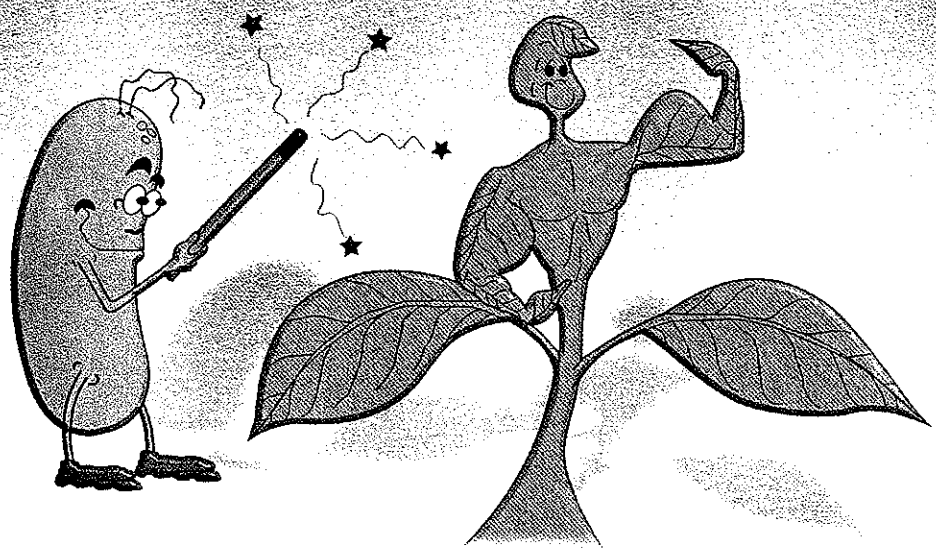
INDUÇÃO DE RESISTÊNCIA EM PLANTAS A PATÓGENOS



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INDUCED SYSTEMIC RESISTANCE IN RICE

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Abstract - The aim of our research is to test whether selected PGPR strains that can induce systemic resistance in dicots are also able to trigger induced systemic resistance (ISR) in rice against major fungal pathogens such as *Magnaporthe grisea* and *Rhizoctonia solani* and if so, to study which bacterial determinants are involved in this process. *Pseudomonas fluorescens* WCS374 and *Pseudomonas aeruginosa* 7NSK2 were the most effective inducers of resistance to *M. grisea.* Surprisingly, root colonization with *Pseudomonas fluorescens* WCS417, a strain that is extensively used to study ISR in Arabidopsis, was not effective and even resulted in increased disease symptoms. *Pseudomonas putida* WCS358, another strain that is highly effective in dicot plants, was only weakly effective in inducing resistance to *M. grisea.* All selected PGPR strains were able to induce resistance to *R. solani* to some extent, but only with *P. fluorescens* WCS374 consistent results were obtained. Bacterial determinants involved in ISR by *P. aeruginosa* 7NSK2 and *P. fluorescens* WCS374 were studied in more detail. For *P. aeruginosa* 7NSK2, only mutations interfering with the production of the phenazine antibiotic pyocyanin production led to a significant decrease in induced

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systemic resistance (ISR) to *M. grisea*, and *in trans* complementation for pyocyanin production restored the ability to elicit ISR. Intriguingly, pyocyanin-deficient mutants, unlike the wild-type, consistently triggered ISR against *R. solani*. Hence, bacterial pyocyanin plays a differential role in 7NSK2-mediated ISR in rice. Application of purified pyocyanin to hydroponically grown rice seedlings induced systemic resistance to *M. grisea* and increased H_2O_2 levels locally on the root surface as well as a biphasic H_2O_2 generation pattern in distal leaves. The cumulative results suggest that reactive oxygen species act as a double-edged sword in the interaction of rice with the hemibiotroph *M. grisea* and the necrotroph *R. solani*. For *P. fluorescens* WCS374, mutants deficient in the production of the fluorescent siderophore pseudobactin were no longer able to induce systemic resistance to *M. grisea* or *R. solani*. Application of purified WCS374 pseudobactin mounted ISR to *M. grisea*, but was less effective against *R. solani*, indicating that in addition to pseudobactin, other bacterial determinants are needed to trigger ISR to *R. solani*. In contract to pyocyanin, WCS374 pseudobactin does not influence the formation of reactive oxygen species in the roots or leaves of rice. Purified pseudobactin obtained from WCS358 was ineffective in triggering ISR to rice blast. How pseudobactins are perceived by plants is presently fully unknown, but there is crop specificity as specific pseudobactins trigger ISR in one plant species but not another.

Introduction

Rice is the most important staple food grain for more than two billion people living in the rural and urban areas of humid and subhumid Asia. Diseases are among the most important limiting factors that affect rice production, causing annual yield loss conservatively estimated at 5% (Mew et al. 2004). More than 70 diseases caused by fungi, bacteria, viruses or nematodes have been recorded on rice (Ou 1985), among which rice blast (*Magnaporthe grisea*) and sheath blight (*Rhizoctonia solani*) are the most serious fungal constraints on high productivity.

The filamentous ascomycete *Magnaporthe grisea* (Hebert) Barr (anamorph *Pyricularia grisea* (Cooke) Sacc.) is the most devastating pathogen of rice worldwide due to its widespread distribution and destructiveness (Talbot 2003). The rice- *M. grisea* interaction is a well-documented gene-for-gene system (Jia et al. 2000; Silue et al. 1992), and the fungus is a hemibiotroph since successful infection requires an initial biotrophic phase in which the pathogen forms bulbous invasive hyphae within apparently healthy plant cells (Koga 1994). Once established in the plant, the fungus switches to necrotrophic growth, killing plant cells and ramifying throughout the tissue. Rice sheath blight is caused by *Rhizoctonia solani* Kühn (sexual stage: *Thanetophorus cucumeris* (Frank) Donk), a soil- and water-borne fungal pathogen enjoying a very wide host range. The pathogen has a necrotrophic lifestyle and is able to produce a host-specific carbohydrate-based phytotoxin (Vidhyasekaran et al. 1997).

Resistant cultivars and application of pesticides have been used for disease control. However, the useful life span of most blast resistant cultivars is only a few years, due to the breakdown of the resistance in face of the high pathogenic variability of the pathogen population (Song and Goodman 2001). Though partial genetic resistance to sheath blight has been reported, no major gene-governed resistance has been found so far despite screening of more than

3000 accessions of germplasm worldwide (Mew et al. 2004). As chemical means of management are often expensive, currently no economically viable or sustainable control measures are available to tackle the diseases. Thus, there is a need to develop alternative disease control strategies providing durable, broad-spectrum resistance. Among such new strategies, induced resistance has emerged as a potential supplement in international crop protection measures.

Induced disease resistance can be defined as the process of active resistance dependent on the host plant's physical or chemical barriers, activated by biotic or abiotic agents (Kloepper *et al.*, 1992). The resulting elevated state of resistance in plant parts distant from the site of primary triggering is variably referred to as systemic acquired resistance (SAR) (Sticher *et al.*, 1997) or induced systemic resistance (ISR). The term SAR is commonly used to denote systemic resistance induced by pathogens. Selected non-pathogenic plant growth-promoting rhizobacteria (PGPR) are also known to induce a systemic resistance (for a review see van Loon et al., 1998). To differentiate this type of induced resistance from pathogen-induced SAR, the term rhizobacteria-mediated ISR is used.

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 (Lavicoli 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).1

In contrast to dicot plants, ISR is much less studied in monocot plants such as rice. In the class of Monocotyledoneae, including the most important agronomic cereals, molecular information on chemically and biologically induced resistance mechanisms is largely missing (Kogel and Langen 2005). One of the most compelling examples of a rice SAR-like response is the enhanced resistance to *M. grisea* that was demonstrated in response to an infection with the non-host pathogen *Pseudomonas syringae* pv. *syringae* (Smith and Metraux 1991). Although the synthetic salicylic acid analogue benzo(1,2,3)-

thiadiazole-7-carbothioc acid (BTH) has been shown to induce disease resistance in rice (Rohilla et al. 2002; Schweizer et al. 1999), reports about the induction of systemic resistance in rice using beneficial microorganisms are scarce. Ccolonization of the rice rhizosphere with the PGPR strains *Pseudomonas fluorescens* PF1 and FP7 enhanced resistance against sheath blight disease (Nandakumar et al. 2001). Someya et al. (2002, 2005) reported induced resistance to rice blast and sheath blight by the antagonistic bacterium *Serratia marcescens* B2. Nothing is known, however, about the bacterial determinants or plant defense pathways involved.

The aim of our research is to test whether selected PGPR strains that can induce systemic resistance in dicots (see Table 1) are also able to trigger ISR in rice against major fungal pathogens such as *Magnaporthe grisea* and *Rhizoctonia solani* and if so, to study which bacterial determinants are involved in this process. In this study we report in more detail about bacterial determinants of *Pseudomonas aeruginosa* 7NSK2 and *Pseudomonas fluorescens* WCS374 involved in ISR in rice. Important metabolites produced by *P. aeruginosa* 7NSK2 are the siderophores pyoverdine and pyochelin, the pyochelin-precursor salicylic acid and the phenazine antibiotic pyocyanin. *P. fluorescens* WCS374 produces the siderophores pseudobactin (= pyoverdine), pseudomonin, and the pseudomonin-precursor salicylic acid. Part of this work has been published (De Vleesschauwer et al., 2006).

Materials and Methods

Bacterial strains and mutants

Bacterial PGPR strains used in this study and their determinants involved in ISR in dicot plants are listed in Table 1. Mutants of *P. aeruginosa* 7NSK2 used in this study are KMPCH, a pyoverdin and pyochelin-negative, but SA-positive mutant; and the pyocyanin-deficient mutants 7NSK2-*phzM* and KMPCH-*phzM* (see

De Vleesschauwer et al., 2006 for more details about these strains). Mutants of *P. fluorescens* 374 used in this study are 374-02 (pseudobactin-deficient), AT12 (pseudobactin and pseudomonin deficient), 4A1 (pseudomonin deficient), BT1 (pseudobactin, pseudomonin and SA deficient).

Table 1. Pseudomonas strains used in this study and their determinants involved in ISR in dicot plants.

Bacterial strain	Plant - Pathogen	Determinant(s) involved in ISR	Reference
P. aeruginosa 7NSK2	Bean - *Colletotrichum lindemuthianum*	Salicylic acid	Bigirimana & Höfte, 2002
	Bean - *Botrytis cinerea*	Salicylic acid	De Meyer & Höfte, 1997
	Tobacco – Tobacco Mosaic Virus	Salicylic acid	De Meyer et al., 1999
	Tomato – *Botrytis cinerea*	Salicylic acid, pyochelin, pyocyanin	Audenaert et al., 2002
	Arabidopsis – *Pseudomonas syringae* pv. *tomato*	unknown	Ran et al., 2005b
P. fluorescens WCS374	Radish – fusarium wilt	Pseudobactin, LPS	Leeman et al., 1995a, 1995b
	Eucalyptis – *Ralstonia solanacearum*	Pseudobactin, unknown determinant(s)	Ran et al., 2005a
P. fluorescens WCS417	Carnation – fusarium wilt	LPS	Van Peer et al., 1992
	Radish – fusarium wilt	LPS, unknown iron-regulated determinant(s)	Leeman et al., 1995a
	Arabidopsis – *Pseudomonas syringae* pv. *tomato*	LPS	Van Wees et al., 1997
P. 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., 2005a

Fungal isolates

Fungal isolates used in this study were *M. grisea* strain VT5M1 and *R. solani* strain MAN-86 (De Vleesschauwer et al., 2006).

Infection assays with bacterial strains

Plants were grown under nonsterile greenhouse conditions in potting soil (Klasmann, substrat no 4, Otrebusy, Germany). Bacterial root colonization was achieved by a combined seed and soil treatment. Briefly, bacteria were routinely grown on King's medium B, scraped off the plates, and suspended in sterile saline (0.85 % NaCl). For seed treatment, rice seeds were surface sterilized with 1% sodium hypochlorite solution and rinsed three times in sterile distilled water. Next, seeds were soaked in a bacterial suspension to a concentration of 5 x 10^7 CFU ml^{-1}, or in the case of control plants in 0.85% NaCl. After five days of incubation, roots of germinated seeds were dipped prior to sowing in a bacterial suspension (5 x 10^7 CFU ml^{-1}) and the potting soil was mixed with bacterial inoculum to a concentration of 5 x 10^7 CFU g^{-1}. In control treatments, roots and soil were treated with equal volumes of sterile demineralised water. Germinated seeds were sown in perforated plastic trays to provide aerobic soil conditions. Ten days after sowing, an additional bacterial application was performed as a soil drench (5 x 10^7 CFU g^{-1}). Twenty eight-day-old plants (4-leaf stage) were challenge inoculated either with *M. grisea* by spraying as described by Ninh Thuan *et al.* (2006) or with *R. solani* using the toothpick inoculation method (Rodrigues *et al.*, 2003).

Hydroponic plant growth

For experiments in which purified pyocyanin was applied to rice seedlings, plants were grown in a hydroponic gnotobiotic system. Surface-sterilized rice seeds were germinated for 5 days on wet filter

paper in Petri dishes. After incubation, germinated seeds were sown in perforated plastic trays filled with sterilized vermiculite, and supplemented with half-strength Hoagland solution. Every three days, 0.5 litre of the half-strength Hoagland solution was added to each tray containing 12 seedlings. In this model, 4 days before challenge inoculation, various concentrations of pyocyanin were applied to the plants by including the desired concentration in the nutrient solution without ethylenediaminetetraacetic acid ferric sodium salt (Acros, Geel, Belgium).

Histochemical detection of H_2O_2

To assess whether pyocyanin was able to produce H_2O_2 in the gnotobiotic system, rice roots were dipped in half-strength Hoagland nutrient solution containing 1 nM pyocyanin for 2 h, rinsed thoroughly with demineralized water and subsequently incubated for 12 h at room temperature in water with 0.01% Triton-X-100 and 3,3'-diaminobenzidine (DAB). DAB (Sigma-Aldrich, Bornem, Belgium) polymerizes in the presence of H_2O_2 to form a brownish-red precipitate that can be visualized. The specificity of the staining was verified by adding 10 mM ascorbic acid.

In planta determination of H_2O_2

The *in planta* accumulation of H_2O_2 was determined following the $TiCl_4$-based technique as described by Mur et al. (2005). H_2O_2 accumulation was expressed relative to values obtained in control samples.

Results and Discussion

Ability of selected PGPR strains to induce systemic resistance to sheath blight and blast in rice

P. fluorescens WCS374 and *P. aeruginosa* 7NSK2 were the most effective inducers of resistance to *M. grisea*. Surprisingly, root colonization with *P. fluorescens* WCS417, a strain that is extensively used to study ISR in Arabidopsis, was not effective and even resulted in increased disease symptoms. *P. putida* WCS358, another strain that is highly effective in dicot plants, was only weakly effective in inducing resistance to *M. grisea* (Table 2). All selected PGPR strains were able to induce resistance to *R. solani* to some extent. Results obtained with *P. aeruginosa* 7NSK2, *P. putida* WCS358 and *P. fluorescens* WCS417, however, were variable. Although small protective effects were observed in single experiments (see Table 2), these strains proved unable to consistently reduce the length of lesions caused by the sheath blight fungus *R. solani* (data not shown). Only with WCS374 consistent results against *R. solani* were obtained.

Table 2. Ability of selected PGPR strains to induce systemic resistance in rice to *Magnaporthe grisea* and *Rhizoctonia solani*.

PGPR strain	*M. grisea* (relative infection, control = 100%)	*R. solani* (lesion length in mm)
Control	100.00 b	67.00 a
P. aeruginosa 7NSK2	65.05 cd	51.38 b
P. putida WCS358	82.16 c	46.59 b
P. fluorescens WCS374	53.40 d	44.35 b
P. fluorescens WCS417	127.50 a	47.80 b

Within columns, data followed by the same letter are not significantly different for P=0.05

Bacterial determinants involved in ISR by P. aeruginosa 7NSK2

The ability of *P. aeruginosa* 7NSK2 to induce resistance was studied in more detail. Figure 1A shows that the pyochelin-negative mutant KMPCH (also pyoverdin deficient) induced resistance to an extent similar to that induced by the wild type, hereby excluding an essential role of the siderophores pyoverdin and pyochelin in ISR in rice to *M. grisea*. Treatment with the pyocyanin-negative mutants 7NSK2-phzM and KMPCH-phzM no longer caused disease reduction, indicating the involvement of the phenazine antibiotic pyocyanin in ISR. *In trans* complementation of 7NSK2-phzM for pyocyanin production (strain 7NSK2-phzMc) restored the capacity to induce resistance to *M. grisea*, confirming the essential role of pyocyanin in 7NSK2-mediated ISR (Fig. 1B).

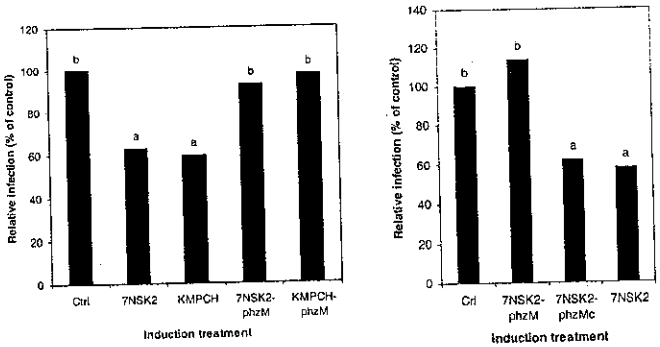

Figure 1. A and 1B. Influence of root treatment with *Pseudomonas aeruginosa* 7NSK2 and various mutants on rice blast (*Magnaporthe grisea*) severity. Mutants derived from strain 7NSK2 have the following characteristics: KMPCH (pyoverdin and pyochelin deficient), 7NSK2-phzM (phzM$^-$, nonproducing pyocyanin), KMPCH-phzM (pyoverdin and pyochelin deficient; phzM$^-$, nonproducing pyocyanin) and 7NSK2-phzMc = strain 7NSK2-phzM complemented with functional phzM gene of 7NSK2, restoring pyocyanin production.

As stated before, *P. aeruginosa* 7NSK2 proved unable to consistently mount ISR to the sheath blight fungus *R. solani* in several preliminary experiments. These data notwithstanding, we tested the same set of mutant strains as described before in a series of infection assays with *R. solani* as challenging pathogen. Nor the wild-type strain 7NSK2 nor the pyochelin-negative mutant KMPCH significantly reduced sheath blight severity (Fig. 2). However, inoculation of the rhizosphere of rice seedlings with the corresponding pyocyanin-deficient strains (7NSK2-phzM and KMPCH-phzM) resulted in significantly higher protection levels to *R. solani* compared to wild type-treated and control plants.

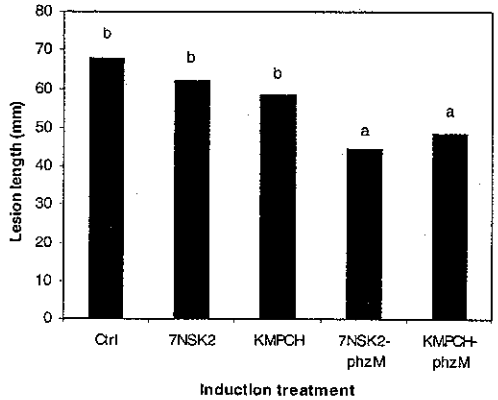

Figure 2. Influence of root treatment with *Pseudomonas aeruginosa* 7NSK2 and various mutants on sheath blight (*Rhizoctonia solani*) severity. Data presented are means from at least three independent experiments with 12 replications per treatment in each experiment. Statistical analysis was performed on pooled data, as interaction between treatment and experiment was not significant at á = 0.05 by analysis of variance. Bars with the same letter are not significantly different by non-parametric Kruskal-Wallis and Mann-Whitney comparisons at P = 0.05. Mutants derived from strain 7NSK2 have the following characteristics: KMPCH (pyoverdin and pyochelin deficient), 7NSK2-phzM (phzM$^-$, nonproducing pyocyanin) and KMPCH-phzM (pyoverdin and pyochelin deficient, phzM$^-$, nonproducing pyocyanin).

The observation that pyocyanin-deficient mutants, unlike wild-type strains, triggered resistance to *R. solani*, whereas the same mutants lost their ability to mount ISR to *M. grisea* (Figs. 1A and 2), suggested that the secretion of pyocyanin might account for the differential effectiveness of 7NSK2-mediated ISR to the latter pathogens. Therefore, we wanted to further explore the role of bacterially produced pyocyanin in 7NSK2-mediated ISR in rice. To this purpose, we applied purified pyocyanin to the roots of rice seedlings in a hydroponic system. No signs of phytotoxicity were observed in leaves of plants after pyocyanin feeding at any of the concentrations tested. In the 25 pM to 100 nM pyocyanin range, ISR to *M. grisea* was evident for all concentrations tested. However, no significant protection could be observed at 50 μM pyocyanin. Conversely, pyocyanin feeding favored subsequent infection by *R. solani*, irrespective of the applied concentration (see De Vleesschauwer et al., 2006). These data suggest a dual role of pyocyanin in 7NSK2-mediated ISR and corroborate the results obtained in the ISR assays with the pyocyanin-negative mutants 7NSK2-phzM and KMPCH-phzM.

Given the fact that pyocyanin has the capacity to undergo redox cycling under aerobic conditions with resulting generation of superoxide and hydrogen peroxide *in vitro* (Hassan and Fridovich 1980), we asked whether pyocyanin also would be capable of producing reactive oxygen species in rice. To this end, we monitored the levels of H_2O_2, which is the major and most long-living reactive oxygen species, both on the roots and in the leaves of hydroponically grown rice seedlings in response to pyocyanin feeding. Detection of H_2O_2 on roots was carried out by means of an endogenous peroxidase-dependent staining procedure with 3,3´-diaminobenzidine (DAB). Roots of rice seedling treated with 100 nM pyocyanin showed strong DAB staining compared to Hoagland-treated control roots. However, DAB staining was not observed on roots in the presence of the H_2O_2 scavenger, ascorbic acid, confirming the specificity of the staining. The *in planta* accumulation of H_2O_2 was determined following the

titanium (IV) chloride method as described by Wu et al. (1995). Inclusion of 100 nM pyocyanin in the nutrient solution revealed a transient rise in H_2O_2 levels in systemic leaves at 8 h post-application compared to control plants, followed by decay to control levels. A second more pronounced rise in H_2O_2 was observed at 48 h post-treatment and persisted for at least 24 h. Taken together, these data clearly demonstrate the ability of bacterial pyocyanin to generate ROS on the root surface of rice seedlings as well as in systemic leaves.

The dual role of the phenazine antibiotic pyocyanin in *P. aeruginosa* 7NSK2-mediated ISR suggests that rice requires distinct mechanisms for defense against *M. grisea* and *R. solani*. On one hand, root treatment with pyocyanin was effective against *M. grisea*, triggering reiterative H_2O_2 microbursts, and causing rapid HR-associated cell death in response to fungal infection, which most likely leads to breakdown of the biotrophic phase of the *M. grisea* infection cycle. On the other hand, treatment with pyocyanin significantly promoted subsequent infection by the necrotrophic pathogen *R. solani* by facilitating pathogen-triggered host cell death. Hence, the oxidative burst and related hypersensitive response might act as a double-edged sword in the interaction of rice with hemibiotrophic (*M. grisea*) and necrotrophic (*R. solani*) pathogens. This conclusion is substantiated with recent research by Ahn et al. (2005), demonstrating the differential beneficial effect of the HR as defense mechanism against *M. grisea* and the necrotrophic rice pathogen *Cochliobolus miyabeanus*. Considering that the effect of the oxidative burst and HR-associated cell death depends on the type of invading pathogen, the widespread cultivation of resistant blast varieties that rely upon major resistance genes may contribute to the increase in sheath blight incidence. In this respect, our recent observation that *R. solani* colonization and sheath blight development is favoured by pre-inoculation with a HR-triggering incompatible *M. grisea* isolate is of particular interest (De Vleesschauwer et al. unpublished results) and might explain why there are no HR-triggering gene-for-gene phenomena known for *R. solani*-rice interactions.

BACTERIAL DETERMINANTS INVOLVED IN ISR BY *P. FLUORESCENS* WCS374

Also the ability of *P. fluorescens* WCS374 to mount ISR in rice was studied in more detail. Figure 3 shows that only mutant 4A1 induced resistance to *M. grisea* to a similar extent as the wild type strain WCS374, excluding a role for pseudomonin in ISR. However, all pseudobactin deficient mutants lost their ability to mount ISR to rice blast. This indicates that the fluorescent pseudobactin siderophore from WCS374 plays an important role in ISR.

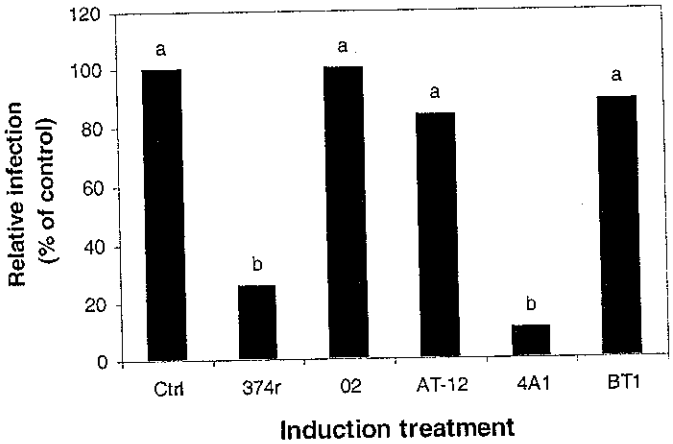

Figure 3. Influence of root treatment with *Pseudomonas fluorescens* WCS374 and various mutants on rice blast (*Magnaporthe grisea*) severity. Data presented are means from one experiment with 24 replications per treatment. Bars with the same letter are not significantly different by non-parametric Kruskal-Wallis and Mann-Whitney comparisons at P = 0.05. Mutants derived from strain WCS374 have the following characteristics: 374-02 (pseudobactin-deficient), AT12 (pseudobactin and pseudomonin deficient), 4A1 (pseudomonin deficient), BT1 (pseudobactin, pseudomonin and SA deficient).

Comparable results were obtained when the same set of WCS374 mutants were tested for their ability to mount ISR to R. solani. The wild type strain WCS374 and its mutant 4A1, were still effective, while all other mutants were unable to induce systemic resistance to sheath blight (Figure 4).

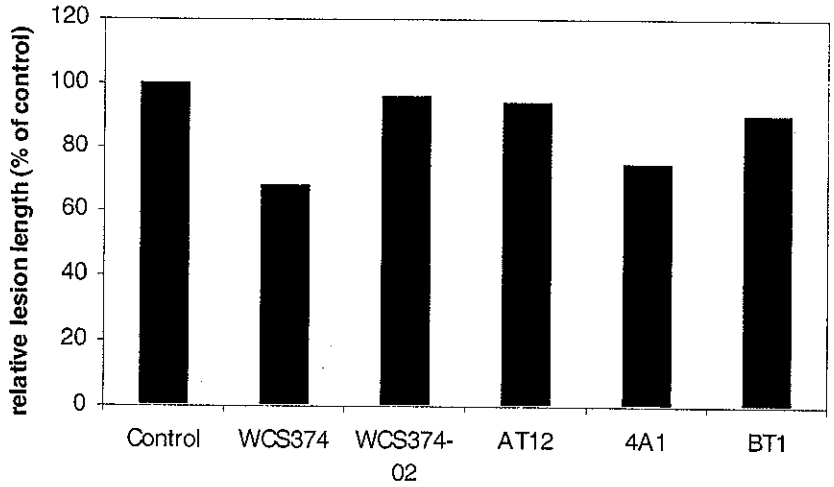

Figure 4. Influence of root treatment with *Pseudomonas fluorescens WCS374* and various mutants on sheath blight (*Rhizoctonia solani*) severity. Data presented are means from two independent experiments with 12 replications per treatment in each experiment. Statistical analysis was performed on pooled data, as interaction between treatment and experiment was not significant at á = 0.05 by analysis of variance. Bars with the same letter are not significantly different by non-parametric Kruskal-Wallis and Mann-Whitney comparisons at P = 0.05. Mutants derived from strain WCS374 have the following characteristics: 374-02 (pseudobactin-deficient), AT12 (pseudobactin and pseudomonin deficient), 4A1 (pseudomonin deficient), BT1 (pseudobactin, pseudomonin and SA deficient).

Since pseudobactin-deficient mutants of *P. fluorescens* WCS374 were unable to trigger ISR to both rice blast and sheath blight, we wanted to further explore the role of this metabolite in ISR in rice. To this purpose, we applied purified pseudobactin from WCS374 to the roots of rice seedlings in a hydroponic system. Feeding rice roots with 70 μg pseudobactin/plant was highly effective in inducing systemic resistance to *M. grisea*, indicating that pseudobactin is the main metabolite involved in ISR to rice blast. However, against *R. solani,* no consistent results were obtained and pseudobactin treatments had only small effects on sheath blight severity, indicating that in addition to pseudobactin, other metabolites may be involved in ISR to sheath blight.

Further studies revealed that WCS374 pseudobactin does not trigger the production of H_20_2 in rice roots or leaves, indicating that WCS374 pseudobactin induces resistance by a mechanism, which is different from pyocyanin-induced resistance. Microscopic observations showed the induction of physical barriers to *M. grisea* in rice leaves upon root treatment with WCS374 pseudobactin.

Interestingly, pseudobactin obtained from *P. putida* WCS358 was ineffective in triggering ISR to rice blast, while this determinant is implicated in ISR in all dicot plants in which WCS358 is effective (see Table 1). The analysis of mutants derived from *P. aeruginosa* 7NSK2 showed that pyoverdin from 7NSK2 does not play a role in ISR to rice blast either. The observation that not all pseudobactins (pyoverdins) induce ISR can be explained by the fact that siderophores produced by different bacteria have very different chemical structures (Höfte 1993). How pseudobactins are perceived by plants is presently fully unknown, but there is crop specificity as specific pseudobactins trigger ISR in one plant species but not another.

Conclusions

In conclusion we can state that selected PGPRs can induce systemic resistance in rice by at least two different mechanisms. Pyocyanin-mediated resistance triggers the production of reactive oxygen species in both the roots and leaves of rice and is effective against *M. grisea* but increases rice sheath blight severity. Resistance mediated by pseudobactin obtained from *P. fluorescens* WCS374 does not trigger the production of reactive oxygen species in the rice plants. This type of resistance is highly effective against *M. grisea*, probably by inducing physical barriers and does not aggravate rice sheath blight severity. The reaction to pseudobactin appears to be crop specific. Our work might contribute to the development of new strategies for disease control in rice.

Acknowledgements

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