting as rhizoremediators (Jacobsen 1997; Somers et al. 2004; Aseri et al. 2008; Glick et al. 2007; Van Loon 2007). Their application as crop inoculants for biofertilization would be an attractive option to reduce the use of chemical fertilizers (Bloembergen and Lugtenberg 2001; Vessey 2003). In addition, PGPR have great adaptation to harsh environments including drought stress (Arshad et al. 2008; Arzanesh et al. 2011), salt stress (Mayak et al. 2004), high temperatures, dryness or heavy rainfalls in tropical countries (Da Mota et al. 2008), and contaminated environments (Burd et al. 2000; Gupta et al. 2002; Dell'Amico et al. 2008), indicating that they could contribute to ameliorate plant crops in areas with poor agricultural potential.

Biocontrol by rhizobacteria could involve PGPR and non-PGPR bacteria in the way that suppression of plant diseases could result in no enhancement of plant growth but only in protection against plant pathogens. Action of bacteria in the rhizosphere is also restricted by their ability to colonize the rhizosphere. Indeed, in practice, we cannot conclude that a bacterium is a PGPR only after its isolation from rhizobacteria and its reintroduction by plant inoculation followed by assessment of its ability to colonize rhizosphere and beneficial effect. This corresponds to only 2–5 % of rhizobacteria (Kloepper and Schroth 1978).

2.2 Root Colonization by Rhizobacteria

Schmidt (1979) proposed the term rhizosphere competence as related to soil microorganisms that show enhanced growth in response to developing plant roots. According to Weller (1988), root colonization is related to bacteria which can colonize the whole root system and survive during several weeks in the presence of the natural microflora. Later, Baker (1991) considered that root colonization is the ability of a microorganism, applied by seed treatment, to colonize the rhizosphere of developing roots. Both terms are used by authors to design the same process, that of the ability of microbial strains to grow and inhabit in the vicinity of roots (rhizosphere) or on the root system. The term rhizosphere effect designs the fact that bacterial density is higher in the rhizosphere in comparison to non-rhizosphere soil (Foster and Rovira 1978). Rhizosphere competence of rhizobacteria is strongly correlated with their ability to use organic acids as carbon sources, and the composition and quantity of root exudates influence also the nature of bacterial activity (Loper and Schroth 1986; Goddard et al. 2001). Abundance and

diversity of microorganisms in the rhizosphere are likely to be related to plant species due to differences in root exudation and rhizodeposition (Marschner et al. 2004; McSpadden Gardener 2004; Lemanceau et al. 1995). The root competence plays a major role in antagonistic activities of some bacteria. Root colonization is so important since poor colonization could cause decreased biocontrol activity. Indeed, population size was reported in many works as correlated to the efficiency of biocontrol activity against plant pathogens (Bull et al. 1991).

The ability to suppress disease by introduced *Pseudomonas* strains relies mainly on their ability to colonize the roots (Chin-A-Woeng et al. 2000) and their rhizosphere population density (Raaijmakers and Weller 1998). For example, the threshold population density required for significant suppression of take-all of wheat by two *Pseudomonas* spp strains; Q2-87 and Q8r1-96 is on average 1.2×10^5 CFU g⁻¹ and 4.6×10^5 CFU g⁻¹ root respectively (Raaijmakers and Weller 1998). Also, due to their lack of motility and consequently rhizosphere colonization, some *Pseudomonas* strains, producing the antibiotic phenazine, failed to suppress soil-borne pathogens (Chin-A-Woeng et al. 2003). Approaches aiming to enhance PGPR root colonization have focused on the effect of abiotic factors (Howie et al. 1987) and biotic factors (Notz et al. 2001): host genotype (Smith and Goodman 1999) and microbial genotypes (Landa et al. 2002, 2003). For instance, it was reported that plant growth promotion observed in tomato was more pronounced with two rhizosphere-competent streptomycetes *S. filipinensis* and *S. atrovirens* isolates than a non-rhizosphere-competent isolate. These two strains produced 1-aminocyclopropane-1-carboxylate (ACC) deaminase and/or indole acetic acid (IAA) (El-Tarabily 2008).

2.2.1 Distribution and Localization of Root Competent Rhizobacteria

For the effective establishment of PGPR beneficial effects, the ability to colonize plant roots by introduced bacteria is an important trait. Attempts to measure external or internal amount of bacteria that colonize root are generally performed after root washing and disinfecting. In these studies, whole root systems or root segments are used. Generally, to determine colonization rate of the bacteria, enumeration of root colonizing bacteria, especially fluorescent pseudomonads, is classically performed by dilution plating (Ongena et al. 2000; Gamalero et al. 2004). Enumeration is performed on nutrient media generally supplemented with antifungal antibiotics such as yeast mannitol agar (YMA) with spectinomycin and kasugamycin (Chebotar et al. 2001) for the isolation of *Bradyrhizobium japonicum*. For the isolation of *Pseudomonads* and fluorescent *Pseudomonas* sp., TSA (Barnett et al. 1999) and King's medium B agar (Raaijmakers and Weller 1998) are used. The most used antibiotics are cycloheximide (which prevents the growth of fungi) and antibacterial compounds such as chloramphenicol and ampicillin (Simon and Ridge 1974;

van Wees et al. 1997). Media like actinomycete isolation agar or Olson medium (Olson 1968) or the rhizospheric soil extract medium (Bouizgarne et al. 2006) supplemented with nalidixic acid and cycloheximide are largely used for the isolation of Streptomyces. Using a selective medium containing cycloheximide and carbenicillin, *Streptomyces lydicus* WYEC 108 were recovered from non-sterile soils. Monitored population of WYEC 108 in both roots and non-rhizospheric soils of pea, cotton and sweet corn planted in amended sterile and non-sterile soils revealed that over 30 days, the population remains stable at 10^5 CFU g⁻¹ root, whereas in non-rhizosphere soil it decreases by 100-fold at least (Yuan and Crawford 1995).

Spontaneous chromosomal mutants or engineered strains with antibiotic resistance are widely used in dilution plating method. The most common resistance used is for rifampicin which is selective for rifampicin-resistant *Pseudomonas* spp. (Geels and Schippers 1983; Raaijmakers et al. 1999; Fließbach et al. 2009) and streptomycin (Asaka and Shoda 1996). Visualization of root colonization was assessed by various techniques: Immunofluorescence microscopy (Troxler et al. 1997; Gamalero et al. 2004), immunofluorescence colony (IFC) staining technique (Schobe and vanVuurde 1997; Raaijmakers et al. 1995) and scanning microscopy (Chin-A-Woeng et al. 1997; Tokala et al. 2002; Gamalero et al. 2004), and confocal laser scanning electron microscopy (Bloembergen et al. 2000; Bolwerk et al. 2003; Gamalero et al. 2004, 2005). In addition to its ability to quantify soil bacteria, bioluminescence genes method (lux gene) also allows to detect genetically engineered soil bacteria (de Weger et al. 1997). All these techniques enabled easier study of PGPR in their natural environment.

Work by Gamalero et al. (2004) concluded that the population dynamics showed spatiotemporal density variation according to the root zone. While *Pseudomonas fluorescens* A6RI density decreased with time in the apex, the elongation, and the young hairy zones, no variation with time was recorded in the hairy zone and the old hairy and the collar zones, and concluded that these variations could be due to patterns of exudates composition and concentrations along the root. *P. polymyxa* was found to be capable of colonizing the root tip and the intercellular spaces outside the vascular cylinder of *Arabidopsis thaliana* and barley (*Hordeum vulgare*) (Timmusk et al. 2005).

Visualization of cellular rhizosphere interactions between antagonistic strains (*Pseudomonas* and *Bacillus*) and *Fusarium oxysporum* f. sp. *radicis-lycopersici*, the causal agent of tomato shoot and root rot, was performed using epifluorescence and confocal laser scanning microscopy (CLSM). By labeling these microorganisms differently with autofluorescent proteins, simultaneous detection enabled deep studies of these interactions in the tomato rhizosphere. According to these researches, biocontrol bacteria were able not only to colonize the tomato roots (Chin-A-Woeng et al. 1997; Bloembergen 2007), but also to colonize fungal hyphae, causing different stress effects to its growth (Bolwerk and Lugtenberg 2005) and actively attacking the pathogen, by producing antibiotic phenazine-1-carboxamide (PCN) (Chin-A-Woeng et al. 1998; Bolwerk et al. 2003).

Generally, population density of actinomycetes is largely higher in rhizosphere in comparison with non-rhizosphere soils (Miller et al. 1989, 1990). SEM studies of the root colonization of *Streptomyces griseoviridis* showed a higher density in the rhizosphere of lettuce than in non-rhizosphere soil (Kortemaa et al. 1994). Also SEM study of *Streptomyces lydicus* WYEC 108 showed a particular interaction between *S. lydicus* strain WYEC 108 and nodules of *Pea*. It appeared to colonize nodulation sites, and then the vegetative hyphae moved onto root hairs and from the external surface of the root cells into the interior of the root cells, intermittently (Tokala et al. 2002). Moreover, PCR-DGGE analysis of DNA from colonized nodules revealed the presence of a *Streptomyces* band in addition to other bands corresponding to the plant and *Rhizobium*. The discovery of a native actinomycete colonizing the surface of a root nodule of a pea plant from an agricultural field in north Idaho demonstrated that this phenomenon could be frequent in nature (Tokala et al. 2002).

2.2.2 Molecular and Biochemical Basis of Root Colonization

However, classical cultivation-based analysis has the disadvantage that only a small proportion of the bacterial populations can be recovered (Amann et al. 1995). More accurate techniques are actually used to quantify bacteria: measuring bacterial activity by thymidine and leucine incorporating techniques (Söderberg and Bååth 1998), immunological techniques such as ELISA (REF) and IFC staining technique (Mahaffee et al. 1997), flow cytometry (Tombolini et al. 1997; Gamalero et al. 2004) and bioluminescent *lux* gene tagged bacteria (Mahaffee et al. 1997; Kragelund et al. 1997), and fluorochrome-labeled RNA-directed probes (Assmus et al. 1995). *Pseudomonas* colonies isolated from the roots of wheat on King B medium and harboring the genes for Phl (2,4-diacetylphloroglucinol) were subsequently quantified by colony hybridization with a Phl-probe followed by polymerase chain reaction (PCR) analysis using Phl-specific primers (Raaijmakers and Weller 1998).

Some of these works showed that higher colonization patterns were found near the collar zone (Kragelund et al. 1997; Gamalero et al. 2004) where root exudation activity is higher (Grayston et al. 1996) in comparison with the apical zone. In addition, these researches agreed that the preferential location of bacteria is situated at the junction between epidermal cells (Chin-A-Woeng et al. 1997; Gamalero et al. 2004, 2005; Lagopodi et al. 2002; Bolwerk et al. 2003) or between and inside the epidermal and cortical cells (Troxler et al. 1997).

In order to study bacterial distribution and organization in the root zones, molecular fingerprinting techniques such as amplified rDNA restriction analysis (ARDRA), whole-cell repetitive sequence-based polymerase chain reaction (rep-PCR), random amplified polymorphic DNA (RAPD) analysis, and restriction fragment length polymorphism (RFLP) allowing to detect bacteria were performed by several authors. Other methods such as the use of genetically engineered bacteria by introduced marker gene or a reporter gene to detect innate activity of the bacterium are used. An example is the use of LacZ which encodes for the β -galactosidases (Kluepfel et al. 1991).

PCR could also detect bacteria by analyzing total rhizosphere DNA or rhizosphere 16S rDNA fragments (Smalla et al. 2001), or amplification of specific genes such as those encoding antibiotics. An example is the use of two oligonucleotide primers Phl2a and Phl2b that targeted the gene for 2,4-diacetylphloroglucinol (Phl) (Raaijmakers and Weller 1998). PCR amplification of target *phlD* genes from 2,4-diacetylphloroglucinol (2,4-DAPG) producers provides a technique sensitive enough to detect log 2.4 cells per sample (McSpadden-Gardener et al. 2001). Colony hybridization followed by PCR analysis was used to determine the frequency of 2,4-DAPG producing wheat root-associated fluorescent *Pseudomonas* in take-all disease suppressive and conductive soils, and showed that in conductive soils these strains were not detected or were detected at densities at least 40-fold lower than those in the suppressive soils. Moreover, in suppressive soils, 2,4-DAPG producing *Pseudomonas* spp. were present on roots of wheat at densities above the threshold required for significant suppression of take-all of wheat (Raaijmakers et al. 1997). Genetic profiles of over a dozen distinct genotypes within a worldwide collection of 2,4-DAPG-producing fluorescent *Pseudomonas* spp. isolated from soils suppressive to *Fusarium* wilt or take-all were analyzed, and isolates belonging to two BOX-PCR genotypes (D-genotype strains and P-genotype strains) were found to be more aggressive colonists of the rhizosphere of pea plants than isolates of other genotypes, suggesting that biosynthetic gene *phlD* profiles were predictive of their rhizosphere competence (Landa et al. 2002). Furthermore, *P. fluorescens* Q8r1-96, a representative D-genotype strain, was a potent competitor toward representatives of the less competent A, B, and L genotypes when coinoculated in a 1:1 ratio in the wheat rhizosphere over several successive cycles (Landa et al. 2003).

Using DGGE fingerprints of PCR-amplified 16S rDNA of whole bacterial communities in three plants: strawberry, potato, and oilseed rape, Smalla et al. (2001) found significant differences in microbial community abundance between soil and rhizosphere communities. Similar results were found for *Bacillus* and *Paenibacillus* which are more abundant in bulk soil than in plant tissues (McSpadden Gardener 2004) using a ribotyping method.

The use of genetically engineered strains defected in some characteristics important in the attachment to plant roots could affect dramatically their effectiveness. It was reported in *Pseudomonas* that flagella (de Weger et al. 1987), pilli (Vesper 1987), O-antigens of lipopolysaccharide (LPS) (de Weger et al. 1989), an agglutinin present in the root (Anderson et al. 1988; Glandorf et al. 1994), and the outer membrane protein OprF (de Mot et al. 1992) are involved in root colonization, particularly in the bacterial attachment to roots. Mutants defective in the synthesis of the O-antigen of LPS are impaired in rhizosphere competence (de Weger et al. 1989; Dekkers et al. 1998). However, genetic determinants involved in the biochemical interactions between root plants and bacteria are poorly understood. On the other hand, flagella and LPS are also reported as bacterial determinants recognized by plants in the process of triggering systemic resistance (Gómez-Gómez et al. 1999; Leeman et al. 1995a, 1995b; Van Peer and Schippers 1992; Duijff et al. 1997; Bakker and Schippers 1995).

Plant-bacteria communication involves diverse signaling molecules. Strains belonging to *Bacillus* were reported as producers of volatile organic compounds

such as acetoin and 2,3-butanediol involved in plant–bacteria communication (Ryu et al. 2003). Aldehydes, ketones, and alcohols had been found to be involved in *A. thaliana* recognition of *Bacillus* strains by triggering morphogenetic changes in root system, consisting of stimulation of primary root growth and/or lateral root development. A good correlation between biomass production and lateral root growth was shown, suggesting that this kind of chemical communication might be of ecological relevance toward enhancing root colonization and reinforcing symbiotic interactions between plants and their associated bacterial populations. However, molecular mechanisms and signaling pathways involved in *A. thaliana* responses to volatile organic compounds remain poorly understood (Gutiérrez-Luna et al. 2010). Despite the well-studied dynamics of root colonization ability of Actinomycetes (Merzaeva and Shirokikh 2006; Franco et al. 2007), little is known about biochemical and molecular traits involved in this interaction as it has been mostly studied on pathogenic actinomycetes, particularly *Clavibacter michiganensis* where bacterial exopolysaccharides have been shown to bind to receptor proteins present on the plasma membrane of potato cells (Shafikova et al. 2003; Bermpohl et al. 1996).

2.3 Rhizobacteria Antagonistic to Plant Disease Agents

2.3.1 Gram Negative Bacteria

The most important group of PGPR among Gram negative bacteria are the genera *Pseudomonas*.

2.3.1.1 Pseudomonas

PGPR effect of *Pseudomonas* was largely reviewed (Lemanceau 1992). Bacteria belonging to *Pseudomonas* were reported as PGPR for many crops of potato (*Solanum tuberosum* L.) (Burr et al. 1978; Schippers et al. 1987), radish (*Raphanus sativus* L.) (Kloepper and Schroth 1978), sugar beet (*Beta vulgaris* L.) (Suslow and Schroth 1982), and lettuce (Chabot et al. 1993).

Strains of fluorescent pseudomonads used in biocontrol have contributed greatly to the understanding of the mechanisms involved in disease suppression. Many of these bacteria could prevent plant diseases by various mechanisms: antibiosis, competition, or parasitism. Within the genus *Pseudomonas*, *Pseudomonas fluorescens* which are ubiquitous rhizosphere inhabitant bacteria are the most studied group (Weller 1988). They were shown to have a higher density and activity in the rhizosphere than in bulk soil. When introduced on seed or planting material, they promote plant growth or control plant diseases by suppressing deleterious rhizosphere microorganisms. They are able to compete aggressively for sites in

the rhizosphere and prevent proliferation of phytopathogens by niche exclusion, production of antibiotics and siderophores, or inducing systemic resistance (De Weger et al. 1986; Haas and Défago 2005; Krishnamurthy and Gnanamanickam 1998); by stimulating plant growth by facilitating either uptake of nutrients from soil (De Weger et al. 1986); or by producing certain plant growth promoting substances (Ryu et al. 2005; Spaepen et al. 2007). Fluorescent pseudomonads have been applied to suppress *Fusarium* wilts of various plant pathogens (Lemanceau and Alabouvette 1993), *Clavibacter michiganensis* subsp. *michiganensis*, causal agent of tomato bacterial canker (Amkraz et al. 2010). In addition, their presence is correlated with disease suppression in some suppressive soils (Kloepper et al. 1980a; Lemanceau et al. 2006). Examples of commercially available biocontrol products from *Pseudomonas* are Bio-save (*P. syringae*) and Spot-Less (*P. aureofaciens* Tx-1).

Van Peer et al. (1991) reported protection of carnation from fusariosis due to phytoalexin accumulation upon treatment with *Pseudomonas* strain WCS417. Other works followed including the use of *P. fluorescens* as an inducing agent to prevent the spread of various plant pathogens (Maurhofer et al. 1994; Duijff et al. 1994a; Leeman et al. 1995b; Pieterse et al. 2000). *P. fluorescens* CHA0 showed ability to protect tobacco against the tobacco necrosis virus concomitant with a systemic accumulation of salicylic acid and associated with the induction of multiple acidic pathogenesis-related proteins, including PR-1a, -1b, and -1c (Maurhofer et al. 1994). Inoculation of *A. thaliana* by *P. fluorescens* WCS417r and of rice by WCS374r conducted to induced systemic resistance (ISR) respectively to *Pseudomonas syringae* pv. *tomato* (Pieterse et al. 2000) and to the leaf blast pathogen *Magnaporthe oryzae* (De Vleeschauwer et al. 2008).

2.3.2 Gram Positive Bacteria

The most important group of PGPR among Gram positive bacteria are *Bacillus*, *Paenibacillus*, and *Actinomycetes*.

2.3.2.1 *Bacillus* and *Paenibacillus*

Different species of *Paenibacillus* can induce plant growth by fixing atmospheric nitrogen (Von Der Weid et al. 2002), and producing auxins (Lebuhn et al. 1997; Bent et al. 2001; Da Mota et al. 2008) and cytokinin (Timmusk et al. 1999). Beneficial effects were reported in lodgepole pine (*Pinus contorta*) (Bent et al. 2001) and spruce (*Picea* sp.) (Shishido et al. 1995) after inoculation of *P. polymyxa* strain. *Bacillus* strains could also repress soil-borne pathogens (Von Der Weid et al. 2005) and induce plant resistance to diseases following root colonization (Timmusk and Wagner 1999).

In the opposite to *Pseudomonas* and other nonspore-forming bacteria, *Bacillus* spp. are able to form endospores that allow them to survive for extended periods

under unfavorable environmental conditions. This trait is relevant in their relative durable viability when stored for a relatively long period (shelf-life). *Bacillus* species have been reported as plant promoting bacteria in a wide range of plants (Deepa et al. 2010; Bai et al. 2003; Kokalis-Burelle et al. 2002; Kloepper et al. 2004). Different *Bacillus* species were reported to be effective biocontrol agents in greenhouse or field trials (Stabb et al. 1994; Kloepper et al. 2004). Isolates of *Bacillus subtilis* inhibited *S. cepivorum* in vitro and were able to suppress the incidence of onion white rot, leading to an increased onion emergence and yield (Utkhede and Rahe 1980). The suppression of onion white rot could be due to a possible antibiotic production and probably also metabolization of onion produced stimulants of sclerotial germination (Utkhede and Rahe 1980). Members of *Bacillus* were reported as producers of antibiotics inhibiting various phytopathogens including *F. oxysporum* f. sp. *ciceri* (Kumar 1999) and *Rhizoctonia solani* (Asaka and Shoda 1996).

Mechanisms involved in *Bacillus* eliciting plant growth promotion include auxin production (Idris et al. 2004; Deepa et al. 2010), increased uptake availability of phosphorus (Idris et al. 2002; Deepa et al. 2010), biocontrol abilities (Asaka and Shoda 1996; Jacobsen et al. 2004), and induction of systemic resistance (Zehnder et al. 2000; Jetiyanon et al. 2003; Bargabus et al. 2003; Kloepper et al. 2004). An example of the use of *Bacillus* as biocontrol PGPR agents include the use of two *B. subtilis* strains (G1 and B3), and two *Bacillus amyloliquefaciens* strains (FZB24 and FZB42) in tobacco, either in the presence or absence of tobacco mosaic virus (TMV). In these experiments, they significantly reduced disease severity. Commercial available biocontrol products include Kodiak (*B. subtilis* strain GB03), Serenade (*B. subtilis* QST 713), YieldShield (*Bacillus pumilus* strain GB34), Companion (*Bacillus subtilis*formis, *B. megaterium*), and EcoGuard (*Bacillus licheniformis* strain SB3086).

Bacillus strains were also reported to be potent inducers of systemic resistance (ISR). Jetiyanon et al. (2003) observed that one PGPR mixture, *B. amyloliquefaciens* strain IN937a and *B. pumilus* strain IN937b, protected plants by inducing systemic resistance against southern blight of tomato caused by *Sclerotium rolfsii*, anthracnose of long cayenne pepper caused by *Colletotrichum gloeosporioides*, and mosaic disease of cucumber caused by cucumber mosaic virus (CMV). In a greenhouse experiment, induced resistance to CMV resulted in 32 % of diseased tomato plants in the most effective PGPR treatments with *B. subtilis* IN937b compared with 88% in the nonbacterized plants (Zehnder et al. 2000). *Bacillus* spp. have been tested in field trials for their capacity to reduce the incidence and severity of the tomato mottle virus (ToMoV) that is transmitted by whiteflies (Murphy et al. 2000; Zehnder et al. 2001) and against CMV (Zehnder et al. 2001).

The ISR displayed against CMV on tomato can be obtained under field conditions albeit at variable extents than that reported from greenhouse experiments (Raupach et al. 1996). *Bacillus thuringiensis* induced accumulation of PR proteins in coffee against *Hemileia vastatrix* (Guzzo and Martins 1996). *B. amyloliquefaciens* strain EXTN-1 induced pathogenesis-related genes including PR-1a against anthracnose disease caused by *Colletotrichum orbiculare* in cucumber (Park et al. 2001; Jeun et al. 2001). In addition to induction of phenylalanine ammonia-lyase (PAL), and

3-hydroxy-3-methylglutaryl CoA reductase (HMGR) genes, EXTN-1 induced transcript accumulation of defense-related genes of PR, particularly PR-1a mRNA, upon challenge inoculation with the Pepper mild mottle virus in tobacco, while EXTN-1 treatment of *Arabidopsis* wild type Col-0 resulted in the activation of PR-1 and the ethylene encoding gene PDF1.2 (Ahn et al. 2002).

2.3.2.2 Actinomycetes

Actinomycetes are Gram-positive bacteria characterized by a genome with high G+C ratio. They are for the most aerobic, but some of them can grow anaerobically. Several Actinomycetes form branching filaments and possess mycelial growth and some species produce external spores.

Despite the fact that actinomycetes are largely spread in the nature especially in telluric ecosystems and that they were strongly studied since they are the origin of numerous antibacterial and antifungal compounds and some are used in biocontrol, only few works are interested in their usefulness as PGPR for plants like wheat (Aldesuquy et al. 1998; Hamdali et al. 2008a) and broccoli (Hasegawa et al. 2008). Recent works demonstrated that plant promotion relies on the ability of the Actinomycetes to solubilize phosphate (El-Tarabily et al. 2008; Hamdali et al. 2008b) or to produce phytohormones (El-Tarabily 2008; Hamdali et al. 2008a), showing the great interest of actinomycetes solubilizing phosphate in soils deficient in available soluble phosphorus (P). In greenhouse experiments, rhizosphere-competent *Micromonospora endolithica* induced increase in available P in the soil, promoted the growth of roots and shoots of bean plants in comparison with a non-phosphate-solubilizing, non-rhizosphere-competent isolate (*M. olivasterospora*) (El-Tarabily et al. 2008). El-Tarabily (2008) reported that the plant growth promotion was most pronounced with one actinomycete strain *S. filipinensis* than with another isolate *S. atrovirens* in greenhouse experiment, probably due to the ability of *S. filipinensis* to produce both ACC deaminase and IAA while *S. atrovirens* produce only ACC deaminase.

It is likely that more interest was addressed to the antibiotic production by actinomycetes or their biopesticide capacities since almost all works were initially interested in these topics. Thus, most studied PGPR actinomycetes possess antibacterial or antifungal activity as they were initially screened for works aiming to suppress a plant disease (de Vasconcellos and Cardoso 2009; El-Tarabily and Sivasithamparam 2006; Hamby and Crawford 2000). Examples of commercial biocontrol products from actinomycetes are Mycostop (*Streptomyces griseoviridis* K61), Actinovate (*Streptomyces lydicus*), and Nogall (*Agrobacterium radiobacter* Strain K1026).

Merriman et al. (1974) reported the use of the PGP *Streptomyces griseus* isolate with biocontrol abilities toward *R. solani* in carrot. Antagonistic Streptomyces were also used to promote the growth of coniferous plants. In Brazil, one *Streptomyces* isolate genetically close to *Streptomyces kasugaensis* able to inhibit the

growth of *Fusarium* and *Armillaria* pine rot showed also plant promotion in growth of *Pinus taeda* seedlings under greenhouse experiment (de Vasconcellos and Cardoso 2009). El-Abyad et al. (1993) described the use of three *Streptomyces* spp., *S. pulcher*, *S. canescens*, and *S. citreofluorescens*, effective in the control of some tomato diseases including those caused by *F. oxysporum* f. sp. *lycopersici*, *Verticillium albo-atrum*, *Alternaria solani*, *Pseudomonas solanacearum*, and *Clavibacter michiganensis* subsp. *michiganensis* in tomato. As seed-coating, tomato growth was significantly improved with the tree antagonistic.

S. violaceusniger YCED9, an antifungal producer (Hamby and Crawford 2000), showed also carrot growth promotion under gnotobiotic conditions. From eight strains shown to be strong *P. ultimum* antagonists, only one (strain WYEC 107) significantly enhanced lettuce growth in the absence of *Pythium ultimum* in glass-house pot studies over a 20-day experiment (Crawford et al. 1993). However another isolated antagonistic strain to *P. ultimum*, *Streptomyces lydicus* strain WYEC 108, with demonstrated PGPR effect in carrots and beets in the absence of fungal pathogen stress (Hamby 2001) had shown in another work an increase in average plant stand, plant length, and plant weight of pea and cotton seedlings grown in either *Pythium ultimum*-infested sterile or non-sterile soils (Yuan and Crawford 1995). In addition to increasing shoots length and plant and root wet weights in pea seedlings in both growth chamber and greenhouse experiments, *Streptomyces lydicus* strain WYEC 108 was also found to increase root nodulation frequency by *Rhizobium* spp. and nodule size and number as shown by the more numerous and vigorous nodules found in *Streptomyces*-colonized plants than in control. Also, an increase in the number of bacteroids per nodule, nitrogenase activity, and nodular assimilation of iron was reported (Tokala et al. 2002). Due to its high potential as fungal antagonist, its good establishment in the rhizosphere of various plants at significant levels (10^4 CFU g⁻¹ of soil), and as it can easily be reisolated for 26 months after inoculation (Crawford et al. 1993), *S. lydicus* strain WYEC 108 led to the formulation and the commercialization of Actinovate[®] and Actino-Iron[®], a well-known biocontrol product (Crawford et al. 2005).

2.4 Bacterial Antagonism: Protection Against Phytopathogens

Generally plant diseases cause 10–20 % loss in production (James 1981). The use of antibacterial and antifungal chemicals is deprecated in view of sustainable agricultural practices. Hence, an alternative to chemical control of plant diseases by the use of bacteria able to antagonize phytopathogenic is considered as a more environmentally friendly process. Biological control of soil-borne pathogens with antagonistic bacteria has been intensively investigated. In this mode of action, direct interaction between PGPR and the endogenous microflora is necessary. PGPR can promote plant growth by suppressing diseases caused by soil-borne pathogens (Van Loon and Glick 2004).

Rhizobacteria can antagonize pathogens through competition, production of antibiotics, or secretion of lytic enzymes (Van Loon and Bakker 2003) that make them a potent tool for reducing damages through preventing deleterious effects of phytopathogens. The main bacteria are representatives of the genera *Pseudomonas*, *Bacillus*, and *Streptomyces*. Numerous studies on bacteria antagonistic to phytopathogens include bacteria such as fluorescent *Pseudomonas* and *Bacillus subtilis* (Kloepper et al. 1989).

2.4.1 Antagonism by Production of Lytic Enzymes

Lytic enzymes are glucanases, proteases (Dunne et al. 1997), cellulases, and chitinases. Bacteria could parasitize disease-causing fungi by the production of these enzymes. Some enzyme producing bacteria are able to destroy oospores of phytopathogenic fungi (El-Tarabily 2006) and affect the spore germination and germ-tube elongation of phytopathogenic fungi (Sneh et al. 1984; Frankowski Lorito et al. 2001). A positive relationship was observed between chitinase production and the antifungal activity of chitinolytic *P. fluorescens* isolates (Velazhahan et al. 1999). Production of extracellular cell wall degrading enzymes has been associated with biocontrol abilities of the producing bacteria (Fridlender et al. 1993; Valois et al. 1996; Singh et al. 1999; El-Tarabily 2006). Tn5 mutants of one of the *Enterobacter* which were deficient in chitinolytic activity were unable to protect plants against the disease (Chernin et al. 1995). In addition, enzyme producing bacteria were successfully used in combination with other biocontrol agents, leading to a synergistic inhibitory effect against pathogen (Dunne et al. 1998; Someya et al. 2007). Table 2.1 gives examples of enzymes produced by biocontrol bacteria.

2.4.2 Antagonism by Antibiosis

Antibiotics produced by bacteria include volatile antibiotics (hydrogen cyanide, aldehydes, alcohols, ketones, and sulfides) and nonvolatile antibiotics: polyketides (diacetyl phloroglucinol; DAPG and mupirocin), heterocyclic nitrogenous compounds (phenazine derivatives: pyocyanin, phenazine-1-carboxylic acid; PCA, PCN, and hydroxy phenazines) (de Souza et al. 2003), and phenylpyrrole antibiotic (pyrrolnitrin) (Ahmad et al. 2008). *Bacillus strains* produce a variety of lipopeptide antibiotics (iturins, bacillomycin, surfactin, and Zwittermicin A).

Introduction of selected antagonistic fluorescent pseudomonads into the rhizosphere can effectively suppress soil-borne plant diseases. *B. subtilis* strain RB14 produces the cyclic lipopeptide antibiotics iturin A and surfactin active against several phytopathogens. *B. subtilis* strains were able to control damping-off of

Table 2.1 Examples of lytic enzymes produced by biocontrol bacteria

Enzyme	Producing bacteria	Target phytopathogen and host plant	References
Chitinases	<i>Aeromonas caviae</i>	<i>Rhizoctonia solani</i> and <i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i> (cotton) and <i>Sclerotium rolfsii</i> (beans)	Inbar and Chet (1991)
	<i>Arthrobacter</i> sp.	<i>Fusarium</i> (carnation)	Koths and Gunner (1967), Sneh (1981)
	<i>Arthrobacter</i>	<i>Fusarium moniliforme</i> var <i>subglutinans</i> (southern pines)	Barrows-Broadbent and Kerr (1981)
	<i>Enterobacter agglomerans</i> , <i>Bacillus cereus</i>	<i>R. solani</i> (cotton)	Chemin et al. (1995, 1997), Pleban et al. (1997)
	<i>Bacillus circulans</i> and <i>Serratia marcescens</i>	<i>Phaeoisariopsis personata</i> (peanut)	Kishore et al. (2005)
	<i>Enterobacter agglomerans</i> , <i>Bacillus cereus</i>	<i>R. solani</i> (cotton)	Chemin et al. (1995, 1997), Pleban et al. (1997)
	<i>Paenibacillus illinoisensis</i>	<i>R. solani</i> (cucumber)	Jung et al. (2003)
	<i>Pseudomonas</i>	<i>Fusarium oxysporum</i> f. sp. <i>cucumerinum</i> (cucumber)	Sneh et al. (1984)
	<i>Serratia plymuthica</i>	<i>Bothrytis cinerea</i> and <i>Sclerotinia sclerotiorum</i> (cucumber)	Kamensky et al. (2003)
	<i>Serratia marcescens</i>	<i>Sclerotium rolfsii</i> (beans) and <i>R. solani</i> (cotton)	Chet et al. (1990)
Glucanases	<i>Streptomyces lydicus</i>	<i>Pythium</i> and <i>Aphanomyces</i>	Mahadevan and Crawford (1997)
	<i>Streptomyces</i> sp.	<i>Phytophthora fragariae</i> (raspberry)	Valois et al. (1996)
	<i>Pseudomonas cepacia</i>	<i>R. solani</i> , <i>Sclerotium rolfsii</i> and <i>Pythium ultimum</i>	Fridlender et al. (1993)
Chitinases and glucanases	<i>Actinoplanes philipinensis</i> and <i>Micromonospora chalcone</i>	<i>Pythium aphanidermatum</i> (cucumber)	El-Tarabily (2006)
	<i>Lysobacter enzymogenes</i>	<i>Pythium</i> (sugar beet)	Palumbo et al. (2005)
	<i>Serratia marcescens</i> , <i>Streptomyces virididiazoticus</i> , <i>Micromonospora carbonacea</i>	<i>Sclerotinia minor</i> (lettuce)	El-Tarabily et al. (2000)
	<i>Streptomyces</i> sp. and <i>Paenibacillus</i> sp.	<i>Fusarium oxysporum</i> f. sp. <i>cucumerinum</i> (cucumber)	Singh et al. (1999)
Chitinases, proteases, and cellulases	<i>Bacillus subtilis</i> , <i>Erwinia herbicola</i> , <i>Serratia plymuthica</i> , <i>Actinomycete</i>	<i>Eutypa lata</i> (grapevine)	Schmidt et al. (2001)
Proteases	<i>Stenotrophomonas maltophilia</i>	<i>Pythium ultimum</i> (sugar beet)	Dunne et al. (1997, 1998)

tomato caused by *Rhizoctonia solani* and was found to produce an antifungal antibiotic inhibiting *F. oxysporum* f. sp. *ciceris*, causal agent of wilt disease in chickpea (Asaka and Shoda 1996; Kumar 1999). Table 2.2 gives some examples of antibiotics produced by biocontrol bacteria.

Most strains of *Pseudomonas* spp. used in biocontrol produce one or several antibiotic compounds with antifungal abilities in vitro (Raaijmakers et al. 2002). Phenazine derivatives produced by fluorescent pseudomonads were reported as implicated in biocontrol of take-all disease (Weller and Cook 1983). *P. fluorescens* 2-79, an in vitro producer of phenazine-1-carboxylate, was reported to suppress *Gaeumannomyces graminis* var. *tritici* (Thomashow and Weller 1988). 2,4-DAPG producers play a key role in the natural suppression of take-all disease of wheat (Raaijmakers et al. 1997, 1999; Raaijmakers and Weller 1998). Suppressive soils to take-all lost its suppressiveness when indigenous DAPG-producing fluorescent *Pseudomonas* spp. was eliminated by pasteurization. Moreover, suppressiveness was able to be transferred to conductive soils when DAPG-producing *Pseudomonas* strains were introduced (Raaijmakers and Weller 1998).

Numerous investigations demonstrate that *Streptomyces* produce numerous secondary metabolites with antibiotic properties. Currently, 42 % of the 23,000 known microbial secondary metabolites are produced by actinobacteria. Actinomycetes and particularly Streptomycetes produce 70–80 % of known bioactive natural products (Berdy 2005). A large number of these antibiotics were exploited in experimental works in laboratories or greenhouses. However, few of these antibiotics are commercialized.

The genus *Streptomyces* is the largest producer of secondary metabolites. The antagonistic properties of *Streptomyces* against numerous phytopathogens including *Alternaria brassicicola*, *Collectotrichum gloeosporioides*, *F. oxysporum*, *Penicillium digitatum*, and *Sclerotium rolfsii* (Khamna et al. 2009) and *F. oxysporum* f. sp. *vasinfectum*, *F. oxysporum* f.sp *lycopersici*, and *F. oxysporum* f. sp. *asparagi*, the causal agents of wilt diseases, are well established. Geldanamycin produced by *Streptomyces hygroscopicus* var. *geldonus* was applied to suppress *Rhizoctonia solani* in soil (Rothrock and Gottlieb 1984). Furthermore, compounds responsible for the antifungal activity of some *Streptomyces* species have been identified: e.g., cycloheximide from *S. griseus*, kasugamycine from *S. kasugaensis*, Blastcidin-S from *S. griseochromogenes*, and Rhizovit from *S. rimosus* etc.

Bacterial strains may protect plants from phytopathogenic fungi due to the volatile antibiotic HCN production (Ahmad et al. 2008). *P. fluorescens* CHAO enhanced root growth and could suppress black root rot of tobacco caused by *Thielaviopsis basicola* under gnotobiotic conditions. CHAO excretes several metabolites with antifungal properties including pyoverdine, DAP, pyoluteorin, and HCN (Ahl et al. 1986; Maurhofer et al. 1995). It was also suggested that HCN might constitute a stress in the plants, provoking an enhancement of their resistance to fungal diseases (Défago et al. 1990). Suppressive effect on black root rot was found to be related to hydrogen cyanide production as demonstrated by the less protective effect of hcn mutant defective in HCN biosynthesis and effective

Table 2.2 Selected examples of antibiotics produced by biocontrol bacteria

Antibiotics	Producing organism	Target organism	References
Bacillomycin	<i>Bacillus</i>	<i>Aspergillus flavus</i>	Moyné et al. (2001)
Kanamine	<i>Bacillus cereus</i>	<i>Phytophthora medicaginis</i>	Miner et al. (1996)
Zwittermycin A	<i>B. cereus</i> and <i>B. thuringiensis</i>	<i>Phytophthora</i>	Silo-Suh et al. (1998)
Iturin	<i>Bacillus</i> spp.	<i>Sclerotinia sclerotiorum</i>	Zhang and Fernando (2004)
	<i>B. cereus</i>	<i>Phytophthora parasitica</i> var. <i>nicotianae</i>	He et al. (1994)
	<i>B. subtilis</i>	<i>Pythium ultimum</i> , <i>R. solani</i> , <i>F. oxysporum</i> , <i>S. sclerotiorum</i> and <i>M. phaseoli</i>	Constantinescu (2001)
	<i>B. subtilis</i>	<i>R. solani</i>	Asaka and Shoda (1996)
Iturin A and Surfactin	<i>Burkholderia cepacia</i>	<i>R. solani</i>	El-Banna and Winkelmann (1988)
Pyrrolnitrin	<i>Pseudomonas fluorescens</i>	<i>Gaumannomyces graminis</i> var. <i>tritici</i>	Tazawa et al. (2000)
2,4-DAPG	<i>Enterobacter agglomerans</i>	<i>R. solani</i>	Howell and Stipanovic (1979)
	<i>Pseudomonas</i>	<i>Agrobacterium tumefaciens</i> , <i>Clavibacterium michiganense</i> , <i>Xanthomonas campestris</i> , <i>Pseudomonas syringae</i> pv. <i>syringae</i>	Chemin et al. (1996)
	<i>P. fluorescens</i>	<i>Xanthomonas oryzae</i> pv. <i>oryzae</i>	Velusamy et al. (2006)
	<i>P. fluorescens</i>	<i>Py. ultimum</i>	Howell and Stipanovic (1980)
Phenazines	<i>P. fluorescens</i>	<i>Gaumannomyces graminis</i> var. <i>tritici</i>	Weller and Cook (1983), Brisbane and Rovira (1988)
Phenazine-1-carboxylate	<i>P. fluorescens</i>	<i>Gaumannomyces graminis</i> var. <i>tritici</i>	Thomasnow et al. (1990)
Phenazine-1-carboxamide	<i>P. aureofaciens</i>	<i>Sclerotinia homeocarpa</i>	Powell et al. (2000)
Viscosinamide	<i>P. chlororaphis</i>	<i>Fusarium oxysporum</i> f. sp. <i>radicis-lycopersici</i>	Chin-A-Woeng et al. (1998), Bolwerk et al. (2003)
Amphisin Geldanamycin	<i>P. fluorescens</i>	<i>R. solani</i>	Nielsen et al. (2002)
	<i>P. fluorescens</i>	<i>Py. ultimum</i>	Thrane et al. (2000)
	<i>P. fluorescens</i>	<i>Py. ultimum</i> and <i>R. solani</i>	Andersen et al. (2003)
	<i>Streptomyces hygroscopicus</i> var. <i>geldonus</i>	<i>R. solani</i>	Rothrock and Gottlieb (1984)
Oligomycin A	<i>Streptomyces libani</i>	<i>Botrytis cinerea</i>	Kim et al. (1999)
Polyoxin D	<i>Streptomyces cacaoi</i> var.	<i>R. solani</i>	Isono et al. (1965)
Kasugamycin	<i>Streptomyces kasugaensis</i>	<i>P. oryzae</i>	Umezawa et al. (1965)

disease suppression when *hcn+* genes were re-introduced into the mutant genome or inserted into the genome of an initially nonactive strain (Voisard et al. 1989).

The role of antibiosis in the protection against phytopathogens was widely reviewed (Raaijmakers et al. 2002). Although there are numerous reports on the lack of correlation between in vitro antibiosis and effectiveness in field pointing out the limitations of using in vitro assays, many others concluded that at least a proportion of laboratory-discovered active isolates could be effective in soil. In general among a group of actinomycetes isolated from soil, while a number of in vitro antagonist actinomycetes may be active in soil, those with no in vitro activity are also inactive in soil (Broadbent et al. 1971). At least, in vitro assays have the advantage of selecting only antibiotic producers and excluding bacteria acting by other mechanisms.

Attempts to establish a causal relationship between antibiotic production revealed in vitro and biocontrol activity had been investigated, three main methods are used: (1) Direct detection and quantification of the antibiotics in the rhizosphere after inoculation through extraction and HPLC purification (Haas and keel 2003). Detection in some cases may be difficult due to the fact that in vitro culture conditions generally differ from those of rhizosphere. Indeed, biotic and abiotic factors including chemical instability of the antibiotic, irreversible binding to soil colloids or organic matter, or microbial decomposition could hamper direct detection of the antibiotic (Thomashow et al. 1997). (2) By use of reporter genes which could “report” the expression of antibiotic biosynthetic genes in the rhizosphere (Haas and keel 2003). This was reported in *Pseudomonas* for the expression of various antibiotics; 2,4-diacetylphloroglucinol (Notz et al. 2001), pyoluteorin (Kraus and Loper 1995), phenazine (Georgakopoulos et al. 1994), and lipopeptide antibiotics (Koch et al. 2002), and (3) genetic manipulation of bacterial antibiotic producers which is a powerful tool to ascertain their role in disease suppression. First work on this topic was performed by Thomashow and Weller (1988) by constructing pseudomonad mutants that lacked phenazine production and showing, thereafter, that these mutants were defective to control a plant disease. Tn5 insertion-derived mutants defective in phenazine synthesis (Phz-) were less effective in biocontrol of take-all disease in comparison with the parental strain of *P. fluorescens*. Moreover, effectiveness of some of these mutants was restored with cloned DNA from the effective parental strain. Mutations in the biosynthetic gene cluster of DAPG reduced biocontrol activity of fluorescent pseudomonads (Keel et al. 1992). Using ARDRA, Sharifi-Tehrani et al. (1998) compared the biocontrol activity of a collection of 2,4-DAPG-producing fluorescent *Pseudomonas* spp. and found that strains producing only 2,4-DAPG were more effective than pyoluteorin- and 2,4-DAPG-producing strains against *Fusarium* crown and root rot of tomato and *Pythium* damping-off of cucumber.

In addition, some works showed that the in vitro activities could not be related to the in situ activity. Spontaneous mutants of two scab-suppressive *Streptomyces* that were defective in in vitro pathogen inhibition activity against *Streptomyces scabies* demonstrated significant scab biocontrol activity, suggesting that the

pathogen inhibition activity detected in vitro may not be an accurate predictor of scab biocontrol (Schottel et al. 2001).

2.4.3 Antagonism by Competition: Siderophore Production

Iron is important for plant health and metabolism. It is found in proteins such as nitrogenase, ferredoxins, cytochromes, and leghemoglobin. PGPR bacteria could perform uptake of iron from soil and provide plant with this element. The most widely studied rhizospheric bacteria with respect to the production of siderophores are fluorescent pseudomonads. Siderophores are low-molecular-mass microbial compounds with high affinity for iron. They possess an iron uptake system (iron-binding ligand) able to chelate Fe^{3+} molecules. They are often induced under limiting Fe^{3+} concentrations to allow bacteria to partially fulfill their iron requirement.

Siderophores represent a large biochemically diverse group produced by either plants or plant associated microorganisms (Loper and Buyer 1991). They include pyoverdins produced by *Pseudomonas*; catechols produced by *Agrobacterium tumefaciens*, *Erwinia chrysanthemi*, and enterobacteriaceae; hydroxamates produced by *Erwinia carotovora*, *Enterobacter cloacae*, and various fungi; and rhizobactin produced by *Rhizobium meliloti*.

Pyoverdine which is a yellow-green, water-soluble fluorescent pigment is the major class of siderophores produced by fluorescent pseudomonad. However, strains of *P. aeruginosa*, *P. syringae*, and *P. putida* could also produce pyoverdine. The chemical structure of pyoverdine has been elucidated and the presence of a chromophore consisting of a 2,3-diamino-6,7-dihydroxyquinoline derivative which is responsible for the fluorescence was reported (Wendenbaum et al. 1983; Leong 1986). Siderophores can be used not only by their producing bacteria (Ongena et al. 1999), but also by other microorganisms. Fluorescent *Pseudomonas* exclusively recognizes the ferric complex of its own PVD. Thus differences in PVD structure affect the biological activity of the siderophores (Hohnadel and Meyer 1988). More than 30 structures of PVDs, differing mainly in their peptide chain, have been described (Budzikiewicz 1997).

Siderophore-producing microbes could contribute to various alterations in plants via the action of siderophores on ferric nutrition (Bar-Ness et al. 1991). Siderophores produced by PGPR could contribute to enhanced growth (Kloepper et al. 1980b). When various plants growing on soil or nutrient solution were supplemented by pyoverdin or ferripyoverdine, they showed, with few exceptions, enhanced chlorophyll content, and enhanced iron content in the roots and ferric reductase activity (Duijff et al. 1994b, c). In other cases, they have reverse action (Becker et al. 1985).

Siderophores are produced by *Pseudomonas* bacteria to compete for iron and consequently impairing growth of soil-borne phytopathogens, and thus are considered as a control mechanism for many pathogens (Duijff et al. 1994a; Schippers

et al. 1987; Bakker 1989). In addition, in vitro assays showed that the inhibition of pathogens based on competition for iron tends to decrease with increasing iron content of the medium (Duijff et al. 1993).

Suppressive soils to fusarium wilts are known to have a very low solubility of ferric iron (Alabouvette et al. 1996). Consequently a strong iron competition occurs in these soils. In addition, the ability to produce siderophores is likely to contribute to the root-colonizing ability of *Pseudomonas* strains, their antagonistic properties, and their usefulness in biocontrol (Leong 1986; Ran et al. 2005).

The role of these microorganisms in disease-suppressive soils particularly to fusarium wilts was shown to be related to siderophore-mediated iron competition. Addition of *Pseudomonas* pyoverdine to soils conducive to fusarium wilts and to *G. graminis* var. *tritici* confer them suppressiveness (Kloepper et al. 1980a). In addition, when soil was treated either by *Pseudomonas* or its pyoverdine, reduced chlamydospore germination of pathogenic *F. oxysporum* was observed (Elad and Baker 1985a), suggesting a possible role of pyoverdines in soil fungistasis and suppressiveness. In addition, some siderophores like pyocyanin and pyoverdine are essential for the induction of systemic resistance (Audenaert et al. 2002; Leeman et al. 1996; Meziane et al. 2005).

Actinomycetes are also reported as siderophore producers (Khamna et al. 2009). Endogenous siderophore (ferrioxamine) and exogenous siderophore (ferrichrome) have been studied in *Streptomyces pilosus* (Muller et al. 1984; Muller and Raymond 1984). *S. lydicus* WYEC108 was found to produce hydroxamate-type siderophores (Tokala et al. 2002). *Streptomyces violaceusniger* strain YCED9 was reported as able to chelate iron under limiting conditions (Buyer et al. 1989).

Evidences for the in situ production of siderophores and their involvement in biocontrol include the following: (1) *Variation in iron availability of the soil*: increasing the iron amount in soil by lowering soil pH through amendment of H_2SO_4 or iron synthetic chelator resulted in loss of suppression of *Fusarium* wilt by *Pseudomonas* (Elad and Baker 1985b). (2) *Addition of siderophores or synthetic chelators to soil*: introduction of pyoverdine in soil resulted in reducing chlamydospore germination of *Fusarium* (Elad and Baker 1985a) and reducing Fusariosis and take-all disease (Kloepper et al. 1980a). Addition of a synthetic chelator; the ferrated form of ethylenediamine-*o*-hydroxyphenyl acetic acid Fe-EDDHA to the nutrient solution for the plants diminished the disease-suppressive effect of *Pseudomonas putida* WCS358 to suppress *Fusarium* wilt of radish caused by *F. oxysporum* f. sp. *raphani* (de Boer et al. 2003), while addition of EDDHA or its ferrated form to conducive soil rendered it suppressive to *Fusarium* wilt of cucumber, flax, and radish (Scher and Baker 1982). (3) *Genetic evidences*: Expression of siderophore biosynthesis genes in the rhizosphere by *P. fluorescens* in which a promoter from a siderophore biosynthesis gene was cloned (reporter gene) (Loper and Lindow 1991) and comparison of the biocontrol abilities of wild-type producing strains and their mutants defective in siderophore production. Bakker et al. (1988) demonstrated that mutants defective in the synthesis of pyoverdine (Pvd-) and able to use pyoverdine produced by a coinoculated wild-type strain showed a great establishment in potato compared with a mutant not able to use pyoverdine.

Wild-types of *Pseudomonas putida* WCS358 which relatively suppress *F. oxysporum* f. sp. *dianthi* in carnation roots were found to depend only on siderophore-mediated competition for iron. Subsequently, its mutant defective in siderophore biosynthesis was ineffective. This fact provides the proof that siderophores were implicated in the suppressiveness of *Fusarium* wilt by this strain (Duijff et al. 1993). Similar results were found in *P. putida* WCS358 for *Fusarium* wilt in radish caused by *F. oxysporum* f. sp. *raphani* (de Boer et al. 2003). However, as disease suppression does not rely only on siderophore production, lack of evidence in the use of mutant-derived strains has been reported. Mutants from *Pseudomonas* sp. WCS417r or *P. putida* strain RE8 defective in siderophore biosynthesis are still able to ensure comparable or a less effective disease suppression in carnation and radish in comparison to wild types due to resistance induction and probably also antibiosis (Duijff et al. 1993; de Boer et al. 2003). In Addition, iron-regulated molecules but non-siderophores could be implicated in disease suppression. In the case of *G. graminis* var. *tritici* (Kloepper et al. 1980a), partial contribution of an iron-regulated nonsiderophore to this suppressiveness is not excluded (Thomashow et al. 1990). Also, Gill and Warren (1988) reported a negatively iron-regulated fungistatic agent to *Pythium ultimum* in iron-limited cultures of *Pseudomonas* sp. NZ130.

2.5 Conclusion

Sustainable agriculture, based on environmentally friendly methods, tends to use bacteria as tools that could by the way reduce the use of chemicals. It is advantageous for sustainable agriculture as introduced bacteria could act as biofertilizers and as biopesticides. In this way, PGPR could constitute a group of bacteria of great importance. Recently, biopesticides are receiving worldwide attention for the sustainability of the agricultural system. Researches had interested the selection of bacteria able to antagonize most deleterious phytopathogens. Unfortunately, most of the works on the biocontrol effect of rhizobacteria were conducted in axenic conditions at laboratory-scale and greenhouse-controlled conditions. Few works were conducted under field conditions. However, the effectiveness of a biocontrol agent depends mainly on its interaction with other microorganisms, the controlled phytopathogen, the plant, and the rhizosphere environment.

Root competence of bacteria with in vitro antagonistic effects toward phytopathogens is one of the most important traits to be considered when bacteria are introduced in native soils where they are subjected to interaction with both roots and other microorganisms. Efficient competition for colonization sites is an important prerequisite for effective biocontrol. In some cases, relatively long time is needed for checking the effectiveness in disease suppression. Also, host crop could affect rhizosphere colonization and competitiveness of antagonistic bacteria as plant response to rhizosphere colonization seems to be bacterium specific. In addition, the sensitivity of phytopathogenic fungi in some cases depends on its

life cycle stage and propagules. For instance, mycelia, zoosporangia, zoospore cysts, and zoospores of *Pythium ultimum* showed difference in sensitivity to 2,4-DAPG (de Souza et al. 2003). For all these reasons, only few examples of biocontrol agents-based products succeeded in field trials and thus have been commercialized for use in agriculture.

A remarkable diversity of metabolites with antibiotic activity is produced by *Pseudomonas*, *Bacillus*, and *Streptomyces* strains. Some of these microorganisms could produce simultaneously more than one compound (for example, *P. fluorescens* strains CHAO and Pf-5) and/or act by more than one mechanism (e.g., antibiosis and competition for nutrients). Rhizobacteria that could also induce systemic resistance confer protection against phytopathogens to plants. DAPG and siderophores such as pyoverdine have been described as inducers of systemic plant resistance. In contrast to antagonism by antibiosis or siderophore production where the population size should be maintained during the biocontrol process, it is sufficient that the plant and the inducing agent be in contact for a limited period and once induced, systemic resistance is expressed systemically throughout the plant and maintained for prolonged periods.

A better understanding of the major mechanism displayed in field soils will suggest what conditions are to be provided in order to optimize the antagonistic activities of inoculant strains. In this optic, controlled root exudation or nutritional amendment could lead to more successful disease management. Bacteria with more than one beneficial effect are of great interest in biocontrol. By combining strains with different disease-suppressive mechanisms, the impact of field fluctuating biotic and abiotic conditions could be minimized as some biocontrol mechanism could be effective even if others are unfunctional. In addition, such combinations could be effective against multiple phytopathogens. However, the use of bioinoculants must be taken with some precautions. Measures must be taken to avoid nontarget effect of the introduced bacteria, to stabilize them in soil systems, and thus to guarantee durability of their beneficial effect and their good performance.

References

- Ahl P, Voisard C, Defago G (1986) Iron bound siderophores, cyanic acid, and antibiotics involved in suppression of *Thielaviopsis basicola* by a *Pseudomonas fluorescens* strain. *J Phytopathol* 116:121–134
- Ahmad F, Ahmad I, Khan MS (2008) Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiol Res* 163:173–181
- Ahn IP, Park K, Kim CH (2002) Rhizobacteria-induced resistance perturbs viral disease progress and triggers defense-related gene expression. *Mol Cell* 13:302–308
- Alabouvette C, Höper H, Lemanceau P, Steinberg C (1996) Soil suppressiveness to diseases induced by soil-borne plant pathogens. In: Stotzky G, Bollag J-M (eds) *Soil biochemistry*. Marcel Dekker, New York, pp 371–413
- Aldequy HS, Mansour FA, Abo-Hamed SA (1998) Effect of the culture filtrates of *Streptomyces* on growth and productivity of wheat plants. *Folia Microbiol* 43:465–470

- Amann RI, Ludwig W, Schleifer K-H (1995) Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiol Rev* 59:143–169
- Amkraz N, Boudyach EH, Boubaker H, Bouizgarne B, Ait Ben Aoumar A (2010) Screening for fluorescent pseudomonades, isolated from the rhizosphere of tomato, for antagonistic activity toward *Clavibacter michiganensis* subsp. *michiganensis*. *World J Microbiol Biotechnol* 26:1059–1065
- Andersen JB, Koch B, Nielsen TH, Sørensen D, Hansen M, Nybroe O, Christophersen C, Sørensen J, Molin S, Givskov M (2003) Surface motility in *Pseudomonas* sp. DSS73 is required for efficient biological containment of the root-pathogenic microfungi *Rhizoctonia solani* and *Pythium ultimum*. *Microbiology* 149:1147–1156
- Anderson AJ, Habibzadegah-Tari P, Pepper CS (1988) Molecular studies on the role of a root surface agglutinin in adherence and colonization by *Pseudomonas putida*. *Appl Environ Microbiol* 54:375–380
- Antoun H, Kloepper JW (2001) Plant growth-promoting rhizobacteria (PGPR). In: Brenner S, Miller JH (eds) *Encyclopedia of genetics*. Academic, New York, pp 1477–1480
- Arshad M, Shaharoon B, Mahmood T (2008) Inoculation with plant growth promoting rhizobacteria containing ACC-deaminase partially eliminates the effects of water stress on growth, yield and ripening of *Pisum sativum* L. *Pedosphere* 18:611–620
- Arzanesht MH, Alikhani HA, Khavazi K, Rahimian HA, Miransari M (2011) Wheat (*Triticum aestivum* L.) growth enhancement by *Azospirillum* sp. under drought stress. *World J Microbiol Biotechnol* 27:197–205
- Asaka O, Shoda M (1996) Biocontrol of *Rhizoctonia solani* damping-off of tomato with *Bacillus subtilis* RB14. *Appl Environ Microbiol* 62:4081–4085
- Aseri GK, Jain N, Panwar J, Rao AV, Meghwal PR (2008) Biofertilizers improve plant growth, fruit yield, nutrition, metabolism and rhizosphere enzyme activities of pomegranate (*Punica granatum* L.) in Indian Thar Desert. *Sci Hortic* 117:130–135
- Assmus B, Hutzler P, Kirchhof G, Amann R, Lawrence JR, Hartmann A (1995) In situ localization of *Azospirillum brasilense* in the rhizosphere of wheat with fluorescently labeled rRNA-targeted oligonucleotide probes and scanning confocal laser microscopy. *Appl Environ Microbiol* 61:1013–1019
- Audenaert K, Pattery T, Comelis P, Hofte M (2002) Induction of systemic resistance to *Botrytis cinerea* in tomato by *Pseudomonas aeruginosa* TNSK2: Role of salicylic acid, pyochelin, and pyocyanin. *Mol Plant Microbe Interact* 15:1147–1156
- Bai Y, Zhou X, Smith DL (2003) Enhanced soybean plant growth resulting from coinoculation of *Bacillus* strains with *Bradyrhizobium japonicum*. *Crop Sci* 43:1774–1781
- Baker R (1991) Induction of rhizosphere competence in the biocontrol fungus *Trichoderma*. In: Keister DL, Cregan PB (eds) *Rhizosphere and plant growth*. Kluwer Academic, Dordrecht, pp 221–228
- Bakker PAHM (1989) Siderophore-mediated plant growth promotion and colonization of roots by strains of *Pseudomonas* spp. Ph.D thesis, Willie Commelin Scholten Phytopathological Laboratory, Department of Plant Pathology, State University Utrecht, Javalaan 20, 3742 Baarn, The Netherlands, pp 100
- Bakker AW, Schippers B (1995) Induction of systemic resistance against *Fusarium* wilt of radish by lipopolysaccharides of *Pseudomonas fluorescens*. *Phytopathology* 85:1021–1027
- Bakker PAHM, Weisbeek PJ, Schippers B (1988) Siderophore production by plant growth promoting *Pseudomonas* spp. *J Plant Nutr* 11:925–933
- Bakker PAHM, Raaijmakers JM, Bloemberg GV, Hofte M, Lemanceau P, Cooke M (2007) New perspectives and approaches in plant growth-promoting rhizobacteria research. *Eur J Plant Pathol* 119:241–242
- Bargabus RL, Zidack NK, Sherwood JE, Jacobsen BJ (2003) Characterisation of systemic resistance in sugar beet elicited by a nonpathogenic, phyllosphere-colonizing *Bacillus mycoides*, biological control agent. *Physiol Mol Plant Pathol* 61:289–298

- Bar-Ness E, Chen Y, Hadar Y, Marschner H, Römheld V (1991) Siderophores of *Pseudomonas putida* as an iron source for dicot and monocot plants. *Plant Soil* 130:231–241
- Barnett SJ, Singleton I, Ryder M (1999) Spatial variation in population of *Pseudomonas corrugata* 2140 and *Pseudomonads* on take-all diseased and healthy root systems of wheat. *Soil Biol Biochem* 31:633–636
- Barrows-Broadbent J, Kerr TK (1981) Inhibition of *Fusarium moniliforme* var. *subglutinans*, the casual agent of pitch canker, by the soil bacterium *Arthrobacter* sp. *Can J Microbiol* 27:20–27
- Becker JO, Hedges RW, Messens E (1985) Inhibitory effect of pseudobactin on the uptake of iron by higher plants. *Appl Environ Microbiol* 49:1090–1093
- Bent E, Tuzun S, Chanway CP, Enebak S (2001) Alterations in plant growth and in root hormone levels of lodgepole pines inoculated with rhizobacteria. *Can J Microbiol* 47:793–800
- Berdy J (2005) Bioactive microbial metabolites. *J Antibiot* 58:1–26
- Bermpohl A, Dreier J, Bahro R, Eichenlaub R (1996) Exopolysaccharides in the pathogenic interaction of *Clavibacter michiganensis* subsp. *michiganensis* with tomato plants. *Microbiol Res* 151:391–399
- Bloemberg GV (2007) Microscopic analysis of plant -bacterium interactions using auto fluorescent proteins. *Eur J Plant Pathol* 119:301–309
- Bloemberg GV, Lugtenberg BJJ (2001) Molecular basis of plant growth promotion and biocontrol by rhizobacteria. *Curr Opin Plant Biol* 4:43–50
- Bloemberg GV, Wijffjes AHM, Lamers GEM, Stuurman N, Lugtenberg BJJ (2000) Simultaneous imaging of *Pseudomonas fluorescens* WCS365 populations expressing three different autofluorescent proteins in the rhizosphere: new perspectives for studying microbial communities. *Mol Plant Microbe Interact* 13:1170–1176
- Bolwerk A, Lugtenberg BJJ (2005) Visualization of interactions of microbial biocontrol agents and phytopathogenic fungus *Fusarium oxysporum* f. sp. *radicis lycopersici* on tomato roots. In: Siddiqui ZA (ed) *PGPR: biocontrol and biofertilization*. Springer, Berlin, pp 217–231
- Bolwerk A, Lagopodi AL, Wijffjes AHM, Lamers GEM, Chin-A-Woeng TFC, Lugtenberg BJJ, Bloemberg GV (2003) Interactions in the tomato rhizosphere of two *Pseudomonas* biocontrol strains with the phytopathogenic fungus *Fusarium oxysporum* f. sp. *radicis-lycopersici*. *Mol Plant Microbe Interact* 11:983–993
- Bouizgarne B, El Hadrami I, Ouhdouch Y (2006) Novel production of isochainin by a strain of *Streptomyces* sp. isolated from rhizosphere soil of the indigenous Moroccan plant *Argania spinosa* L. *World J Microbiol Biotechnol* 22:423–429
- Brisbane PG, Rovira AD (1988) Mechanisms of inhibition of *Gaeumannomyces graminis* var. *tritici* by fluorescent pseudomonads. *Plant Pathol* 37:104–111
- Broadbent P, Baker KF, Waterworth Y (1971) Bacteria and actinomycetes antagonistic to fungal root pathogens in Australian soils. *Aust J Biol Sci* 24:925–944
- Budzikiewicz H (1997) Siderophores of fluorescent pseudomonads. *Z Naturforsch C* 52:713–720
- Bull CT, Weller DM, Thomashow LS (1991) Relationship between root colonization and suppression of *Gaeumannomyces graminis* var. *tritici* by *Pseudomonas fluorescens* strain 2–79. *Phytopathology* 81:954–959
- Burd GI, Dixon DG, Glick BR (2000) Plant growth-promoting bacteria that decrease heavy metal toxicity in plants. *Can J Microbiol* 46:237–245
- Burr TJ, Schroth MN, Suslow T (1978) Increased potato yields by treatment of seed pieces with specific strains of *Pseudomonas fluorescens* and *P. putida*. *Phytopathology* 68:1377–1383
- Buyer JS, Sikora LJ, Chaney RL (1989) A new growth medium for the study of siderophore-mediated interactions. *Biol Fertil Soils* 8:97–101
- Chabot R, Antoun H, Cescas M (1993) Stimulation de la croissance du maïs et de la laitue romaine par des microorganismes dissolvant le phosphore inorganique. *Can J Microbiol* 39:941–947
- Chebottar VK, Asis CA Jr, Akao S (2001) Production of growth-promoting substances and high colonization ability of rhizobacteria enhance the nitrogen fixation of soybean when coinoculated with *Bradyrhizobium japonicum*. *Biol Fertil Soils* 34:427–432
- Chernin L, Ismailov Z, Haran S, Chet I (1995) Chitinolytic *Enterobacter agglomerans* antagonistic to fungal plant pathogens. *Appl Environ Microbiol* 61:1720–1726

- Chernin L, Brandis A, Ismailov Z, Chet I (1996) Pyrrolnitrin production by an *Enterobacter agglomerans* strain with a broad spectrum of antagonistic activity towards fungal and bacterial phytopathogens. *Curr Microbiol* 32:208–212
- Chernin LS, Fuente LDL, Sobolov V, Haran S, Vorgias CE, Oppenheim AB, Chet I (1997) Molecular cloning, structural analysis, and expression in *Escherichia coli* of a chitinase gene from *Enterobacter agglomerans*. *Appl Environ Microbiol* 63:834–839
- Chet I, Ordentlich A, Shapira R, Oppenheim A (1990) Mechanisms of biocontrol of soil-borne plant pathogens by rhizobacteria. *Plant Soil* 129:85–92
- Chin-A-Woeng TFC, de Priester W, van der Bij AJ, Lugtenberg BJJ (1997) Description of the colonization of a gnotobiotic tomato rhizosphere by *Pseudomonas fluorescens* biocontrol strain WCS365, using scanning electron microscopy. *Mol Plant Microbe Interact* 10:79–86
- Chin-A-Woeng TFC, Bloemberg GV, van der Bij AJ, van der Drift KMG, Schripsema J, Kroon B, Scheffer RJ, Keel C, Bakker PAHM, Tichy HV, de Bruijn FJ, Thomas-Oates JE, Lugtenberg BJJ (1998) Biocontrol by phenazine-1-carboxamide-producing *Pseudomonas chlororaphis* PCL1391 of tomato root rot caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici*. *Mol Plant Microbe Interact* 11:1069–1077
- Chin-A-Woeng TFC, Bloemberg GV, Mulders IHM, Dekkers LC, Lugtenberg BJJ (2000) Root colonization by phenazine-1-carboxamide-producing bacterium *Pseudomonas chlororaphis* PCL1391 is essential for biocontrol of tomato root rot. *Mol Plant Microbe Interact* 12:1340–1345
- Chin-A-Woeng TFC, Bloemberg GV, Lugtenberg BJJ (2003) Phenazines and their role in biocontrol by *Pseudomonas* bacteria. *New Phytol* 157:503–523
- Constantinescu F (2001) Extraction and identification of antifungal metabolites produced by some *B. subtilis* strains. *Analele Institutului de Cercetari Pentru Cereale Protectia Plantelor* 31:17–23
- Crawford DL, Lynch JM, Whipps JM, Ousley MA (1993) Isolation and characterization of actinomycete antagonists of a fungal root pathogen. *Appl Environ Microbiol* 59:3899–3905
- Crawford DL, Kowalski M, Roberts MA, Merrel G, Deobald LA (2005) Discovery, development and commercialization of a microbial antifungal biocontrol agent *Streptomyces lydicus* WYEC 108: history of a decade long endeavour. *Soc Ind Microbiol News* 55:88–95
- Da Mota FF, Gomes EA, Seldin L (2008) Auxin production and detection of the gene coding for the auxin efflux carrier (AEC) protein in *Paenibacillus polymyxa*. *J Microbiol* 56:275–264
- de Boer M, Bom P, Kindt F, Keurentjes JJB, van der Sluis I, van Loon LC, Bakker PAHM (2003) Control of *Fusarium* wilt of radish by combining *Pseudomonas putida* strains that have different disease-suppressive mechanisms. *Phytopathology* 93:626–632
- de Mot R, Proost P, van Damme J, Vander Leyden J (1992) Homology of the root adhesin of *Pseudomonas fluorescens* OE 28.3 with porin F of *P. aeruginosa* and *P. syringae*. *Mol Gen Genet* 231:489–493
- de Souza JTA, Arnould C, Deulvot C, Lemanceau P, Gianinazzi-Pearson V, Raaijmakers JM (2003) Effect of 2,4-diacetylphloroglucinol on *Pythium*: cellular responses and variation in sensitivity among propagules and species. *Phytopathology* 93:966–975
- de Vasconcellos RLF, Cardoso EJB (2009) Rhizospheric Streptomycetes as potential biocontrol agents of *Fusarium* and *Armillaria* pine rot and as PGPR for *Pinus taeda*. *Biocontrol* 54:807–816
- De Vleeschauwer D, Djavaheri M, Bakker PAHM, Höfte M (2008) *Pseudomonas fluorescens* WCS374r-induced systemic resistance in rice against *Magnaporthe oryzae* is based on pseudobactin-mediated priming for a salicylic acid-repressible multifaceted defense response. *Plant Physiol* 148:1996–2012
- De Weger LA, Van Bostel R, Van Der Burg B, Gruters RA, Geels FP, Schippers B, Lugtenberg B (1986) Siderophores and outer membrane proteins of antagonistic, plant- growth-stimulating, rootcolonizing *Pseudomonas* spp. *J Bacteriol* 165:585–594
- de Weger LA, van der Vlugt CIM, Wijffjes AHM, Bakker PAHM, Schippers B, Lugtenberg BJJ (1987) Flagella of a plant growth stimulating *Pseudomonas fluorescens* strain are required for colonization of potato roots. *J Bacteriol* 169:2769–2773

- de Weger LA, Bakker PAHM, Schippers B, van Loosdrecht MCM, Lugtenberg BJJ (1989) *Pseudomonas* spp. with mutational changes in the Oantigenic side chain of their lipopolysaccharide are affected in their ability to colonize potato roots. In: Lugtenberg BJJ (ed) Signal molecules in plants and plant-microbe interactions. Springer, Berlin, pp 197–202
- de Weger LA, Kuipe I, van der Bij AJ, Lugtenberg BJJ (1997) Use of a lux-based procedure to rapidly visualize root colonization by *Pseudomonas fluorescens* in the wheat rhizosphere. *Antonie Leeuwenhoek* 72:365–372
- Deepa CK, Dastager SG, Pandey A (2010) Plant growth-promoting activity in newly isolated *Bacillus thioeparus* (NII-0902) from Western ghat forest, India. *World J Microbiol Biotechnol* 26:2277–2283
- Défago G, Haas D, Berling CH, Burger U, Keel C, Voisard C, Wirthner P, Wuthrich B (1990) Suppression of black root rot of tobacco and other root diseases by strains of *Pseudomonas fluorescens*: potential applications and mechanisms. In: Hornby D (ed) Biological control of soil-borne plant pathogens. CAB International, Wallingford, pp 93–108
- Dekkers LC, van der Bij AJ, Mulders IHM, Phoelich CC, Wentwoord RAR, Glandorf DCM, Wijffelman CA, Lugtenberg BJJ (1998) Role of the O-antigen of lipopolysaccharide, and possible roles of growth rate and NADH: ubiquinone oxidoreductase (nuo) in competitive tomato root-tip colonization by *Pseudomonas fluorescens* WCS365. *Mol Plant Microbe Interact* 11:763–771
- Dell'Amico E, Cavalca L, Andreoni V (2008) Improvement of *Brassica napus* growth under cadmium stress by cadmium-resistant rhizobacteria. *Soil Biol Biochem* 40:74–84
- Duijff BJ, Meijer JW, Bakker PAHM, Schippers B (1993) Siderophore-mediated competition for iron and induced resistance in the suppression of *Fusarium* wilt of carnation by fluorescent *Pseudomonas* spp. *Neth Plant Pathol* 99:277–289
- Duijff BJ, Bakker PAHM, Schippers B (1994a) Suppression of *Fusarium* wilt of carnation by *Pseudomonas putida* WCS358 at different levels of disease incidence and iron availability. *Biocontrol Sci Technol* 4:279–288
- Duijff BJ, Bakker PAHM, Schippers B (1994b) Ferric pseudobactin 358 as an iron source for carnation. *J Plant Nutr* 17:2069–2078
- Duijff BJ, De Kogel WJ, Bakker PAHM, Schippers B (1994c) Influence of pseudobactin 358 on the iron nutrition of barley. *Soil Biol Biochem* 26:1681–1994
- Duijff BJ, Gianinazzi-Pearson V, Lemanceau P (1997) Involvement of the outer-membrane lipopolysaccharides in the endophytic colonization of tomato roots by biocontrol *Pseudomonas fluorescens* WCS417r. *New Phytol* 135:325–334
- Dunne C, Crowley JJ, Moënne-Loccoz Y, Dowling DN, de Bruijn FJ, O'Gara F (1997) Biological control of *Pythium ultimum* by *Stenotrophomonas maltophilia* W81 is mediated by an extracellular proteolytic activity. *Microbiology* 143:3921–3391
- Dunne C, Moënne-Loccoz Y, McCarthy J, Higgins P, Powell J, Dowling DN, O'Gara F (1998) Combining proteolytic and phloroglucinol-producing bacteria for improved biocontrol of *Pythium*-mediated damping-off of sugar beet. *Pathology* 47:299–307
- El-Abyad MS, El-Sayed MA, El-Shanshoury AR, El-Sabbagh SM (1993) Towards the biological control of fungal and bacterial diseases of tomato using antagonism *Streptomyces* spp. *Plant Soil* 149:185–195
- Elad Y, Baker R (1985a) The role of competition for iron and carbon in suppression of chlamydo-spore germination of *Fusarium* spp by *Pseudomonas* spp. *Phytopathology* 75:1053–1059
- Elad Y, Baker R (1985b) Influence of trace amounts of cations and siderophore-producing pseudomonads on chlamydo-spore germination of *Fusarium oxysporum*. *Phytopathology* 75:1047–1052
- El-Banna N, Winkelmann G (1988) Pyrrolnitrin from *Burkholderia cepacia*: antibiotic activity against fungi and novel activities against streptomycetes. *J Appl Microbiol* 85:69–78
- El-Tarabily KA (2006) Rhizosphere-competent isolates of *Streptomyces* and non-streptomycete *Actinomycetes* capable of producing cell-wall degrading enzymes to control *Pythium aphanidermatum* damping-off disease of cucumber. *Can J Bot* 84:211–222

- El-Tarabily KA (2008) Promotion of tomato (*Lycopersicon esculentum* Mill.) plant growth by rhizosphere competent 1- aminocyclopropane-1- carboxylic acid deaminase-producing streptomycete *Actinomycetes*. Plant Soil 308:161–174
- El-Tarabily KA, Sivasithamparam K (2006) Non-streptomycete actinomycetes as biocontrol agents of soil-borne fungal plant pathogens and as plant growth promoters. Soil Biol Biochem 38:1505–1520
- El-Tarabily KA, Soliman MH, Nassar AH, Al-Hassani HA, Sivasithamparam K, McKenna F, Hardy GESJ (2000) Biological control of Sclerotinia minor using a chitinolytic bacterium and Actinomycetes. Plant Pathol 49:573–583
- El-Tarabily KA, Nassar AH, Sivasithamparam K (2008) Promotion of growth of bean (*Phaseolus vulgaris* L.) in a calcareous soil by a phosphate-solubilizing, rhizosphere- competent isolate of *Micromonospora endolithica*. Appl Soil Ecol 39:161–171
- Fließbach A, Winkler M, Lutz MP, Oberholzer H-R, Mäder P (2009) Soil Amendment with *Pseudomonas fluorescens* CHA0: Lasting effects on soil biological properties in soils low in microbial biomass and activity. Microb Ecol 57:611–623
- Foster RC, Rovira AD (1978) The ultrastructure of the rhizosphere of *Trifolium subterraneum* L. In: Loutit MW, Miles JAR (eds) Microbial ecology. Springer, Berlin, pp 278–290
- Franco C, Michelsen P, Percy N, Conn V, Listiana E, Moll S, Loria R, Coombs J (2007) Actinobacterial endophytes for improved crop performance. Australas Plant Pathol 36:524–531
- Frankowski Lorito M, Scala F, Schmidt R, Berg G, Bahl H (2001) Purification and properties of two chitinolytic enzymes of *Serratia plymuthica* HRO-C48. Arch Microbiol 176:421–426
- Fridlender M, Inbar J, Chet I (1993) Biological control of soilborne plant pathogens by a β -1, 3 glucanase producing *Pseudomonas cepacia*. Soil Biol Biochem 25:1211–1221
- Gamalero E, Lingua G, Capri FG, Fusconi A, Berta G, Lemanceau P (2004) Colonization pattern of primary tomato roots by *Pseudomonas fluorescens* A6RI characterized by dilution plating, flow cytometry, fluorescence, confocal and scanning electron microscopy. FEMS Microbiol Ecol 48:79–87
- Gamalero E, Lingua G, Tombolini R, Avidano L, Pivato B, Berta G (2005) Colonization of tomato root seedling by *Pseudomonas fluorescens* 92 rkG5: spatio-temporal dynamics, localization, organization, viability, and culturability. Microbiol Ecol 50:289–297
- Geels FP, Schippers B (1983) Selection of antagonistic fluorescent *Pseudomonas* spp. and their root colonization and persistence following treatment of seed potatoes. Phytopathol J 108:193–206
- Georgakopoulos DG, Hendson M, Panopoulos NJ, Schroth MN (1994) Cloning of a phenazine biosynthetic locus of *Pseudomonas aureofaciens* PGS12 and analysis of its expression in vitro with the ice nucleation reporter gene. Appl Environ Microbiol 60:2931–2938
- Gill PR, Warren GJ (1988) An iron-antagonized fungistatic agent that is not required for iron assimilation from a fluorescent rhizosphere pseudomonad. J Bacteriol 170:163–170
- Glandorf DCM, van der Sluis I, Anderson AJ, Bakker PAHM, Schippers B (1994) Agglutination, adherence, and root colonization by fluorescent pseudomonads. Appl Environ Microbiol 60:1726–1733
- Glick BR, Todorovic B, Czarny J, Cheng Z, Duan J, McConkey B (2007) Promotion of plant growth by bacterial ACC deaminase. Crit Rev Plant Sci 26:227–242
- Goddard VJ, Bailey MJ, Darrah P, Lilley AK, Thompson IP (2001) Monitoring temporal and spatial variation in rhizosphere bacterial population diversity: a community approach for the improved selection of rhizosphere competent bacteria. Plant Soil 232:181–193
- Gómez-Gómez L, Felix G, Boller T (1999) A single locus determines sensitivity to bacterial flagellin in *Arabidopsis thaliana*. Plant J 18:277–284
- Grayston SJ, Vaughan D, Jones D (1996) Rhizosphere carbon flow in trees, in comparison with annual plants: the importance of root exudation and its impact on microbial activity and nutrient availability. Appl Soil Ecol 5:29–56
- Gupta A, Meyer JM, Goel R (2002) Development of heavy metal-resistant mutants of phosphate solubilizing *Pseudomonas* sp. NBRI 4014 and their characterization. Curr Microbiol 45:323–327

- Gutiérrez-Luna FM, López-Bucio J, Altamirano-Hernández J, Valencia-Cantero E, de la Cruz HR, Macías-Rodríguez L (2010) Plant growth-promoting rhizobacteria modulate rootsystem architecture in *Arabidopsis thaliana* through volatile organic compound emission. *Symbiosis* 51:75–83
- Guzzo SD, Martins EMF (1996) Local and systemic induction of β -1, 3-glucanase and chitinase in coffee leaves protected against *Hemileia vastatrix* by *Bacillus thuringiensis*. *J Phytopathol* 144:449–454
- Haas D, Défago G (2005) Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nat Rev Microbiol* 3:307–319
- Haas D, Keel C (2003) Regulation of antibiotic production in root-colonizing *Pseudomonas* spp. and relevance for biological control of plant disease. *Annu Rev Phytopathol* 41:117–153
- Hamby MK (2001) M.S. thesis. University of Idaho, Moscow
- Hamby MK, Crawford DL (2000) The enhancement of plant growth by selected *Streptomyces* species. In: 100th General meeting of American Society for Microbiology, Los Angeles, CA. Abstract 567
- Hamdali H, Bouizgarne B, Hafidi M, Lebrihi A, Virolle MJ, Ouhdouch Y (2008a) Screening for rock phosphate solubilizing *Actinomycetes* from Moroccan phosphate mines. *Appl Soil Ecol* 38:12–19
- Hamdali H, Hafidi M, Virolle MJ, Ouhdouch Y (2008b) Rock phosphate-solubilizing *Actinomycetes*: screening for growth promoting activities. *World J Microbial Biotechnol* 24:2565–2575
- Hasegawa S, Meguro A, Shimizu M, Nishimura T, Toyoda K, Shiraishi T, Kunoh H (2008) Two bioassay methods to evaluate root-accelerating activity of *Streptomyces* sp. *MBR52* metabolites. *Actinomycetologica* 22:42–45
- He H, Silo-Suh LA, Handelsman J, Clardy J (1994) Zwittermicin A, an antifungal and plant protection agent from *Bacillus cereus*. *Tetrahedron Lett* 35:2499–2502
- Hiltner L (1904) Über neue Erfahrungen und Probleme auf dem Gebiet der Bodenbakteriologie und unter besonderes Berücksichtigung der Grundungen und Brauche. *Arb Dtsch Landwirt Ges Berl* 98:59–78
- Hohnadel D, Meyer JM (1988) Specificity of pyoverdine-mediated iron uptake among fluorescent *Pseudomonas* strains. *J Bacteriol* 170:4865–4873
- Howell CR, Stipanovic RD (1979) Control of *Rhizoctonia solani* on cotton seedlings with *Pseudomonas fluorescens* and with an antibiotic produced by the bacterium. *Phytopathology* 69:480–482
- Howell CR, Stipanovic RD (1980) Suppression of *Pythium ultimum*-induced damping off of cotton seedlings by *Pseudomonas fluorescens* and its antibiotic, pyoluteorin. *Phytopathology* 70:712–715
- Howie WJ, Cook RJ, Weller DM (1987) Effects of soil matrix potential and cell motility on wheat root colonization by fluorescent pseudomonads suppressive to take-all. *Phytopathology* 77:286–292
- Idris EES, Makarewicz O, Farouk A, Rosner K, Greiner R, Bochow H, Richter T, Borriß R (2002) Extracellular phytase activity of *Bacillus amyloliquefaciens* FZB45 contributes to its plant-growth-promoting effect. *Microbiology* 148:2097–2109
- Idris EES, Bochow H, Ross H, Borriß R (2004) Use of *Bacillus subtilis* as biocontrol agent. Phytohormone like action of culture filtrates prepared from plant growth-promoting *Bacillus amyloliquefaciens* FZB24, FZB42, FZB45 and *Bacillus subtilis* FZB37. *J Plant Dis Prot* 111:583–597
- Inbar J, Chet I (1991) Evidence that chitinase produced by *Aeromonas caviae* is involved in the biological control of soil-borne plant pathogens by this bacterium. *Soil Biol Biochem* 23:973–978
- Isono K, Nagatsu J, Kawashima Y, Suzuki S (1965) Studies on polyoxins, antifungal antibiotics. Part I. Isolation and characterization of polyoxins A and B. *Agric Biol Chem* 29:848–854

- Jacobsen CS (1997) Plant protection and rhizosphere colonization of barley by seed inoculated herbicide degrading *Burkholderia* (*Pseudomonas*) *cepacia* DBO1 (pRO101) in 2, 4-D contaminated soil. *Plant Soil* 189:139–144
- Jacobsen BJ, Zidack NK, Larson BJ (2004) The role of *Bacillus*-based biological control agents in integrated pest management systems: plant diseases. *Phytopathology* 94:1272–1275
- James WC (1981) Estimated losses of crops from plant pathogens. In: Pimentel D (ed) *Handbook of pest management in agriculture*, vol 1. CRC, Boca Raton, FL, pp 79–94
- Jetiyanon K, Fowler WD, Kloepper JW (2003) Broad-spectrum protection against several pathogens by PGPR mixtures under field conditions in Thailand. *Plant Dis* 87:1390–1394
- Jeun YC, Park KS, Kim H (2001) Different mechanisms of induced systemic resistance and systemic acquired resistance against *Colletotrichum orbiculare* on the leaves of cucumber plants. *Mycobiology* 29:19–26
- Jung WJ, An KN, Jin YL, Park RD, Lim KT, Kim KY, Kim TH (2003) Biological control of damping off caused by *Rhizoctonia solani* using chitinase producing *Paenibacillus illinoisensis* KJA-424. *Soil Biol Biochem* 35:1261–1264
- Kamensky M, Ovdais M, Chet I, Chernin L (2003) Soil-borne strain IC14 of *Serratia plymuthica* with multiple mechanisms of antifungal activity provides biocontrol of *Botrytis cinerea* and *Sclerotinia sclerotiorum* diseases. *Soil Biol Biochem* 35:323–331
- Keel C, Schnider U, Maurhofer M, Voisard C, Laville J, Burger U, Wirthner P, Haas D, Défago G (1992) Suppression of root diseases by *Pseudomonas fluorescens* CHA0: importance of the bacterial secondary metabolite 2, 4-diacetylphloroglucinol. *Mol Plant-Microbe Interact* 5:4–13
- Kennedy AC (2005) Rhizosphere. In: Sylvia DM, Fuhrmann JJ, Hartel PG, Zuberer DA (eds) *Principles and applications of soil microbiology*, 2nd edn. Pearson, Prentice Hall, Upper Saddle River, NJ, pp 242–262
- Khamna S, Yokota A, Lumyong S (2009) *Actinomycetes* isolated from medicinal plant rhizosphere soils: diversity and screening of antifungal compounds, indole-3-acetic acid and siderophore production. *World J Microbiol Biotechnol* 25:649–655
- Kim BS, Moon SS, Hwang BK (1999) Isolation, identification and antifungal activity of a macrolide antibiotic, oligomycin A, produced by *Streptomyces libani*. *Can J Bot* 77:850–858
- Kishore GK, Pande S, Podile AR (2005) Biological control of late leaf spot of peanut (*Arachis hypogaea*) with chitinolytic bacteria. *Phytopathology* 95:1157–1165
- Kloepper JW (2003) A review of mechanisms for plant growth promotion by PGPR. In: Reddy MS, Anandaraj M, Eapen SJ, Sarma YR, Kloepper JW (eds) 6th International PGPR workshop (Abstracts and short papers), 5–10 Oct 2003, Indian Institute of Spices Research, Calicut, India, pp 81–92
- Kloepper JW, Schroth MN (1978) Plant growth-promoting rhizobacteria on radishes. In: *Proceedings of the 4th international conference on plant pathogenic bacteria*, vol 2. Station de Pathologie Végétale et de Phytobactériologie, INRA, Angers, France, pp 879–882
- Kloepper JW, Leong J, Teintze M, Schroth MN (1980a) *Pseudomonas* siderophores: a mechanism explaining disease-suppressive soils. *Curr Microbiol* 4:317–320
- Kloepper JW, Leong J, Teintze M, Schroth MN (1980b) Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. *Nature* 286:835–836
- Kloepper JW, Lifshitz R, Zablotticz RM (1989) Free-living bacterial inocula for enhancing crop productivity. *Trend Biotechnol* 7:39–43
- Kloepper JW, Ryu C-M, Zhang S (2004) Induced systemic resistance and promotion of plant growth by *Bacillus* spp. *Phytopathology* 94:1259–1266
- Kluepfel DA, Kline EL, Skipper HD, Hughes TA, Gooden DT, Drahos DJ, Barry GF, Hemming BC, Brandt EJ (1991) The release and tracking of genetically engineered bacteria in the environment. *Phytopathology* 81:348–352
- Koch B, Nielsen TH, Sorensen D, Andersen JB, Christophersen C, Molin S, Givskov M, Sorensen J, Nybroe O (2002) Lipopeptide production in *Pseudomonas* sp. strain DSS73 is regulated by components of sugar beet seed exudate via the Gac two-component regulatory system. *Appl Environ Microbiol* 68:4509–4516

- Kokalis-Burelle N, Vavrina CS, Roskopf EN, Shelby RA (2002) Field evaluation of plant growth promoting rhizobacteria amended transplant mixes and soil solarization for tomato and pepper production in Florida. *Plant Soil* 238:257–266
- Kortema H, Rita H, Hahtela K, Smolander A (1994) Root colonization ability of antagonistic *Streptomyces griseoviridis*. *Plant Soil* 163:77–83
- Koths JS, Gunner HR (1967) Establishment of a rhizosphere microflora on carnation as a means of plant protection in steamed greenhouse soils. *Am Soc Hortic Sci* 91:617–626
- Kragelund L, Hosbond C, Nybroe O (1997) Distribution of metabolic activity and phosphate starvation response of *lux* tagged *Pseudomonas fluorescens* reporter bacteria in the barley rhizosphere. *Appl Environ Microbiol* 63:4920–4928
- Kraus J, Loper JE (1995) Characterization of a genomic region required for production of the antibiotic pyoluteorin by the biological control agent *Pseudomonas fluorescens* Pf-5. *Appl Environ Microbiol* 61:849–854
- Krishnamurthy K, Gnanamanickam SS (1998) Induction of systemic resistance and salicylic acid accumulation in *Oryza sativa* L. in the biological suppression of rice blast cause by treatments with *Pseudomonas* spp. *World J Microbiol Biotechnol* 14:935–937
- Kumar BSD (1999) Fusarial wilt suppression and crop improvement through two rhizobacterial strains in chick pea growing in soils infested with *Fusarium oxysporum* f. sp. *ciceris*. *Biol Fertil Soils* 29:87–91
- Lagopodi AL, Ram AF, Lamers GEM, Punt P, van den Hondel CAM, Lugtenberg B, Bloemberg GV (2002) Confocal laser scanning microscopical analysis of tomato root colonization and infection by *Fusarium oxysporum* f. sp. *radicis lycopersici* using the green fluorescent protein as a marker. *Mol Plant Microbe Interact* 15:172–179
- Landa BB, Mavrodi OV, Raaijmakers JM, McSpadden-Gardener BB, Thomashow LS, Weller DM (2002) Differential ability of genotypes of 2,4-diacetylphloroglucinol producing *Pseudomonas fluorescens* to colonize the roots of pea. *Appl Environ Microbiol* 68:3226–3237
- Landa BB, Mavrodi DM, Thomashow LS, Weller DM (2003) Interactions between strains of 2, 4- diacetylphloroglucinol-producing *Pseudomonas fluorescens* in the rhizosphere of wheat. *Phytopathology* 93:982–994
- Lazarovits G, Nowak J (1997) Rhizobacteria for improvement of plant growth and establishment. *HortScience* 32:188–192
- Lebuhn M, Heulin T, Hartmann A (1997) Production of auxin and other indolic and phenolic compounds by *Paenibacillus polymyxa* strains isolated from different proximity to plant roots. *FEMS Microbiol Ecol* 22:325–334
- Leeman M, van Pelt JA, Den Ouden FM, Heinsbroek M, Bakker PAHM, Schippers B (1995a) Induction of systemic resistance against *Fusarium* wilt of radish by lipopolysaccharides of *Pseudomonas fluorescens*. *Phytopathology* 85:1021–1027
- Leeman M, Van Pelt JA, Den Ouden FM, Heinsbroek M, Bakker PAHM, Schippers B (1995b) Induction of systemic resistance by *Pseudomonas fluorescens* in radish cultivars differing in susceptibility to *Fusarium* wilt, using a novel bioassay. *Eur J Plant Pathol* 101:655–664
- Leeman M, den Ouden FM, van Pelt JA, Dirkx FPM, Steijl H, Bakker PHAM, Schippers B (1996) Iron availability affects induction of systemic resistance to *Fusarium* wilt of radish by *Pseudomonas fluorescens*. *Phytopathology* 86:149–155
- Lemanceau P (1992) Effets bénéfiques de rhizobactéries sur les plantes: exemple des *Pseudomonas* spp. *Fluorescents*. *Agron* 12:413–437
- Lemanceau P, Alabouvette C (1993) Suppression of fusarium wilts by fluorescent pseudomonas: mechanisms and applications. *Biocontrol Sci Technol* 3:219–234
- Lemanceau P, Corberand T, Gardan L, Latour X, Laguerre G, Boeufgras JM, Alabouvette C (1995) Effect of two plant species, flax (*Linum usitatissimum* L.) and tomato (*Lycopersicon esculentum* Mill.), on the diversity of soilborne populations of fluorescent pseudomonads. *Appl Environ Microbiol* 61:1004–1012
- Lemanceau P, Maurhofer M, Défago G (2006) Contribution of studies on suppressive soils to the identification of bacterial biocontrol agents and to the knowledge of their modes of action. In: Gnanamanickam SS (ed) *Plant-associated bacteria*. Springer, New York, pp 231–267

- Leong J (1986) Siderophores: their biochemistry and possible role in the biocontrol of plant pathogens. *Annu Rev Phytopathol* 24:187–209
- Loper JE, Buyer JS (1991) Siderophores in microbial interactions on plant surfaces. *Mol Plant Microbe Interact* 4:5–13
- Loper JE, Lindow SE (1991) A biological sensor for available iron in the rhizosphere. In: Keel C, Koller B, Défago G (eds) *Plant growth-promoting rhizobacteria: progress and prospects*. IOBC/WPRS Bulletin XIV, pp 177–181
- Loper JE, Schroth MN (1986) Importance of siderophores in microbial interactions in the rhizosphere. In: Swinburne TR (ed) *Iron siderophores and plant disease*. Plenum, New York, pp 85–98
- Lynch JM (1990) Introduction: some consequences of microbial rhizosphere competence for plant and soil. In: Lynch JM (ed) *The rhizosphere*. Wiley, Chichester, pp 1–10
- Mahadevan B, Crawford DL (1997) Properties of the chitinase of the antifungal biocontrol agent *Streptomyces lydicus* WYEC108. *Enzyme Microb Technol* 20:489–493
- Mahaffee WF, Bauske EM, van Vuurde JWL, van der Wolf M, van den Brink M, Kloepper JW (1997) Comparative analysis of antibiotic resistance, immunofluorescent colony staining, and a transgenic marker (bioluminescence) for monitoring the environmental fate of a rhizobacterium. *Appl Environ Microbiol* 63:1617–1622
- Marschner P, Crowley D, Yang CH (2004) Development of specific rhizosphere bacterial communities in relation to plant species, nutrition and soil type. *Plant Soil* 261:199–208
- Maurhofer M, Hase C, Meuwly P, Metraux JP, Defago G (1994) Induction of systemic resistance of tobacco to tobacco necrosis virus by the root-colonizing *Pseudomonas fluorescens* strain CHA0: influence of the *gacA* gene and of pyoverdine production. *Phytopathology* 84:139–146
- Maurhofer M, Keel C, Haas D, Défago G (1995) Influence of plant species on disease suppression by *Pseudomonas fluorescens* strain CHA0 with enhanced antibiotic production. *Plant Pathol* 44:40–50
- Mayak S, Tirosh T, Glick BR (2004) Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. *Plant Physiol Biochem* 42:565–572
- McSpadden Gardener BB (2004) Ecology of *Bacillus* and *PaeniBacillus* spp. in agricultural systems. *Phytopathology* 94:1252–1258
- McSpadden-Gardener BB, Mavrodi DV, Thomashow LS, Weller DM (2001) A rapid polymerase chain reaction-based assay characterizing rhizosphere populations of 2, 4-diacetylphloroglucinol-producing bacteria. *Phytopathology* 91:44–54
- Merriman PR, Price RD, Kollmorgen JF, Piggott T, Ridge EH (1974) Effect of seed inoculation with *Bacillus subtilis* and *Streptomyces griseus* on the growth of cereals and carrots. *Aust J Agric Res* 25:219–226
- Merzaeva OV, Shirokikh IG (2006) Colonization of plant rhizosphere by *Actinomycetes* of different genera. *Microbiology* 75:226–230
- Meziane H, Van der Sluis I, Van Loon LC, Hofte M, Bakker PAHM (2005) Determinants of *Pseudomonas putida* WCS358 involved in inducing systemic resistance in plants. *Mol Plant Pathol* 6:177–185
- Miller JJ, Liljeroth E, Henken G, van Veen JA (1989) Fluctuations in the fluorescent pseudomonad and *Actinomycetes* populations of rhizosphere and rhizoplane during the growth of spring wheat. *Can J Microbiol* 36:254–258
- Miller JJ, Liljeroth E, Willemsen-de Klein MJEIM, van Veen JA (1990) The dynamics of *Actinomycetes* and fluorescent pseudomonads in wheat rhizoplane and rhizosphere. *Symbiosis* 9:389–391
- Milner JL, Silo-Suh L, Lee JC, He H, Clardy J, Handelsman J (1996) Production of kanosamine by *Bacillus cereus* UW85. *Appl Environ Microbiol* 62:3061–3065
- Moyne AL, Shalby R, Cleveland TE, Tuzun S (2001) Bacillomycin, D, an iturin with antifungal activity against *Aspergillus flavus*. *J Appl Microbiol* 90:622–629
- Muller G, Raymond KN (1984) Specificity and mechanism of ferrioxamine mediated iron transport in *Streptomyces pilosus*. *J Bacteriol* 160:304–312

- Muller G, Matzanke BF, Raymond KN (1984) Iron transport in *Streptomyces pilosus* mediated by ferrichrome siderophores, rhodotorulic acid, and enantio- rhodotorulic acid. *J Bacteriol* 160:313–318
- Murphy JF, Zehnder GW, Schuster DJ, Sikora EJ, Polstan JE, Kloepper JW (2000) Plant growth-promoting *rhizobacteria* mediated protection in tomato against tomato mottle virus. *Plant Dis* 84:779–784
- Nielsen TH, Sorensen D, Tobiasen C, Andersen JB, Christophersen C, Givskov M, Sorensen J (2002) Antibiotic and biosurfactant properties of cyclic lipopeptides produced by fluorescent *Pseudomonas* spp. from the sugar beet rhizosphere. *Appl Environ Microbiol* 68:3416–3423
- Notz R, Maurhofer M, Schnider-Keel U, Duffy B, Haas D, Défago G (2001) Biotic factors affecting expression of the 2,4-diacetylphloroglucinol biosynthesis gene *phlA* in *Pseudomonas fluorescens* biocontrol strain CHA0 in the rhizosphere. *Phytopathology* 91:873–881
- Olson EH (1968) *Actinomycetes* isolation agar (Difco Supplementary Literature). Difco Laboratory, Detroit
- Ongena M, Daayf F, Jacques P, Thonart P, Benhamou N, Paulitz TC, Cornelis P, Koedam N, Belanger RR (1999) Protection of cucumber against *Pythium* root rot by fluorescent pseudomonads: predominant role of induced resistance over siderophores and antibiosis. *Plant Pathol* 48:66–76
- Ongena M, Daayf F, Jacques P, Thonart P, Banhamou N, Paulitz TC, Belanger RR (2000) Systemic induction of phytoalexins in cucumber in response to treatment with fluorescent *Pseudomonads*. *Plant Pathol* 49:523–530
- Palumbo JD, Yuen GY, Jochum CC, Tatum K, Kobayashi DY (2005) Mutagenesis of beta-1,3-glucanase genes in *Lysobacter enzymogenes* strain C3 results in reduced biological control activity toward *Bipolaris* leaf spot of tall fescue and *Pythium* damping-off of sugar beet. *Phytopathology* 95:701–707
- Park KS, Ahn IP, Kim H (2001) Systemic resistance and expression of the pathogenesis-related genes mediated by the plant growth-promoting rhizobacterium *Bacillus amyloliquefaciens* EXTN-1 against anthracnose disease in cucumber. *Mycobiology* 29:48–53
- Pieterse CMJ, Van Pelt JA, Ton J, Parchmann S, Mueller MJ, Buchala AJ, Métraux J-P, Van Loon LC (2000) Rhizobacteria-mediated induced systemic resistance (ISR) in *Arabidopsis* requires sensitivity to jasmonate and ethylene but is not accompanied by an increase in their production. *Physiol Mol Plant Pathol* 57:123–34
- Pleban S, Chernin L, Chet I (1997) Chitinolytic activity of an endophytic strain of *Bacillus cereus*. *Lett Appl Microbiol* 25:284–288
- Powell JF, Vargas JM, Nair MG, Detweiler AR, Chandra A (2000) Management of dollar spot on creeping bentgrass with metabolites of *Pseudomonas aureofaciens* (TX-1). *Plant Dis* 84:19–24
- Raaijmakers JM, Weller DM (1998) Natural plant protection by 2, 4- Diacetylphloroglucinol-producing *Pseudomonas* spp. in take-all decline soils. *Mol Plant Microbe Interact* 11:144–152
- Raaijmakers JM, Leeman M, van Oorschot MMP, van der Sluis I, Schippers B, Bakker PAHM (1995) Dose–response relationships in biological control of fusarium wilt of radish by *Pseudomonas* spp. *Phytopathology* 85:1075–1081
- Raaijmakers J, Weller DM, Thomashow LS (1997) Frequency of antibiotic-producing *Pseudomonas* spp. in natural environments. *Appl Environ Microbiol* 63:881–887
- Raaijmakers JM, Bonsall RF, Weller DM (1999) Effect of population density of *Pseudomonas fluorescens* on production of 2, 4-diacetylphloroglucinol in the rhizosphere of wheat. *Phytopathology* 89:470–475
- Raaijmakers JM, Vlami M, de Souza JT (2002) Antibiotic production by bacterial biocontrol agents. *Anton van Leeuwenhook* 81:537–547
- Ran L, Xiang M, Zhou B, Bakker PAHM (2005) Siderophores are the main determinants of fluorescent *Pseudomonas* strains in suppression of grey mould in *Eucalyptus urophylla*. *Acta Phytopathol Sinica* 35:6–12
- Raupach GS, Liu L, Murphy JF, Tuzun S, Kloepper JW (1996) Induced systemic resistance in cucumber and tomato against *Cucumber mosaic cucumovirus* using plant growth-promoting rhizobacteria (PGPR). *Plant Dis* 80:891–894

- Rothrock CS, Gottlieb D (1984) Role of antibiosis in antagonism of *Streptomyces hygroscopicus* var. *geldanus* to *Rhizoctonia solani* in soil. *Can J Microbiol* 30:1440–1447
- Ryu C-M, Farag MA, Hu C-H, Reddy MS, Wei H-X, Pare PW, Kloepper JW (2003) Bacterial volatiles promote growth in *Arabidopsis*. *Proc Natl Acad Sci USA* 100:4927–4932
- Ryu C-M, Hu CH, Locy RD, Kloepper JW (2005) Study of mechanisms for plant growth promotion elicited by rhizobacteria in *Arabidopsis thaliana*. *Plant Soil* 268:285–292
- Scher FM, Baker R (1982) Effect of *Pseudomonas putida* and a synthetic iron chelator on induction of soil suppressiveness to *Fusarium* wilt pathogens. *Phytopathology* 72:1567–1573
- Schippers B, Bakker AW, Bakker PAHM (1987) Interactions of deleterious and beneficial rhizosphere microorganisms and the effect of cropping practices. *Annu Rev Phytopathol* 25:339–358
- Schmidt EL (1979) Initiation of plant root microbe interactions. *Annu Rev Microbiol* 33:355–376
- Schmidt CS, Lorenz D, Wolf GA (2001) Biological control of the grapevine dieback fungus *Eutypa lata* I: screening of bacterial antagonists. *J Phytopathol* 149:427–435
- Schober RBM, vanVuurde JWL (1997) Detection and enumeration of *Erwinia carotovora* subsp. *atroseptica* using spiral plating and immunofluorescence colony staining. *Can J Microbiol* 43:847–853
- Schottel JL, Shimizu K, Kinkel LL (2001) Relationships of in vitro pathogen inhibition and soil colonization to potato scab biocontrol by antagonistic *Streptomyces* spp. *Biol Control* 20:102–112
- Shafikova TN, Romanenko AS, Borovskii GB (2003) Plasma membrane receptors for exopolysaccharides of the ring rot causal agent in potato cells. *Russ J Plant Physiol* 50:220–223
- Sharifi-Tehrani A, Zala M, Natsch A, Moënne-Loccoz Y, Défago G (1998) Biocontrol of soil-borne fungal plant diseases by 2,4- diacetylphloroglucinol-producing fluorescent pseudomonads with different restriction profiles of amplified 16S rDNA. *Eur J Plant Pathol* 104:631–643
- Shishido M, Loeb BM, Chanway CP (1995) External and internal root colonization of lodgepole pine seedlings by two growth-promoting *Bacillus* strains originated from different root microsites. *Can J Microbiol* 41:707–713
- Silo-suh LA, Stab VE, Raffel SR, Handelsman J (1998) Target range of Zwittermicin A, an Aminopolyol antibiotic from *Bacillus cereus*. *Curr Microbiol* 37:6–11
- Simon E, Ridge H (1974) The use of ampicillin in a simplified selective medium for the isolation of fluorescent pseudomonads. *J Appl Bacteriol* 37:459–460
- Singh PP, Shin YC, Park CS, Chung YR (1999) Biological control of *Fusarium* wilt of cucumber by chitinolytic bacteria. *Phytopathology* 89:92–99
- Smalla K, Wieland G, Buchner A, Zock A, Parzy J, Kaiser S, Roskot N, Heuer H, Berg G (2001) Bulk and rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis: plant dependent enrichment and seasonal shifts revealed. *Appl Environ Microbiol* 67:4742–4751
- Smith KP, Goodman RM (1999) Host variation for interactions with beneficial plant-associated microbes. *Annu Rev Phytopathol* 37:473–491
- Sneh B (1981) Use of rhizosphere chitinolytic bacteria for biological control of *Fusarium oxysporum* f. sp. *dianthi* in carnation. *Phytopathol Z* 100:251–256
- Sneh B, Dupler M, Elad Y, Baker R (1984) Chlamydospore germination of *Fusarium oxysporum* f. sp. *cucumerinum* as affected by fluorescent and lytic bacteria from *Fusarium*-suppressive soil. *Phytopathology* 74:1115–1124
- Söderberg KH, Bååth E (1998) Bacterial activity along a young barley root measured by the thymidine and leucine incorporating techniques. *Soil Biol Biochem* 30:1259–1268
- Somers E, Vanderleijden J, Srinivasan M (2004) Rhizosphere bacterial signalling: a love parade beneath our feet. *Crit Rev Microbiol* 30:205–240
- Someya N, Tsuchiya K, Yoshida T, Noguchi MT, Akutsu K, Sawada H (2007) Co-inoculation of an antibiotic-producing bacterium and a lytic enzyme-producing bacterium for the biocontrol of tomato wilt caused by *Fusarium oxysporum* f. sp. *lycopersici*. *Biocontrol Sci* 12:1–6

- Spaepen S, Vanderleyden J, Remans R (2007) Indole-3-acetic acid in microbial and microorganism-plant signaling. *FEMS Microbiol Rev* 31:425–448
- Stabb E, Jacobson LM, Handelsman J (1994) Zwittermicin A-producing strains of *Bacillus cereus* from diverse soils. *Appl Environ Microbiol* 60:4404–4412
- Suslow TV, Schroth MN (1982) Rhizobacteria of sugar beets: effects of seed application and root colonization on yield. *Phytopathology* 72:199–206
- Tazawa J, Watanabe K, Yoshida H, Sato M, Homma Y (2000) Simple method of detection of the strains of fluorescent *Pseudomonas* spp. producing antibiotics, pyrrolnitrin and phloroglucinol. *Soil Microorg* 54:61–67
- Thomashow LS, Weller DM (1988) Role of a phenazine antibiotic from *Pseudomonas fluorescens* in biological control of *Gaeumannomyces graminis* var. *tritici*. *J Bacteriol* 170:3499–3508
- Thomashow LS, Weller DM, Bonsall RF, Pierson LS (1990) Production of the antibiotic phenazine-1-carboxylic acid by fluorescent *Pseudomonas* species in the rhizosphere of wheat. *Appl Environ Microbiol* 56:908–912
- Thomashow LS, Bonsall RF, Weller DM (1997) Antibiotic production by soil and rhizosphere microbes in situ. In: Hurst CJ, Knudsen GR, McInerney MJ, Stetzenbach LD, Walter MV (eds) *Manual of environmental microbiology*. ASM Press, Washington, DC, pp 493–499
- Thrane C, Nielsen TH, Nielsen MN, Olsson S, Sorensen J (2000) Viscosinamide producing *Pseudomonas fluorescens* DR54 exerts biocontrol effect on *Pythium ultimum* in sugar beet rhizosphere. *FEMS Microbiol Ecol* 33:139–146
- Timmusk S, Wagner EGH (1999) The plant-growth-promoting rhizobacterium *Paenibacillus polymyxa* induces changes in *Arabidopsis thaliana* gene expression: a possible connection between biotic and abiotic stress responses. *Mol Plant Microbe Interact* 12:951–959
- Timmusk S, Nicander B, Granhall U, Tillberg E (1999) Cytokinin production by *Paenibacillus polymyxa*. *Soil Biol Biochem* 31:1847–1852
- Timmusk S, Grantcharova N, Wagner EG (2005) *Paenibacillus polymyxa* invades plant roots and forms biofilms. *Appl Environ Microbiol* 71:7292–7300
- Tokala RK, Strap JL, Jung CM, Crawford DL, Salove MH, Deobald LA, Bailey JF, Morra MJ (2002) Novel plant-microbe rhizosphere interaction involving *Streptomyces lydicus* WYEC108 and the pea plant (*Pisum sativum*). *Appl Environ Microbiol* 68:2161–2171
- Tombolini R, Unge A, Davey ME, de Bruijn F, Jansson K (1997) Flow cytometric and microscopic analysis of GFP-tagged *Pseudomonas fluorescens* bacteria. *FEMS Microbiol Ecol* 22:17–28
- Troxler J, Berling C-H, Moënné-Loccoz Y, Keel C, Défago G (1997) Interactions between the biocontrol agent *Pseudomonas fluorescens* CHA0 and *Thielaviopsis basicola* in tobacco roots observed by immunofluorescence microscopy. *Plant Pathol* 46:62–71
- Umezawa H, Okami T, Hashimoto T, Suhara Y, Hamada M, Takeuchi T (1965) A new antibiotic, kasugamycin. *J Antibiot Ser A* 18:101–103
- Utkhede RS, Rahe JE (1980) Biological control of onion white rot. *Soil Biol Biochem* 12:101–104
- Valois D, Fayad K, Barasbiye T, Garon T, Dery C, Brzezinski R, Beaulieu C (1996) Glucanolytic *Actinomyces* antagonistic to *Phytophthora fragariae* var. *rubi*, the causal agent of raspberry root rot. *Appl Environ Microbiol* 62:1630–1635
- Van Loon LC (2007) Plant responses to plant growth-promoting rhizobacteria. *Eur J Plant Pathol* 119:243–254
- Van Loon LC, Bakker PAHM (2003) Signalling in rhizobacteria-plant interactions. In: De Kroon H, Visser EJW (eds) *Root ecology (Ecological studies)*, vol 168. Springer, Berlin, pp 297–330
- Van Loon LC, Glick BR (2004) Increased plant fitness by rhizobacteria. In: Sandermann H (ed) *Molecular ecotoxicology of plants*. Springer, Berlin, pp 177–205
- Van Peer R, Schippers B (1992) Lipopolysaccharides of plant growth promoting *Pseudomonas* sp. strain WCS417r induce resistance in carnation to *Fusarium* wilt. *Neth J Plant Pathol* 98:129–139

- Van Peer R, Niemann GJ, Schippers B (1991) Induced resistance and phytoalexin accumulation in biological control of fusarium wilt of carnation by *Pseudomonas* sp. Strain WCS417r. *Phytopathology* 81:728–734
- Van Wees SCM, Pieters CMJ, Trisjssenaar A, Van't Westende YAM, Hartog F, van Loon LC (1997) Differential induction of systemic resistance in *Arabidopsis* by biocontrol bacteria. *Mol Plant Microbe Interact* 10:716–724
- Velazhahan R, Samiyappan R, Vidhyasekaran P (1999) Relationship between antagonistic activities of *Pseudomonas fluorescens* isolates against *Rhizoctonia solani* and their production of lytic enzymes. *Z Pflanz Pflanz* 106:244–250
- Velusamy P, Immanuel JE, Gnanamanickam SS, Thomashow L (2006) Biological control of rice bacterial blight by plant-associated bacteria producing 2,4-diacetylphloroglucinol. *Can J Microbiol* 52:56–65
- Vesper SJ (1987) Production of pili (fimbriae) by *Pseudomonas fluorescens* and a correlation with attachment to corn roots. *Appl Environ Microbiol* 53:1397–1405
- Vessey JK (2003) Plant growth promoting rhizobacteria as biofertilizers. *Plant Soil* 255:571–586
- Voisard C, Keel C, Haas D, Defago G (1989) Cyanide production by *Pseudomonas fluorescens* helps suppress black root rot of tobacco under gnotobiotic conditions. *EMBO J* 8:351–358
- Von Der Weid I, Duarte G, Van Elsas JD, Seldin L (2002) *Paenibacillus brasiliensis* sp. nov., a novel nitrogen-fixing species isolated from the maize rhizosphere in Brazil. *Int J Syst Evol Microbiol* 52:2147–2153
- Von Der Weid I, Artursson V, Seldin L, Jansson JK (2005) Antifungal and root surface colonization properties of GFP tagged *Paenibacillus brasiliensis* PB177. *World J Microbiol Biotechnol* 2(1):1591–1597
- Weller DM (1988) Biological control of soilborne plant pathogens in the rhizosphere with bacteria. *Annu Rev Phytopathol* 26:379–407
- Weller DM, Cook RJ (1983) Suppression of take-all of wheat by seed treatments with fluorescent pseudomonads. *Phytopathology* 73:463–469
- Wendenbaum S, Demange P, Dell A, Meyer JM, Abdallah MA (1983) The structure of pyoverdine, the siderophores of *Pseudomonas aeruginosa*. *Tetrahedron Lett* 24:4877–4880
- Yuan WM, Crawford DL (1995) Characterization of *Streptomyces lydicus* WYEC108 as a potential biocontrol agent against fungal root and seed rots. *Appl Environ Microbiol* 61:3119–3128
- Zehnder GW, Yao C, Murphy JF, Sikora EJ, Kloepper JW (2000) Induction of resistance in tomato against *Cucumber mosaic cucumovirus* by plant growth-promoting rhizobacteria. *Biocontrol* 45:127–137
- Zehnder GW, Murphy JF, Sikora EJ, Kloepper JW (2001) Application of rhizobacteria for induced resistance. *Eur J Plant Pathol* 107:39–50
- Zhang Y, Fernando WGD (2004) Zwittermicin A detection in *Bacillus* spp. controlling *Sclerotinia sclerotiorum* on canola. *Phytopathol* 94:S116

Bacteria in Agrobiolology: Disease Management

Maheshwari, D.K. (Ed.)

2013, XIII, 495 p., Hardcover

ISBN: 978-3-642-33638-6