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Competition for Iron and Induced Systemic Resistance by Siderophores of Plant Growth Promoting Rhizobacteria

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6.1
Introduction

Most aerobic and facultative anaerobic microorganisms produce low molecular weight Fe^{3+} specific ligands, so-called siderophores, under conditions of low iron availability. The siderophores sequester ferric ions in the environment and the ferric siderophores are taken up in the microbial cells after specific recognition by membrane proteins (Höfte 1993). The production of siderophores is an important trait of so-called plant growth promoting rhizobacteria (PGPR) in their ability to suppress soil-borne plant pathogens (Kloepper et al. 1980). Competition for ferric iron between the PGPR and the plant deleterious microorganisms is considered the mode of action of these siderophores (Buysens et al. 1996; Loper and Buyer 1991; Raaijmakers et al. 1995; Schippers et al. 1987). However, it has been reported that disease suppression also occurs when the PGPR and the pathogen are inoculated and remain spatially separated, thus avoiding direct interactions. In this case the protective effect has to be plant mediated and this phenomenon was named induced systemic resistance (Van Loon et al. 1998). For instance, when *Pseudomonas fluorescens* strain WCS417 remained confined to the carnation root system and *Fusarium oxysporum* f.sp. *dianthi* was slash-inoculated into the stem, it was found that the bacteria were still protective (Van Peer et al. 1991). On cucumber similar observations were made for PGPR strains applied to the roots, and subsequent challenge inoculation of the leaves with the anthracnose fungus *Colletotrichum orbiculare* (Wei et al. 1991). The inducing rhizobacteria trigger a reaction in the plant roots leading to a signal that spreads systemically throughout the plant, finally resulting in enhanced

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defensive capacity to subsequent pathogen infections. The protective action of PGPR against soil-borne pathogens in the rhizosphere is thus extended to a defence-stimulating effect in aboveground tissues against foliar pathogens. This enhanced defensive capacity was expressed in roots as well as in leaves, adding the mechanism of ISR to the list of mechanisms of PGPR effective against soil-borne pathogens (Leeman et al. 1995b). In view of the role of iron-regulated metabolites in suppression of soil borne diseases by PGPR, their possible involvement in ISR has been subject of numerous studies.

6.2 Role of Siderophores and Iron-Regulated Compounds in ISR

Bacterial determinants of ISR that have been identified so far are lipopolysaccharides (LPS) (Leeman et al. 1995b; Van Peer and Schippers 1992), flagella (Meziane et al. 2005), the antibiotics 2,4-diacetylphloroglucinol (Iavicoli et al. 2003; Weller et al. 2004) and pyocyanin (Audenaert et al. 2002), the volatile 2,3-butanediol (Ryu et al. 2004), N-alkylated benzylamine (Ongena et al. 2005) and iron-regulated compounds (Bakker et al. 2003). In this review, we will focus on the compounds produced upon iron-limitation (Table 6.1).

6.2.1 Pseudomonas aeruginosa 7NSK2

Pseudomonas aeruginosa 7NSK2 is a PGPR isolated from the roots of barley (Iswandi et al. 1987). Under iron-limiting conditions, this strain produces three siderophores, pyoverdine, pyochelin and its precursor salicylic acid (SA) and can induce resistance to plant diseases caused by *Botrytis cinerea* on bean and tomato (De Meyer and Höfte 1997; De Meyer et al. 1999b), *Colletotrichum lindemuthianum* on bean (Bigirimana and Höfte 2002) and Tobacco Mosaic Virus on tobacco (De Meyer et al. 1999a). Interestingly, exogenously applied SA induces a systemic resistance in many plant species (Sticher et al. 1997), and therefore this metabolite may be of importance in ISR triggered by strain 7NSK2.

Under iron-limitation, SA-deficient mutants of this strain were not able to induce resistance to the pathogens mentioned above in a pyoverdine-negative or pyoverdine-positive background indicating that SA or pyochelin is essential for ISR in bean, tomato and tobacco. In tomato and tobacco, it was shown that 7NSK2 induces resistance via the SA-dependent signal transduction pathway, since 7NSK2 no longer induced resistance in *NahG* tobacco (De Meyer et al. 1999a) and *NahG* tomato plants (Audenaert et al. 2002). Plants carrying the bac-

Table 6.1. Examples of bacterial strains for which iron-chelating or iron-regulated compounds are involved in induced systemic resistance

Bacterial strain	Plant – pathogen	Determinant(s) involved in ISR	Reference
Pseudomonas aeruginosa 7NSK2	Bean – *Colletotrichum lindemuthianum*	Salicylic acid	Bigirimana and Höfte (2002)
	Bean – *Botrytis cinerea*	Salicylic acid	De Meyer and Höfte (1997)
	Tomato – *Botrytis cinerea*	Salicylic acid, pyochelin, pyocyanin	Audenaert et al. (2002).
	Tobacco – Tobacco Mosaic Virus	Salicylic acid	De Meyer et al. (1999a)
Pseudomonas fluorescens CHA0	Tobacco – Tobacco mosaic virus	Pyoverdine	Maurhofer et al. (1994)
	Arabidopsis – *Peronospora parasitica*	2,4-diacetylphloroglucinol	Iavicoli et al. (2003)
Pseudomonas fluorescens WCS374	Radish – fusarium wilt	Pseudobactin, LPS	Leeman et al. (1995b, 1996)
	Rice – *Pyricularia grisea*	Pyocyanin	De Vleesschauwer et al. (2006)
	Eucalyptus – *Ralstonia solanacearum*	Pseudobactin, unknown determinant(s)	Ran et al. (2005)
	Rice – *Rhizoctonia solani*	Salicylic acid	De Vleesschauwer et al., unpublished
Pseudomonas fluorescens WCS417	Carnation – fusarium wilt	LPS	Van Peer and Schippers (1992)
	Radish – fusarium wilt	LPS, unknown iron-regulated determinant(s)	Leeman et al. (1996)
	Arabidosis – *Pseudomonas syringae* pv. tomato	LPS	Van Wees et al. (1997)
Pseudomonas putida WCS358	Arabidopsis – *Pseudomonas syringae* pv. tomato	Pseudobactin, flagella, LPS	Bakker et al. (2003); Meziane et al. (2005)
	Tomato – *Botrytis cinerea*	Pseudobactin	Meziane et al. (2005)
	Bean – *Botrytis cinerea*	Pseudobactin, LPS	Meziane et al. (2005)
	Bean – *Colletotrichum lindemuthianum*	Pseudobactin, LPS	Meziane et al. (2005)
	Eucalyptus – *Ralstonia solanacearum*	Pseudobactin, LPS	Ran et al. (2005)
Pseudomonas putida BTP1	Bean – *Botrytis cinerea*	N-alkylated benzylamine	Ongena et al. (2005)
Serratia marcescens 90-166	Cucumber – *Colletotrichum orbiculare*	Catechol-type siderophore	Press et al. (2001)

terial *NahG* gene, encoding the enzyme salicylate hydroxylase, which converts SA into the non-inducing product catechol, no longer express SA induced resistance (Gaffney et al. 1993). For *P. aeruginosa* KMPCH, a pyochelin-negative and SA-positive mutant of 7NSK2, it was illustrated that bacterial SA induced phenylalanine ammonia lyase (PAL) activity in bean leaves upon root colonization increased in bean leaves upon root colonization with KMPCH (De Meyer and Höfte 1997). On tomato roots, KMPCH produced SA and induced PAL activity, but surprisingly, this was not the case for the wild type strain 7NSK2 (Audenaert et al. 2002). *P. aeruginosa* 7NSK2 is also able to induce resistance to *Pseudomonas syringae* pv. *syringae* in *Arabidopsis thaliana*. Mutants of 7NSK2 deficient in pyoverdine, pyocheline or SA production were as effective as the wild-type strain in inducing resistance indicating that in *Arabidopsis* these compounds are not necessary for the induction of ISR. Interestingly, strain 7NSK2 still induced resistance to *P. syringae* pv. *syringae* in *NahG Arabidopsis* plants (Ran 2005).

Besides siderophores, *P. aeruginosa* 7NSK2 also produces pyocyanin (5-methyl-1-hydroxyphenazinium betaine), a blue phenazine compound that is considered to be a virulence factor in clinical isolates of *P. aeruginosa* (Britigan et al. 1992, 1997). Abeysinge (1999) has shown that high concentrations of purified pyocyanin (0.1 mM) can induce resistance to *B. cinerea* in bean. Mutant PHZ1 is not able to produce pyocyanin due to an insertion in the *phzM* gene that encodes an *O*-methyltransferase. In infection experiments, PHZ1 did not induce resistance in tomato to *B. cinerea*. In addition, a pyochelin and SA negative mutant 7NSK2-562 did not induce resistance either, although it over-produced pyocyanin. Induced resistance was restored, when both mutants were co-inoculated on tomato roots or when PHZ1 was complemented for pyocyanin production. These results indicate that in strain 7NSK2 pyocyanin and pyochelin (or salicylic acid) act synergistically in induced resistance, probably by generating the very reactive OH-radical on plant roots (Audenaert et al. 2002). We also constructed the *phzM* mutation in mutant KMCPH. Surprisingly, mutant KMPCH-*phzM* lost its ability to induce resistance to *B. cinerea* in bean and tomato (Audenaert et al., unpublished) indicating that also in strain KMPCH, SA and pyocyanin, rather than SA alone are the determinants for induced resistance in bean and tomato.

Studies about bacterial determinants involved in ISR have mainly been carried out in dicot plants. Recently, we started work on ISR in our lab using the monocot rice as a model plant. We were interested to see whether the same bacterial determinants are involved in ISR in mono- and dicotyledon plants. As challenging pathogens we used the major pathogens of rice: *Pyricularia grisea*, the causal agent of rice blast; *Xanthomonas oryzae* pv. *oryzae*, the causal agent of bacterial blight and *Rhizoctonia solani*, the causal agent of sheath blight. *P. aeruginosa* 7NSK2 was able to induce resistance to rice blast, but was not effective against sheath blight or blight. We tested all available mutants of 7NSK2 for their ability to induce resistance to blast and sheath blight. Pyocyanin appeared to be the main metabolite responsible for induced resistance to blast, while there was no role for SA or pyochelin. SA-deficient mutants were in general even more ef-

fective in inducing resistance than the wild type strain (De Vleesschauwer et al., in preparation). The situation appeared to be entirely different for sheath blight. While the wild type strain 7NSK2 was not effective against *R. solani*, the pyocyanin mutants 7NSK2-*phzM* and KMPCH-*phzM* were able to induce resistance (De Vleesschauwer et al. 2006). Transient generation of H_2O_2 by redox-active pyocyanin in planta most likely accounts for the dual role of the latter compound in 7NSK2-mediated ISR in rice since exogenous application of sodium ascorbate alleviated the opposite effects of pyocyanin on *P. grisea* and *R. solani* pathogenesis (De Vleesschauwer et al. 2006). Resistance could also be induced with pure SA applied to rice roots in a gnotobiotic system (De Vleesschauwer et al., in preparation).

We conclude that while in bean, tomato and tobacco SA/pyochelin and pyocyanin act synergistically to induce resistance, in the monocot rice SA or pyocyanin alone are sufficient to induce resistance. The bacterial metabolite involved, however, depends on the challenging pathogen. It is known that pyocyanin can undergo redox-cycling, resulting in the generation of superoxide and H_2O_2 (Britigan et al. 1997). These active oxygen species (AOS) are apparently sufficient to induce resistance to blast in rice. In dicot plants such as bean and tomato, however, these AOS have to be converted to the very reactive OH-radical through the Haber-Weiss reaction in the presence of an iron-chelating compound such as Fe-pyochelin or Fe-SA.

6.2.2
Pseudomonas fluorescens CHA0 and P. fluorescens P3

P. fluorescens CHA0 is an effective biocontrol agent of various soil borne pathogens (see Haas and Defago 2005 for a review). This strain produces pyoverdine, salicylic acid and various antimicrobial compounds such as hydrogen cyanide, 2,4-diacetylphloroglucinol (DAPG) and pyoluteorin. *P. fluorescens* CHA0 can induce resistance against leaf necrosis caused by Tobacco Necrosis Virus (TNV) in tobacco plants. A *gacA* mutant, in which the production of hydrogen cyanide, DAPG and pyoluteorin is blocked, had the same capacity to induce resistance against TNV as did the wild type strain. The pyoverdine-negative mutant CHA400, however, was significantly less able to protect tobacco leaves against TNV (Maurhofer et al. 1994). The transposon insertion in mutant CHA400 was not localized and it is not clear whether the pyoverdine mutation is the only mutation in strain CHA400. More recently, ISR with *P. fluorescens* CHA0 was studied in *Arabidopsis thaliana* with *Peronospora parasitica* as the challenging pathogen. In this study it was shown that DAPG is required for the induction of ISR to *Peronospora parasitica*, since only mutations interfering with DAPG, including the *gacA* mutation, led to a significant decrease in ISR (Iavicoli et al. 2003). In this study, mutant CHA400 was as effective as the wild-type strain. Similarly, ISR in *A. thaliana* against *P. syringae* pv *tomato* by *P. fluorescens* Q2-87

is triggered by DAPG (Weller et al. 2004). In the latter case the possible involvement of siderophores was not studied.

Introduction of the SA biosynthesis genes *pchBA* from *P. aeruginosa* PAO1 (Serino et al. 1995) into *P. fluorescens* P3, which does not produce SA, rendered this strain capable of SA production in vitro and significantly improved its ability to induce systemic resistance in tobacco against TNV (Maurhofer et al. 1998).

6.2.3
Pseudomonas fluorescens WCS417 and WCS374

P. fluorescens WCS417 was one of the first PGPR strains for which ISR was found to be an important mode of action. When strain WCS417 was applied to roots of carnation it protected the treated plants significantly from wilting caused by *F. oxysporum* f.sp. *dianthi* that was slash inoculated into the stem (Van Peer et al. 1991). Purified lipopolysaccharides of this strain also induced resistance in carnation (Van Peer and Schippers 1992), suggesting LPS as a trigger of ISR in this plant species. The pseudobactin (pyoverdin) siderophore of WCS417 seems not to be involved in ISR, since a pseudobactin negative Tn5 insertion mutant was as effective as the parental strain in protecting carnation from fusarium wilt (Duijff et al. 1993). The involvement of LPS and siderophores in ISR against fusarium wilt were investigated further for *P. fluorescens* strains WCS417 and WCS374 in a bioassay with radish specifically designed to study induced resistance (Leeman et al. 1995a). The purified LPS of both strains triggered ISR in radish; moreover, mutants of the strains lacking the 0-antigenic side chain were not able to induce resistance against fusarium wilt (Leeman et al. 1995b). However, the latter experiments were performed under conditions of high iron availability for the PGPR strains, thereby excluding a possible role for siderophores, which are produced only upon iron limitation. When iron availability for the bacteria was lowered, strains WCS374 and WCS417 reduced disease to a much lower level compared to high iron availability, and more striking, the 0-antigen mutants of both strains did trigger ISR under conditions of low iron availability (Leeman et al. 1996). These results suggest that iron-regulated metabolites of the strains are also involved in triggering ISR. Purified pseudobactin of WCS374, but not that of WCS417, induced ISR when applied to the roots of radish. Interestingly, pseudobactin mutants of both strains were as effective as the parental strains, with again additional ISR induction activity when iron availability was lowered. Both strains produce SA at low iron availability and this metabolite was suggested to be a determinant of WCS374 and WCS417 triggering ISR in radish (Leeman et al. 1996). However, in *Arabidopsis thaliana* WCS417 can induce resistance whereas WCS374 cannot, despite the fact that WCS374 produces six to seven times more SA than does WCS417. In the *A. thaliana* system ISR by WCS417 is independent of SA accumulation in the plant (Pieterse et al. 1996 1998), excluding a role for bacterially produced SA. The LPS of WCS417 does seem to be involved in ISR *in A. thaliana* (Van Wees et al. 1997). So far the

involvement of pseudobactin in ISR by WCS417 in Arabidopsis has not been investigated. The inability of WCS374 to trigger ISR in *Arabidopsis* may be due to the observation that upon iron limitation this strain produces not only pseudobactin and SA, but also pseudomonine, a siderophore containing a SA moiety (Mercado-Blanco et al. 2001). Possibly in the Arabidopsis rhizosphere all SA produced by WCS374 is channelled into pseudomonine that cannot trigger ISR in this plant species. This hypothesis will be investigated using purified pseudomonine and mutants defective in pseudomonine production.

Strain WCS417 induces resistance in all plant species it has been tested in (Van Loon et al. 1998). However, recently it was reported that this strain could not trigger ISR in *Eucalyptus urophylla* (Ran et al. 2005) or rice (De Vleesschauwer, unpublished). *P. fluorescens* WCS374 does induce ISR against *Ralstonia solanacearum* in Eucalyptus and whereas a pseudobactin mutant was as effective as the parental strain in disease suppression, purified pseudobactin did trigger ISR. These results suggest that ISR by WCS374 in *E. urophylla* is triggered by its pseudobactin siderophore and as yet unknown additional determinant(s) (Ran et al. 2005). Interestingly, WCS374 could also induce systemic resistance in rice against both rice blast and sheath blight. A pseudobactin mutant lost its ability to suppress disease, while purified pseudobactin from strain WCS374 triggered ISR to rice blast and sheath blight (De Vleesschauwer, unpublished).

6.2.4
Pseudomonas putida WCS358

Strain WCS358 was originally isolated from potato tuber surface and the involvement of its fluorescent pseudobactin siderophore in disease suppression has been studied in a variety of plant-pathogen systems. Mutants defective in pseudobactin biosynthesis were isolated and analysed after Tn5 transposon mutagenesis (Marugg et al. 1985). Mutants were compared to the parental strain regarding their ability to increase potato plant growth in pot experiments and in the field (Schippers et al. 1987), and to suppress fusarium wilt in carnation (Duijff et al. 1993), and radish (Raaijmakers et al. 1995). In all cases it was observed that the parental strain was more effective than the mutant strain suggesting that pseudobactin is the key biocontrol compound in WCS358. Effective competition for ferric iron was suggested to be the main mode of action of WCS358 (Bakker et al. 1993). Mutants defective in pseudobactin biosynthesis were isolated and analysed after Tn5 transposon mutagenesis (Marugg et al. 1985). Mutants were compared to the parental strain regarding their ability to increase potato plant growth in pot experiments and in the field (Schippers et al. 1987), and to suppress fusarium wilt in carnation (Duijff et al. 1993), and radish (Raaijmakers et al. 1995). In all cases it was observed that the parental strain was more effective than the mutant strain suggesting that pseudobactin is the key biocontrol compound in WCS358. Effective competition for ferric iron was suggested to be the main mode of action of WCS358. Effective competition for ferric iron was suggested to be the main mode of action of WCS358 (Bakker et al. 1993) or radish (Leeman et al. 1995a), *P. putida* WCS358 cannot trigger ISR in carnation (Van Wees et al. 1997), *E. urophylla* (Ran et al. 2005) bean, and tomato (Meziane et al. 2005). In these plants species the involvement of the siderophore of WCS358 as a trigger of ISR was studied using both purified pseudobactin and mutants defective in pseudobactin biosynthesis. Whereas in Arabidopsis the purified siderophore induces ISR, the pseudobactin mutant was still as effective as the wild type strain (Bakker et al. 2003; Meziane et al. 2005). In addition to the siderophore, the LPS and the flagella of WCS358 also play a role in trig-

gering ISR, indicating redundancy for ISR triggering traits of this strain in the A. thaliana - P. syringae pv tomato model system. In Eucalyptus the situation is different; the mutant no longer induces resistance and the purified siderophore does trigger ISR, suggesting that pseudobactin is the sole determinant of ISR in this plant species ISR (Ran et al. 2005). In tomato a similar situation was observed, ISR against B. cinerea was triggered by the purified siderophore and the pseudobactin mutant no longer induced resistance (Meziane et al. 2005). In bean more than one determinant seems to be involved in WCS358 mediated ISR against B. cinerea and C. lindemuthianum. Both the pseudobactin mutant and the purified compound induced resistance; an additional role for LPS was suggested in this case (Meziane et al. 2005). Thus, for strain WCS358 multiple traits can be involved in ISR depending on the host plant. In previous studies in which a role for siderophore mediated competition for iron by this strain was suggested the possible involvement of ISR should be evaluated.

6.2.5
Pseudomonas putida BTP1

P. putida BTP1, obtained from barley roots, was originally selected for its specific features regarding pyoverdine-mediated iron transport (Jacques et al. 1995). P. putida BTP1 was shown to enhance the resistance of cucumber to root rot caused by Pythium aphanidermatum (Ongena et al. 1999). Results from split root experiments suggested that the protective effect was due to ISR, since systemic accumulation of antifungal compounds was observed in the host plant after treatment with BTP1 or with M3, its siderophore deficient mutant (Ongena et al. 1999). P. putida BTP1 is also able to protect bean against leaf infection with Botrytis cinerea. Biocontrol assays carried out with cell-free culture fluids of BTP1 clearly indicated that ISR was mostly induced by one or several metabolite(s) excreted by the strain under iron-limited in vitro growth conditions. In vivo assays with samples from successive fractionation steps of the BTP1 supernatant led to the isolation of fractions containing one main metabolite that retained most of the resistance-inducing activity in bean (Ongena et al. 2002). Recently, this metabolite was structurally characterized as an N-trialkylated benzylamine derivative (Ongena et al. 2005). Although the production of this metabolite is iron-regulated in strain BTP1, the compound itself has apparently no siderophore activity.

6.2.6
Serratia marcescens 90-166

Serratia marcescens 90-166 can induce resistance to fungal, viral and bacterial pathogens in cucumber such as Colletotrichum orbiculare, Fusarium oxysporum

f. sp. cucumerinum, Cucumber Mosaic Virus, P. syringae pv. lachrymans, and Erwinia tracheiphila. Strain 90-166-mediated ISR is dependent on iron concentration. The capacity of strain 90-166 to induce resistance is diminished under iron-replete conditions. In addition, suppression of cucumber anthracnose by strain 90-166 was significantly improved when the iron concentration of the planting mix was decreased by addition of the iron-chelator EDDHA (Press et al. 2001). S. marcescens 90-166 is known to produce SA, but mutants deficient in SA production retained ISR activity in cucumber against C. orbiculare (Press et al. 1997). In addition to SA, S. marcescens produces a catechol-type siderophore, which is probably identical to enterobactin. A siderophore-deficient mutant of S. marcescens was identified, in which a homologue of the entA gene is inactivated. EntA encodes 2,3-dihydro-2,3-dihydroxybenzoate dehydrogenase, an enzyme in the enterobactin biosynthesis pathway. The entA mutant of S. marcescens 90-166 was no longer able to induce resistance to C. orbiculare in cucumber. It was observed that there was a significant decrease in internal root population sizes of the entA mutant of S. marcescens compared with wild type strain 90-166. Press et al. (2001) hypothesized that siderophore production by strain 90-166 serves to detoxify the active oxygen species produced by the plant in response to the bacterium as was reported for Erwinia amylovora siderophores (Dellagi et al. 1998). The lack of enterobactin production in the entA mutant may render this strain more susceptible to active oxygen species and result in lowered internal populations. It was not determined, however, whether changes in internal colonization by the entA mutant contributed to changes in the ISR phenotype of this strain (Press et al. 2001).

6.3
Conclusions

The production of siderophores occurs under conditions of iron-limitation. Such conditions are likely to prevail in the rhizosphere (Loper and Henkels 1999), and siderophore mediated competition for iron is one of the mechanisms of bacterial antagonism against soil-borne pathogens (Loper and Buyer 1991). However, siderophore production can also trigger ISR and it can therefore play a dual role in disease suppression by depriving resident pathogens from iron locally and by inducing resistance in the plant systemically. The observation that not all siderophores induce ISR can be explained by the fact that siderophores produced by different bacteria have very different chemical structures (Höfte 1993). How siderophores are perceived by plants is presently completely unknown, but there is crop specificity as specific siderophores trigger ISR in one plant species but not another.

Several ISR-eliciting PGPR are able to produce SA in vitro, whereas others are not. Induction of systemic resistance in NahG-transformed plants demonstrated that ISR against TMV and Botrytis cinerea in tobacco and tomato by

7NSK2 (De Meyer et al. 1999a; Audenaert et al. 2002) and in Arabidopsis against *P. syringae* pv. *maculicola* by *B. pumilus* SE34 (Ryu et al. 2003) depends on SA accumulation in the plant. In other cases, PGPR still effectively induce ISR in *NahG* plants. Moreover, mutants of *S. marcescens* 90-166 that do not produce SA were as effective as the parental strain in triggering ISR in tobacco against *P. syringae* pv. *tomato* and in cucumber against *C. orbiculare* (Press et al. 1997). Thus, the importance of bacterially produced SA in PGPR-mediated ISR appears to be limited.

Whereas SA triggers a signal transduction pathway in the plant that depends on SA, ISR triggered by several PGPR strains is independent of SA but relies on jasmonic acid (JA) and ethylene signalling in the plant (Pieterse et al. 1996, 1998). Interestingly, simultaneous activation of the SA dependent and the JA/ethylene dependent pathways leads to an enhanced level of protection against pathogens (Van Wees et al. 2000). Whereas there is a wide range of pathogens against which both SA dependent and JA/ethylene dependent induced resistance are effective, some are only affected by one type of induced resistance (Ton et al. 2002). Therefore simultaneous activation of the SA dependent and independent pathways may increase the range of pathogens that are effectively suppressed after treatment with PGPR.

Redundancy in ISR eliciting determinants in PGPR on the one hand hampers studies to elucidate the involvement of these determinants; on the other hand it may give ISR robustness. If one determinant fails to elicit ISR or is not produced under certain conditions, other traits can still be effective. In these cases it would be favourable if the different traits were also differentially regulated.

Increased knowledge on the variety of bacterial determinants of ISR and their regulation in the rhizosphere will not only increase our fundamental understanding of interactions in this highly dynamic environment, but it will also increase possibilities to apply this mode of action of PGPR in crop protection strategies.

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