SCREENING SYSTEMIC RESISTANCE-INDUCING FLUORESCENT PSEUDOMONADS FOR CONTROL OF BACTERIAL BLIGHT OF COTTON CAUSED BY *XANTHOMONAS CAMPESTRIS* pv. *MALVACEARUM*

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SUMMARY

Iron-regulated metabolites, such as siderophores are important determinants of fluorescent pseudomonads in induction of systemic resistance (ISR) of plants. In this work, King's medium B supplemented with 120 ppm 8-hydroxyquinoline (8-HQ) chelator was used for screening of pseudomonads from the rhizosphere of cotton with a high capacity to produce siderophores. Use of this medium resulted in isolates with a high ISR capability equal or greater than the reference strain *P. aeruginosa* 7NSK2. All strains except 13Q and EQ significantly decreased the percentage of infected area on leaves. Siderophore production by both the strains 35Q and 16Q, that were the fastest growing strains on KB media supplemented with 8HQ, was significantly higher than that of the reference strain 7NSK2. In hydroponic cultivation assays, ISR ability of 35Q depended on one or a complex of several iron regulated metabolite/s, as its ISR was significantly reduced in pots with iron excess. Nonetheless, ISR by 35Q was not completely abolished under iron excess suggesting a possible role for other determinants.

Key words: screening, ISR, PGPR, peroxidase, phenylalanine ammonia lyase, 8-hydroxyquinoline.

INTRODUCTION

Fluorescent pseudomonads of the class of plant growth-promoting rhizobacteria (PGPR) are known to improve growth in different crops (Kloepper and Schroth, 1981; Gutterson, 1990). Earlier studies were focused on the suppression of soil-borne pathogens by certain strains of *Pseudomonas* spp. (Weller, 1988; Capper and Higgins, 1993), but some of these biological control strains can also reduce disease caused by foliar pathogens by triggering a plant-mediated resistance mechanism called induced systemic resistance (ISR). Such resistance is effective against a broad spectrum of pathogens. For example, In *Arabidopsis*, ISR triggered by the root-colonizing bacterial strain *Pseudomonas fluorescens* WCS417r is effective against several pathogens including the fungal root pathogen *Fusarium oxysporum* f. sp. *raphani*, the bacterial leaf pathogens *Pseudomonas syringae* pv. *tomato* DC3000 (Pst) and *Xanthomonas campestris* pv. *campestris*, the oomycete *Hyaloperonospora parasitica*, and the fungus *Alternaria brassicicola* (Pieterse *et al.*, 1996; Ton *et al.*, 2002). The signalling pathway controlling rhizobacteria-mediated ISR clearly differs from pathogen-induced systemic acquired resistance (SAR) in that it is not associated with the accumulation of salicylic acid and induction of PRs, and is one of the mechanisms by which rhizobacteria, especially fluorescent pseudomonads, can suppress disease (Pieterse *et al.*, 1996; Bakker *et al.*, 2007).

The onset of ISR is thought to be the result of perception of one or more eliciting compounds (determinants) of rhizobacteria by the plant root surface. Upon binding by a receptor, transduction of signals produced and mediated by the plant would lead to the ISR state. A large number of determinants for ISR have been described (reviewed by Bakker, *et al.*, 2007 for pseudomonads and Kloepper *et al.*, 2004 for *Bacillus* spp.). Among these, iron-regulated metabolites such as pseudobactin siderophore (Maurhofer *et al.*, 1994; Leeman *et al.*, 1996; Meziane *et al.*, 2005; Ran *et al.*, 2005), a *N*-alkylated benzylamine derivative (Ongena *et al.*, 2005), salicylic acid (De Meyer *et al.*, 1999) and pyochelin siderophore (Audenaert *et al.*, 2002) have been widely investigated and their substantial role in ISR proven in many cases.

Production of these metabolites depends on the concentration of iron in the environment and under conditions of low iron availability, most rhizobacteria can be stimulated to produce them. Plant growth stimulation by pseudomonads is prevented when the soil is enriched with Fe(III) (Kloepper *et al.*, 1980). Purified siderophores exhibit a disease suppressive effect that is very similar to that of the producer strain (Kloepper *et al.*, 1980; Neilands and Leong, 1986).

In this study, we used King's medium B supplement-

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ed with 120 ppm 8-hydroxyquinoline (8-HQ) chelator to screen pseudomonads from the rhizosphere of cotton for high siderophore production. Because of its high stability constant (K= 10^{33}-10^{34}) (Sillen and Martell, 1971), this chelator reduces available iron in medium. Fast growing isolates on this medium were selected for evaluation of their effect on cotton plant growth and their resistance to bacterial blight.

MATERIAL AND METHODS

Selection of a dosage of 8-HQ for preparation of a semi-selective medium. In this study, we used reference strain *Pseudomonas aeruginosa* 7NSK2 for selection of a discriminative dosage of 8-HQ for preparation of semi-selective medium. A suspension containing 8×10^2 cells l^{-1} in sterile distilled water was prepared from a 48 h culture of strain 7NSK2 on TSBA medium. Thereafter, 100 µl of this suspension was plated on KB media containing increasing concentrations of the iron chelator 8-HQ (Merck, Germany), i.e., 0, 40, 60, 80, 100, 120 and 200 ppm or on KB media containing increasing concentrations of 8-HQ + equimolar amounts of $FeCl_3$. Plates were incubated at 26°C and after 38 h the number of developed colonies were counted and dose-response curves and effective concentration (EC_{50}) determined. The 8-HQ was dissolved in 70% ethanol and suitable quantities from this stock solution were added to the KB medium. Where necessary, additional amounts of 70% ethanol were supplied so that all media contained equal amounts of 70% ethanol. To investigate the possible toxic effect of 8HQ for strain 7NSK2, equimolar amounts of $FeC1_3$ were added to KB media containing increasing concentrations of the 8-HQ mentioned above.

Field sampling and isolation of bacteria. In July 2008, 100 cotton plants were collected from two fields of Varamin Cotton Research Centre in Tehran province (Iran). These sites were selected because they have been planted continuously with cotton for at least 20 years. Plants were gently removed from soil and transported to the laboratory in plastic bags. Adhering soil was carefully brushed off the roots followed by gentle washing in sterile water. One gram of roots was placed in 9 ml of 0.1 M phosphate buffer (pH 7) supplemented with 0.025% Tween 20 and vortexed for 5 min. Thereafter 100 µl of first to fourth dilutions were transferred to KB medium or KB supplemented with 120 ppm 8-HQ. Fluorescent pseudomonad-like colonies that developed on these plates were purified.

Greenhouse experiments. The 19 strains of Pseudomonas colonies with fast growth on 8-HQ-KB were selected for greenhouse studies. *P. aeruginosa* 7NSK2 and *P. fluorescens* CHA0 were used as reference strains. Strains were grown on KB agar plates for 30 h at 26°C. Bacteria scraped from the agar were suspended in 0.1 M phosphate buffer (pH 7), and washed twice by centrifugation (for 10 min at 6,000 rpm). Washed pellets were suspended in 1% methylcellulose solution and optical density of these suspensions adjusted to 0.6 at 620 nm by a spectrophotometer (PG instruments T70+, UK**).** Acid-delinted and neutralized cotton seeds were disinfected for 3 min in a 0.5% (w/v) sodium hypochlorite solution and rinsed 4 times with sterile distilled water. Seeds were subsequently dipped for 45 min in cell suspensions. Seeds mock-treated with 1% methylcellulose solution without bacteria were kept as a control. The seeds were air-dried at 27°C for 30 min and sown in plastic pots (8 cm diameter, 8.9 cm height). The soil used was sterilized sandy clay loam from cotton fields.

Plants were grown in a greenhouse maintained at 26-28ºC and 65-75% relative humidity (RH). At 25 days after sowing, plants were inoculated with *Xanthomonas campestris* pv. *malvacearum* (*Xcm*) by spraying leaves with a suspension containing 10^6 cells ml^{-1}, when the stomata were open (in the morning). After inoculation, plants were maintained at day and night temperatures of 31 and 26°C, respectively and at 95-100% RH for two days. High levels of moisture were provided by an ultrasonic humidifier. After 48 h the RH and temperature of the greenhouse were returned to previous conditions. After 15 days three leaves per pot were selected and the percentage infected area calculated. There were three pots per treatment and three plants per pot.

Siderophore production by selected strains. Production of pseudobactin-type (Pyoverdine) siderophores by selected strains was assessed according to Meyer and Abdallah (1978). Ten ml of succinate minimal medium (0.4% succinic acid, 0.6% K_2HPO_4, 0.02% KH_2PO_4, 0.02% $MgSO_4.7H_2O$, 0.01% ammonium sulphate, pH 7.2) was inoculated with 30 µl of actively growing cells (10^5 cells ml^{-1}) and after 40 h at 26ºC, the culture supernatants were used for measurement of pseudobactin. Pseudobactin concentration was calculated using the absorption maximum and the molar absorption coefficient (λ_{max} = 400 nm and Σ= 20 000 M^{-1} cm^{-1}).

ISR of 35Q under low iron availability. Under conditions of low iron availability, most aerobic and facultative anaerobic microorganisms, including fluorescent *Pseudomonas* spp., produce several siderophores to sequester ferric ions in the environment and the ferrated siderophores are taken up by the microbial cells through specific recognition by membrane proteins (Höfte, 1993). Thus, this assay was designed to investigate the possible role of siderophores in ISR by this strain. Acid-delinted and disinfected cotton seeds were pre-germinated on moist filter paper for 48 h at 26ºC and inoculated with cell suspensions of strain 35Q as mentioned above. The seeds were then sown in plastic pots containing autoclaved perlite and irrigated with

Hoagland nutrient solution (Hoagland and Arnon, 1938). In the case of treatments with low iron, pots were irrigated with nutrient solution not containing iron. Inoculation of leaves with *Xcm* and greenhouse conditions in this experiment were similar to those mentioned above. Two days after inoculation of *Xcm*, one gram of leaves from each treatment was collected for analysis of defence-related enzymes.

Assays for peroxidase (PO) and phenylalanine ammonia lyase (PAL). Leaf tissues were immediately homogenized with 2 ml 0.1 M sodium phosphate buffer, pH 7.0 at 4ºC. The homogenate was centrifuged for 20 min at 12,000 rpm. Protein extracts prepared from leaves were used for the estimation of PO and PAL activity (Anand *et al.*, 2007).

PO activity was assayed spectrophotometrically (Hammerschmidt, 1982). The 2.1 ml reaction mixture in 3 ml cuvette consisted of 1.5 ml of 0.05 M pyrogallol, 100 µl of enzyme extract and 0.5 ml of 1% H_2O_2. H_2O_2 was added last to initiate the reaction and the change in absorbance was recorded at 420 nm for 1 min. The enzyme activity was expressed as change in absorbance $min^{-1}g^{-1}$ fresh weight of enzyme extract.

PAL activity was determined as the rate of conversion of L-phenylalanine to *trans*-cinnamic acid at 290 nm as described by Dickerson *et al.* (1984) with minor modifications. The assay mixture consisted of 200 µl crude extract and 1800 µl of 6 µM l-phenylalanine in 1000 µl of 500 mM Tris-HCl buffer (pH 8·5). The mixture was incubated at 37°C for 1 h and measured spectrophotometrically at 290 nm. The amount of *trans*-cinnamic acid synthesized was calculated using its extinction coefficient of 9630 M^{-1} cm^{-1}. Enzyme activity was expressed on a fresh weight basis (nMol min^{-1} g^{-1}).

Gel electrophoresis for detection of peroxidase isoforms. For native anionic polyacrylamide gel electrophoresis, a 12% acrylamide resolving gel and 6% acrylamide stacking gel were prepared. After electrophoresis for 4 h at 85 V and 10ºC, the gel was incubated for 5 min in the solution containing 0.05 M pyrogallol, then 33 ml H_2O_2 solution was added under constant shaking until bands appeared.

Data analysis. Data were processed by analysis of variance, followed by the Duncan multiple range test (< = 0.05), with SAS 9.1 software (SAS institute, USA). Probit analysis was used to measure EC_{50} with SPSS 9.

RESULTS

Selection of a dosage of 8HQ for preparation of selective medium. The number of colonies of *P. aeruginosa* 7NSK2 that developed on 8HQ-KB decreased with increasing concentrations of 8HQ in the medium (Fig. 1). The EC_{50} value of 7NSK2 was around 130 ppm 8HQ. When these increasing concentrations were supplemented with equimolar amounts of iron, no reduction in the number of CFUs was observed. It appears that the inhibition property of this compound is mediated by chelating of iron and not by toxicity of 8HQ or its Fe(III) chelates.

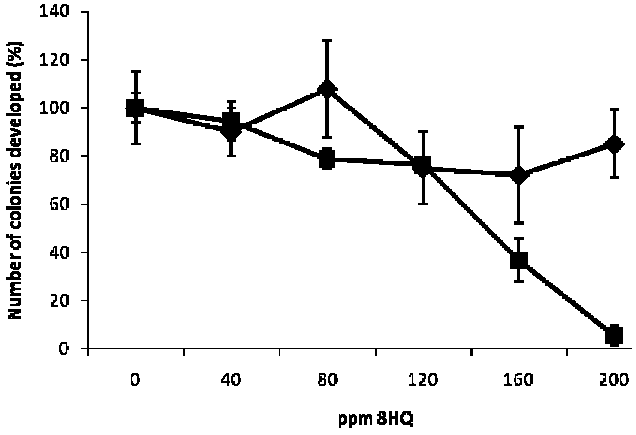

Fig. 1. Number of colonies of strain 7NSK2 on KB medium containing increasing concentrations of 8HQ (squares) and on medium with increasing concentrations of 8HQ + equimolar amounts of iron (diamonds). Data are means from three independent plates. Bars indicate standard error.

We used 120 ppm of 8HQ for preparation of selective medium for isolation of pseudomonads with high ability to produce siderophores. High concentrations of 8HQ were not applied because some valuable isolates may not grow on such concentrations.

Field sampling and isolation of bacteria. The number and variety of colonies that developed on KB medium were high and selection and isolation of pseudomonads on this medium was difficult. In contrast on the media with 120 ppm 8HQ the number of colonies was usually less than 50. Notably, in this medium 98% of colonies were pseudomonads with strong fluorescence under UV light at 366 nm. This medium therefore facilitates the screening process and it can be efficient tool for high throughput prequalification of ISR fluorescent pseudomonads.

Greenhouse experiments. All strains, except for 13Q and EQ, significantly decreased the percent of infected area on leaves (Table 1). Ability of strain CHA0 to induce systemic resistance was low but its growth promotion was considerable. Resistance induced by most of the strains was higher than that of 7NSK2. This strain is well known as an efficient biocontrol strain, inducing resistance against different plant pathogens (De Meyer *et al.*, 1999; Audenaert *et al.*, 2002; De Vleesschauwer *et*

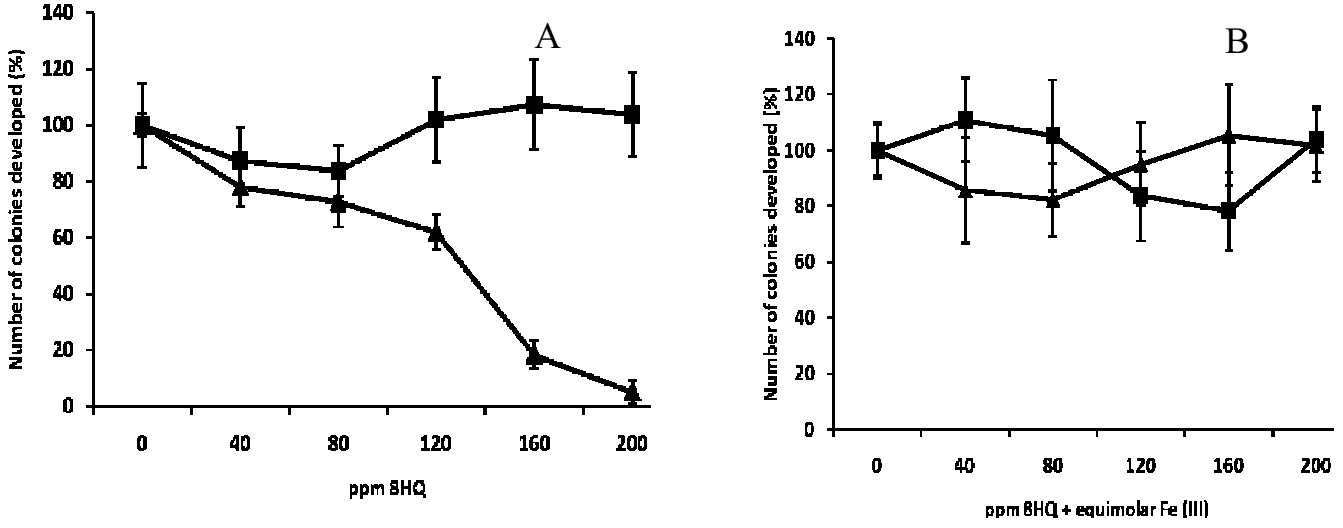

Fig. 2. Number of colonies of strains 16Q (squares) and 35Q (triangles) on KB medium with increasing concentrations of 8HQ (A) and on medium with increasing concentrations of 8HQ + equimolar amounts of iron (B). Data are means from three independent plates. Bars indicate standard error.

al., 2006). Strain 10A drastically decreased the percentage of infected area on leaves but it had a negative effect on cotton plants as the fresh weight of plants treated with this strain was 59.5% lower than those of infected control plants (Table 1).

Dosage response curves of 16Q and 35Q on 8HQKB. Growth of 35Q depended on the concentration of 8HQ and its dose-response curve was similar to that of 7NSK2 (Fig. 1 and 2). The number of 16Q colonies developed did not decrease at high concentra-

Table 1. Effect of isolates on plant fresh weight and percentage infected area on leaves. Isolates applied at time of sowing as seed bacterization and at 25 days as leaf spray. Fifteen days after inoculation of pathogen, plants were collected for measurement of infected area and fresh weight.

Isolates	Percent of infected area on leaves[b]	Fresh weight[c]
P. aeruginosa 13Q	10.51a	4.3bcdef
P. aeruginosa EQ	9.97a	5.1abcde
CX[a]	9.10a	4.2bcdef
P. fluorescens 60	6.00b	5.6abcde
*P. fluorescens*CHA0	5.70b	5.7abcde
P. aeruginosa 17Q	5.35b	3.7bcdef
P. fluorescens 147	4.72b	4.3bcdef
P. aeruginosa 34Q	4.36bc	5.7abcde
P. aeruginosa 42Q	4.35bc	2.8ef
P. aeruginosa 26Q	4.28bc	4.3bcdef
P. aeruginosa 2Q	4.00bcd	5.1abcde
P. fluorescens 120	3.90bcd	6.4bc
P. putida 113	3.78bcd	6.3abc
P. aeruginosa 7NSK2	3.74bcd	5.9abcd
P. aeruginosa CQ	3.67bcd	4.4bcdef
P. aeruginosa 32Q	3.63bcd	5.1abcde
P. aeruginosa AQ	3.55bcd	5.0abcde
P. aeruginosa 135	3.53bcd	3.4cdef
P. aeruginosa 35Q	1.88cde	5.6abcde
P. aeruginosa 16Q	1.75ed	4.9abcde
P. aeruginosa 10A	1.04e	1.7f
P. aeruginosa 6Q	1.04e	3.0def
Control	0.00f	4.6abcde

[a]Inoculated control; [b]three pots, three leaves per pot; [c]three pots, three plants per pot.
Values followed by the same letter are not significantly different at 5%, as determined by analysis of variance followed by Duncan's test.

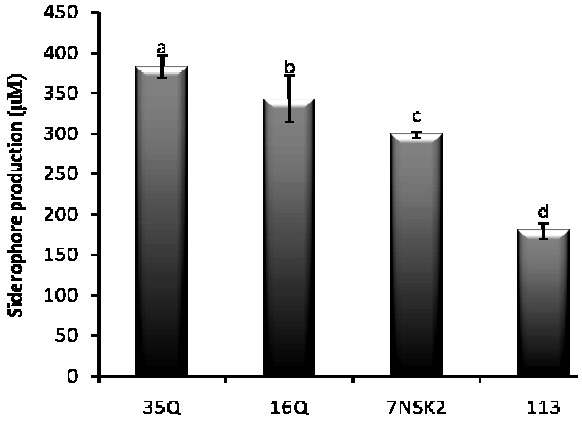

Fig. 3. Siderophore production by several isolates under low iron conditions in succinate medium. All values were significantly different at 5%, as determined by variance analysis followed by Duncan's test. Bars indicate standard error.

tions of 8HQ (Fig. 2A). No reduction in numbers of CFUs was observed with either strain on KB media with increasing concentrations of 8HQ supplemented with equimolar Fe(III) (Fig. 2B).

Siderophore production by selected strains. In this experiment siderophore production of strains 35Q and 16Q (the fastest growing strains on 8-HQ+KB), was investigated. Since 7NSK2 is a powerful producer of pseudobactin, this strain was used as reference. Siderophore production by both 35Q and 16Q was significantly higher than that of 7NSK2 (Fig. 3). The final concentration of siderophore produced by 35Q reached a maximum of 381.7 µM which is considerably more than that of reference strain 7NSK2 in this study and in reports on other *Pseudomonas* strains (Djavaheri, 2007; Diaz de Villegas *et al*., 2002). Siderophore production by strain 113 was lower than with other strains. This low concentration was expected because it was not able to grow on KB medium with high concentrations of 8-HQ (e.g. 120 ppm).

ISR of 35Q under low iron availability. Strain 35Q showed clear growth promotion activity in this assay, especially under low iron (Fig. 4). There were no significant differences between the disease resistance of control plants grown with or without excess Fe (Fig. 4A). Plants treated with 35Q showed significantly more disease resistance in both conditions, but disease resistance of plants with low iron availability was significantly higher than those of plants with Fe excess. Furthermore, dry and fresh weight of plants treated with 35Q with iron deficiency, was higher than those of plants with Fe excess but in the case of plants without rhizobacterium, presence of iron increased the fresh and dry weight of plants (Fig. 4A). At 48 h after inoculation of plants with *Xcm*, PO activity of plants treated with 35Q with iron deficiency was higher than those of plants with iron excess (Fig. 5), when it was 6.5 and 26.76 units higher than the plants treated with 35Q and nonbacterized plants with Fe excess, respectively. Increase of PAL activity in bacterized plants with iron deficiency was significantly higher than those of plants with iron excess.

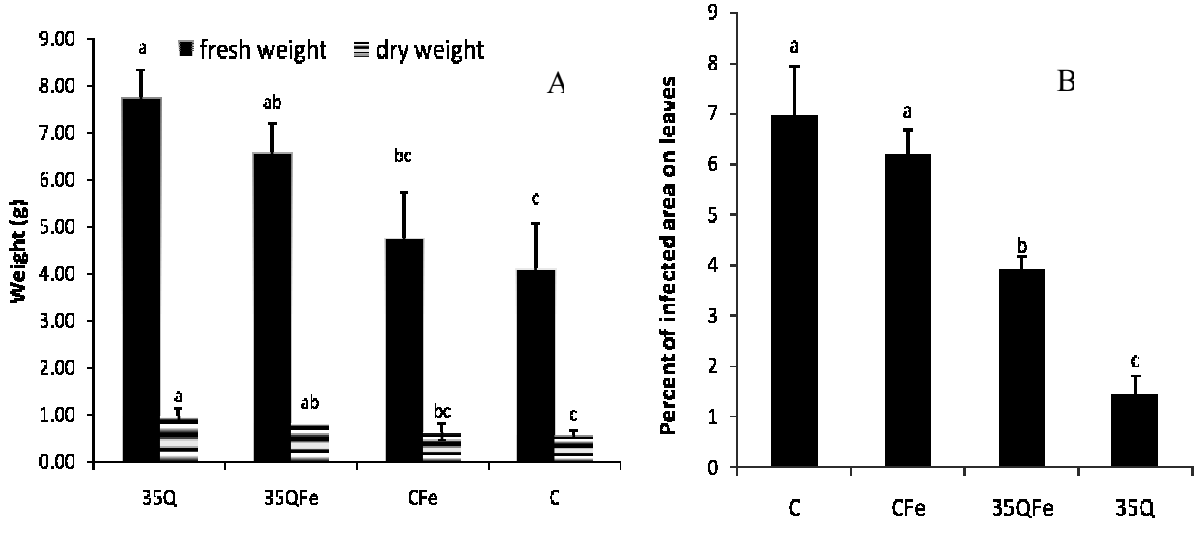

Fig. 4. (A) Effect of 35Q on fresh and dry weight of plants with (35QFe) or without excess iron (35Q). (B) Disease suppression by 35Q under conditions of iron deficiency and iron excess. C: infected control plants with iron deficiency, CFe: plants with excess iron. Values followed by the same letter were not significantly different at 5%, as determined by variance analysis followed by Duncan's test. Bars indicate standard error.

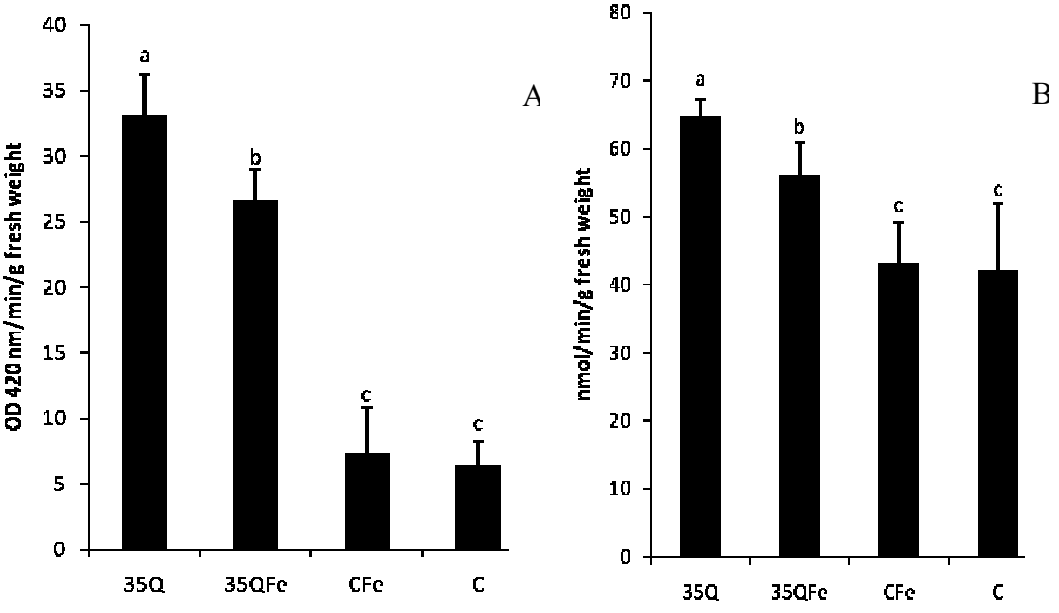

Fig. 5. (A) PO (A) and PAL (B) activity in leaves of plants treated with 35Q with (35QFe) or without excess iron (35Q) 48 h after inoculation of pathogen. C: infected control plants with iron deficiency, CFe: plants with excess iron. Values followed by the same letter were not significantly different at 5%, as determined by variance analysis followed by Duncan's test. Bars indicate standard error.

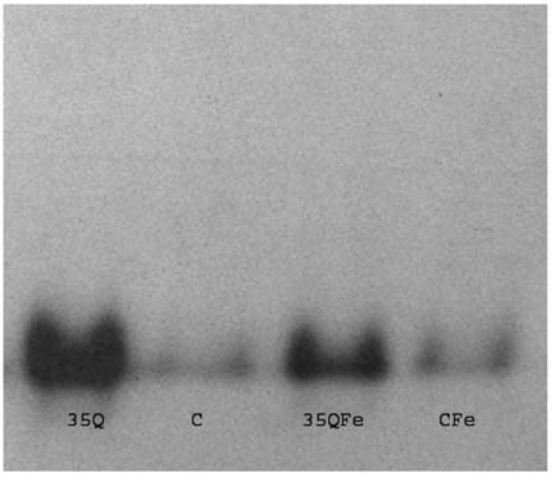

Fig. 6. Native 12% polyacrylamide gel electrophoresis analysis for PO isozyme profile induced by 35Q in leaves of plants treated with 35Q with (35QFe) or without excess iron (35Q) 48 h after inoculation of pathogen. C: infected control plants with iron deficiency, CFe: plants with excess iron.

DISCUSSION

In recent years the concept of induced resistance has attracted much attention as a possible strategy in integrated plant disease management (Bakker *et al.*, 2007). Since the siderophores produced by fluorescent pseudomonads are important metabolites for induction of systemic resistance, in this study we used KB medium with very low iron availability to screen isolates for there potential to produce high levels of siderophore. To modify KB medium and lower its iron availability, the medium was supplemented with 120 ppm 8-HQ. Commercial 8-HQ is as an antiseptic, disinfectant, and pesticide (Phillips, 1956), but in this study, its inhibiting action to 7NSK2 and 35Q was abolished by equimolar Fe(III) added to the 8-HQ+KB media (Fig. 1 and 2). Therefore it appears that inhibition property of this compound is mediated by iron chelation and not by toxicity of 8-HQ or its Fe(III) chelates. Use of this medium resulted in strains with a high ISR capability equal or greater than the reference strain 7NSK2. This strain is a well known ISR bacterium (De Meyer *et al.*, 1999; Audenaert *et al.*, 2002; De Vleesschauwer, 2006). Out of 19 strains, 35Q was selected because of its high growth promotion and ISR ability for other greenhouse studies to determine its possible mechanism in induction of plant resistance. The dose-response curve of this strain was almost similar to that of strain 7NSK2 but pseudobactin production by 35Q was 28.13% greater than that of 7NSK2.

Another strain that performed well in ISR was 16Q, but fresh and dry weight of plants treated with this strain were low. Its pseudobactin production was significantly lower than that of 35Q (Fig. 3), but its growth was not abolished at high concentrations of 8-HQ (Fig. 2A). It seems that the Fe(III) binding constant (log K) of its siderophore is higher than that of 35Q.

Strain 10A drastically decreased the percentage of infected area on leaves but it had a negative effect on the plant and reduced fresh weight. This strain had high in-

hibition activity against *Rhizoctonia solani* AG4 (data not shown). Perhaps it produces an antibiotic with a phytotoxic effect that reduces plant growth or maybe the level of antibiotic produced by this strain is too high. Although some antibiotics produced by rhizobacteria, such as 2,4-diacetylphloroglucinol (DAP) or phenazine-1-carboxylic acid (PCA) are important in disease suppression, they can be toxic to plants at high concentrations and could induce SAR in the same way as a pathogen causing localized necrosis (Maurhofer, 1995).

To study the possible role of siderophores in ISR by a selected strain (35Q), hydroponic experiments with cotton plants of defined nutrition were designed. In this assay, bacterized plants with low iron availability showed high levels of disease resistance (Fig. 4), and fresh weights from these treatments were significantly higher than those of other treatments. On the other hand, 48 h after inoculation of plants with *Xcm*, levels of defence-related enzymes in these plants were significantly higher than those of other treatments. These results suggest that the production of one or more iron-regulated metabolites increased under low-iron conditions, and could play an important role in induction of plant resistance by this strain. Since strain 35Q is a powerful pseudobactin producer, this metabolite is a strong candidate as a determinant of this strain for induction of plant resistance. The ability of this metabolite to induce plant resistance has been proved in many cases (Maurhofer *et al.*, 1994; Leeman *et al.*, 1996; Meziane *et al.*, 2005; Ran *et al.*, 2005). However ISR by 35Q was not completely abolished in the plants with iron excess, suggesting a possible role for another or other determinant/s.

REFERENCES

Akkopru A., Demir S., 2005. Biological control of *Fusarium* wilt in tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* by AMF *Glomus intraradices* and some rhizobacteria. *Journal of Phytopathology* **153**: 544-550.

Anand T., Chandrasekaran A., Raguchander T., Prakasam V., Samiyappan R., 2007. Chemical and biological treatments for enhancing resistance in chilli against *Colletotrichum capsici* and *Leveillula taurica*. *Archives of Phytopathology and Plant Protection* **1**: 1-19.

Åström B., 1990. Interactions between plants and deleterious rhizosphere bacteria. Importance of plant genotype and possible mechanisms involved. Dissertation, Swedish University of Agricultural Sciences, Uppsala, Sweden, ISSN 0348-3428.

Audenaert K., Pattery T., Cornelis P., Höfte M., 2002. Induction of systemic resistance to *Botrytis cinerea* in tomato by *Pseudomonas aeruginosa* 7NSK2: Role of salicylic acid, pyochelin, and pyocyanin. *Molecular Plant-Microbe Interactions* **15**: 1147-1156.

Bakker P.A.H.M., Pieterse C.M.J., Van Loon L.C., 2007. Induced systemic resistance by fluorescent *Pseudomonas* spp. *Phytopathology* **97**: 239-243.

Capper A.L., Higgins K.P., 1993. Application of *Pseudomonas fluorescens* isolates to wheat as potential biocontrol agents against take-all. *Plant Pathology* **42**: 560-567.

De Meyer G., Capieau K., Audenaert K., Buchala A., Métraux J.P., Höfte M., 1999. Nanogram amounts of salicylic acid produced by the rhizobacterium *Pseudomonas aeruginosa* 7NSK2 activate the systemic acquired resistance pathway in bean. *Molecular Plant-Microbe Interactions* **12**: 450-458.

Diaz de Villegas M.E., Villa P., Frías A., 2002. Evaluation of the siderophores production by *Pseudomonas aeruginosa* PSS. *Microbiologia* **4**: 112-117.

De Vleesschauwer D., Cornelis P., Höfte M., 2006. Redox-Active pyocyanin secreted by *Pseudomonas aeruginosa* 7NSK2 triggers systemic resistance to *Magnaporthe grisea* but enhances *Rhizoctonia solani* susceptibility in rice. *Molecular Plant-Microbe Interactions* **19**: 1406-1419.

Dickerson D.P., Pascholati S.F., Hagerman A.E., Butler L.G., Nicholson R.L., 1984. Phenylalanine ammonia-lyase and hydroxycinnamate: CoA ligase in maize mesocotyls inoculated with *Helminthosporium maydis* or *Helminthosporium carbonum*. *Physiological Plant Pathology* **25**:111-123.

Djavaheri M., 2007. Iron-regulated metabolites of plant growth-promoting *Pseudomonas fluorescens* WCS374: Their role in induced systemic resistance. PhD Thesis. Utrecht University, The Netherlands.

Gutterson N., 1990. Microbial fungicides: Recent approaches to elucidating the mechanisms. *Critical Reviews in Biotechnology* **10**: 69-91.

Hammerschmidt R., Nuckles E.M., Kuc J., 1982. Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. *Physiological Plant Pathology* **20**:73-82.

Hoagland D.R., Arnon D.I., 1938. The water culture method for growing plants without soil. *California Agricultural Experiment Station Bulletin* **347**: 36-39.

Höfte M., 1993. Classes of microbial siderophores. In: Barton L.L., Hemming B.C. (eds). Iron Chelation in Plants and Soil Microorganisms, pp 3-26. Academic Press, San Diego. CA, USA.

Kloepper J.W., Schroth M.N., 1981. Plant growth promoting rhizobacteria and plant growth under gnotobiotic conditions. *Phytopathology* **71**: 642-646.

Kloepper J.W., Leong J., Teintze M., Schroth M.N., 1980. *Pseudomonas* siderophores: A mechanism explaining disease-suppressive soils. *Current Microbiology* **4**:317-320.

Leeman M., Den Ouden F.M., Van Pelt J.A., Dirkx F.P.M., Steijl H., Bakker P.A.H.M., Schippers B., 1996. Iron availability affects induction of systemic resistance against *Fusarium* wilt of radish by *Pseudomonas fluorescens*. *Phytopathology* **86**: 149-155.

Maurhofer M., Keel C., Haas D., Defago G., 1995. Influence of plant species on disease suppression by *Pseudomonas fluorescens* strain CHA0 with enhanced antibiotic production. *Plant Pathology* **44**: 40–50.

Maurhofer M., Hase C., Meuwly P., Métraux J.P., 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.

Meyer J.M., Abdallah M.A., 1978. The fluorescent pigment of *Pseudomonas fluorescens:* Biosynthesis, purification and physicochemical properties. *Journal of Genetic Microbiology.* **118**: 125-129.

Meziane H., Van der Sluis I., Van Loon L.C., Höfte M., Bakker P.A.H.M., 2005. Determinants of *Pseudomonas putida* WCS358 involved in inducing systemic resistance in plants. *Molecular Plant Pathology* **6**: 177-185.

Neilands J.B., Leong S.A., 1986. Siderophores in relation to plant growth and disease. *Annual Review of Plant Physiology* **37**: 187-208.

Ongena M., Jourdan E., Schäfer M., Kech C., Budzikiewicz H., Luxen A., Thonart P., 2005. Isolation of an N-alkylated benzylamine derivative from *Pseudomonas putida* BTP1 as elicitor of induced systemic resistance in bean. *Molecular Plant-Microbe Interactions* **18**: 562-569.

Phillips J.P., 1956. The Reactions of 8-Quinolinol. *Chemical Reviews* **56**: 271-297.

Pieterse C.M.J., Van Wees S.C.M., Hoffland E., Van Pelt J.A., Van Loon L.C., 1996. Systemic resistance in Arabidopsis induced by biocontrol bacteria is independent of salicylic acid accumulation and pathogenesis-related gene expression. *Plant Cell* **8**: 1225-1237.

Ran L.X., Li Z.N., Wu G.J., Van Loon L.C., Bakker P.A.H.M., 2005. Induction of systemic resistance against bacterial wilt in *Eucalyptus urophylla* by fluorescent *Pseudomonas* spp. *European Journal of Plant Pathology* **113**: 59-70.

Sillen L.G., Martell A.E., 1971. Stability Constants of Metal Ion Complexes (Supplement). The Chemistry Society, London, UK.

Ton J., Pieterse C.M.J., Van Loon L.C., 1999. Identification of a locus in Arabidopsis controlling both the expression of rhizobacteria-mediated induced systemic resistance (ISR) and basal resistance against *Pseudomonas syringae* pv. *tomato*. *Molecular Plant-Microbe Interactions* **12**: 911-918.

Van Loon L.C., Bakker P.A.H.M, Pieterse C.M.J., 1998. Systemic resistance induced by rhizosphere bacteria. *Annual Review of Phytopathology* **36**: 453-483.

Weller D.M., 1988. Biological control of soil borne plant pathogens in the rhizosphere with bacteria. *Annual Review of Phytopathology* **26**: 379-407.

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