J. Biol. Control, 21(1): 97-104, 2007

Role of antagonistic bacteria in suppression of bakanae disease of rice caused by *Fusarium moniliforme* Sheld

M. NAGARAJ KUMAR, G. S. LAHA and C. S. REDDY
Department of Plant Pathology
Directorate of Rice Research (ICAR)
Rajendranagar, Hyderabad 500 030, Andhra Pradesh
E-mail: lahags@yahoo.co.in

ABSTRACT: **Nine selected bacteria were tested for their antagonistic activity against *Fusarium moniliforme*, causing bakanae disease of rice. The bacteria significantly suppressed the growth of the fungus in dual culture. The bacteria produced varying amounts of lytic enzymes (chitinase and b-1-3-glucanase), siderophores, salicylic acid and hydrogen cyanide. In greenhouse experiments, the antagonist B-44 was most effective in reducing the bakanae disease infected seedlings followed by PF-9 and PF-13.**

KEY WORDS: Antagonistic bacteria, Bakanae disease, *Fusarium moniliforme*, rice

INTRODUCTION

Bakanae disease caused by *Fusarium moniliforme* Sheld. {teleomorph: *Gibberella fujikuroi* (Swada) Ito} has become an important disease of rice in many rice growing areas (Singh and Sunder, 1997). Diseased seedlings are lanky, pale yellow in colour and taller than the healthy ones. Such seedlings die either before or after transplanting. Diseased plants have less tillers and their leaves dry out one after another, wilt and die within few weeks. Under favorable conditions the yield loss due to this disease may go up to 90 per cent (Singh and Sunder, 1997). The pathogen is seed borne as well as soil borne in nature. However, the seed borne infection is more important as soil borne inoculum is rapidly reduced with time (Ou, 1985). Host plant resistance offers an important method of management of the disease (Krishnaveni *et al.*, 2002). However, the most common method of managing the disease is seed treatment with fungicide. But, the excessive use of chemicals is not desirable and resistance of this pathogen to various fungicides has also been reported (Ogawa, 1988). There are many reports of success in biological control of plant pathogens using fungal and bacterial antagonists (Reddy *et al.*, 2005). Fluorescent pseudomonads have been successfully used for the control of *Fusarium moniliforme* (Rosales and Mew, 1997; Ahamed and Srivastava, 2002). The present study reports the efficacy of antagonistic bacteria (*Pseudomonas fluorescens* and *Bacillus subtilis*) against bakanae disease of rice.

MATERIALS AND METHODS

i. Organisms

The fungus *Fusarium moniliforme* was isolated from infected rice plant and maintained

on potato dextrose agar (PDA) slants at 4 °C. Antagonistic bacteria were isolated from different sources *viz.*, farmyard manure, rice seeds, rice phyllosphere and rice rhizosphere using Pseudomonas Agar Fluorescein (PAF) medium. Both fluorescent and non-fluorescent colonies were picked up and maintained in PAF slants at 4 °C.

ii. Dual culture

The antagonism of bacterial strains to *F. moniliforme* was tested *in vitro* by dual culture technique on PAF and PDA medium. Bacterial antagonists were streaked on both sides of the plate at 1 cm away from periphery. The plates were then co-inoculated at the centre with a 5 mm diameter plug of 7-day-old growth of *F. moniliforme*. Plates inoculated with fungus only served as control. Observations were recorded on the radial growth of the fungus after 7 days of incubation at 28 ± 2 °C.

Testing for production of lytic enzymes and antimicrobial compounds

iii. Chitinase

Bacterial antagonists were grown at room temperature (28 ± 2° C) for 96 h on a rotary shaker in 250 ml conical flasks containing 50 ml of chitin-peptone medium (glucose 0.5 %, peptone 0.2 %, colloidal chitin 0.2%, K_2HPO_4 0.1 %, MgSO4,7H2O 0.05 % and NaCl 0.05 %, pH 6.8) (Lim *et al.*, 1991). The cultures were centrifuged at 10,000 rpm for 10 min at 4° C and the supernatant was used as enzyme source. Colloidal chitin was prepared from chitin flakes according to Dhingra and Sinclair (1985). The reaction mixture contained 0.25 ml of enzyme solution, 0.3 ml of 1 M sodium acetate buffer (pH 5.3) and 0.5 ml of colloidal chitin (0.1 %). The reaction mixture was incubated at 50°C for 4 h in a water bath. Chitinase activity was determined by measuring the release of reducing sugars by the method of Nelson (1944). One unit of chitinase was determined as 1 nmol of GlcNAc released per minute per mg of protein. Protein content in all the samples was determined as described by Lowry *et al.* (1951) using bovine serum albumin as the standard.

iv. b-1-3-glucanase

The enzyme degrades b-1-3-glucan, which is one of the major constituents of fungal cell wall. Powder of dry mycelium of *F. moniliforme* was used as the source of b-1-3-glucan. Bacterial antagonists were grown at room temperature (28±2° C) for 96 h on a rotary shaker in 250 ml conical flasks containing 50 ml of peptone medium containing dry mycelial powder (0.2 %). The cultures were then centrifuged at 10,000 rpm for 10 min at 4°C and the supernatant was used as enzyme source. The reaction mixture contained 0.25 ml of enzyme solution, 0.3 ml of 0.1 M phosphate buffer (pH 5.5) and 0.5 ml of 0.2 % suspension of dry mycelial powder (Lim *et al.*, 1991). The reaction mixture was incubated at 40°C for 2 h in a water bath. b-1,3-glucanase activity was determined as 1 nmol of glucose released per minute per mg of protein. Protein content in all the samples was determined as described by Lowry *et al.* (1951) using bovine serum albumin as the standard.

v Salicylic Acid

Bacterial antagonists were grown at room temperature (28 ± 2 °C) for 48 h on a rotary shaker in 250 ml conical flasks containing 50 ml of the King's B broth medium. Cells were then collected by centrifugation at 10,000 rpm for 10 min and 4 ml of cell free culture filtrate was acidified with 1 N HCl to pH 2.0 and SA was extracted in chloroform (2x2 ml). To the pooled chloroform extracts, 4 ml of distilled water and 5 ml of 2 M $FeCl_3$ were added. The absorbance of the purple iron-SA complex, which was developed in the aqueous phase, was read at 527 nm in a Spectrophotometer. A standard curve was prepared with SA dissolved in King's B broth medium. The quantity of SA in the culture filtrate was expressed as mg ml^{-1} (Meyer *et al.*, 1992).

vi. Siderophore

Bacterial antagonists were grown in King's B broth for 3 days at room temperature (28 ± 2 °C) and centrifuged at 10,000 rpm for 10 min and the supernatant was collected. The pH of the supernatant was adjusted to 2.0 with 1N HCl and equal quantity of ethyl acetate was added in a

separating funnel, mixed well and ethyl acetate fraction was collected. Five ml of ethyl acetate fraction was mixed with 5 ml of Hathway's reagent (The reagent was prepared by adding 1 ml of 0.1 M ferric chloride in 0.1 N HCl to 100 ml of distilled water, and to this 1 ml of 0.1 M potassium ferricyanide was added). The absorbance for dihydroxy phenols was read at 700 nm in a Spectrophotometer (Reeves *et al.*, 1983). A standard curve was prepared using dihydroxy benzoic acid. The quantity of siderophore synthesized was expressed as m mol benzoic acid ml^{-1} of culture filtrate.

vii. Hydrogen cyanide

Production of cyanide as one of the volatile metabolites by the bacterial antagonists was tested using the method of Lorck (1948). Bacteria were inoculated on PAF or PAF medium supplemented with glycine (4.4 g/litre). Sterilized filter paper strips soaked with freshly prepared picric acid solution (2.5 g picric acid, 12.5 g sodium carbonate and 1000 ml sterile distilled water) were carefully placed in the upper lids of the Petri-plates. The plates were then sealed with parafilm and incubated at 28 °C for 48-72 hours. A change of colour from yellow to light brown, brown or reddish brown was recorded as weak, moderate or strong HCN production, respectively. The effect of volatile secondary metabolites produced by bacterial antagonists on the growth of *F. moniliforme* was studied by paired Petri-plate technique. Bacteria were inoculated on PAF or PAF supplemented with glycine (4.4 g/litre). The fungus was inoculated on PDA. Each PDA plate with the fungus was paired with a Petri-dish containing the bacteria (lower) and sealed with parafilm. Plates inoculated with fungus only, paired with un-inoculated PAF or PAF with glycine served as control. The diameter of the fungal growth was measured after 7 days of incubation at 28 °C.

viii. Glasshouse evaluation

For glasshouse evaluation of antagonistic bacteria against bakanae disease, the fungus *F. moniliforme* was mass multiplied in sterilized rice husk: oat mixture (1:3) for 15 days (Sunder and Satyavir, 1998). The inoculum was then mixed with the soil (25g/pot) one day before sowing. The bacterial antagonists were multiplied on PAF medium. Seed bacterization was carried out by soaking the seeds (cultivar-TN1) with bacterial cells suspended in one percent (W/V) carboxy methyl cellulose (Na- salt) for 4 hours. The seeds were then air dried on laboratory benches overnight. Seeds without bacteria served as control. Seeds were sown @ 15 seeds/pot and three replications were kept for each treatment. Plants were also grown on pots where no fungal inoculum was added. To compare the efficacy of antagonistic bacteria with fungicide, seeds were also treated with fungicide carbendazim @ 2 g/ kg of seeds. Pots were watered daily. Observations were taken 45 days after sowing by recording the number of infected seedlings.

RESULTS AND DISCUSSIONS

Out of one hundred and thirteen different bacteria isolated from four different sources, *viz.* farmyard manure, rice seeds, rice rhizosphere and rice phyllosphere, nine bacteria were selected for their antagonistic activity against *F. moniliforme*. The selected bacterial isolates PF-2, PF-6, PF-7, PF-9 and B-7 were isolated from farmyard manure, PF-13 and PF-C-1 from rice rhizosphere and B-44 and B-45 were from rice phyllosphere. Out of these, six were fluorescent pseudomonads (PF-2, PF-6, PF-7, PF-9, PF-13 and PF-C-1). The fluorescent pseudomonads were identified according to Stolp and Gadkari (1981). All the fluorescent bacterial antagonists were gram negative, rod shaped and all produced yellowish green pigment on King's B medium (King *et al.*, 1954). All were gelatin liquefiers and oxidase- and arginine dihydrolase positive and were identified as *Pseudomonas fluorescens*. The bacilli (B-7, B-44 and B-45) were identified according to Schaad (1988). All the bacilli were gram positive, produced spores (central in position), grew at 45°C, pH 5.7 and 7 percent Sodium chloride concentration. They utilized citrate, produced acid from arabinose, xylose and mannitol and hydrolysed starch. All of them were positive for Voges Proskauer test and negative for anaerobic growth in glucose broth. All the bacilli were identified as *Bacillus subtilis*. There was a wide variation among the bacteria in their

Table 1. *In vitro* inhibition of growth of *F. moniliforme* by antagonistic bacteria

Bacterial strains	Inhibition of radial growth (%)	
	PAF	PDA
Pseudomonas fluorescens -PF-2	72.6 (58.5)[a]	52.1 (46.2)[ab]
Pseudomonas fluorescens -PF-6	62.4 (52.2)[b]	19.8 (26.1)[c]
Pseudomonas fluorescens -PF-7	53.8 (47.2)[c]	27.2 (30.8)[c]
Pseudomonas fluorescens -PF-9	61.6 (51.7)[b]	48.6 (44.2)[ab]
Pseudomonas fluorescens -PF-13	63.2 (52.7)[b]	49.7 (44.8)[ab]
Pseudomonas fluorescens -PF-C-1	46.9 (43.3)[d]	32.2 (34.2)[bc]
Bacillus subtilis-B-45	48.3 (44.0)[d]	51.9 (46.1)[ab]
Bacillus subtilis-B-44	57.7 (49.4)[bc]	58.4 (49.9)[a]
Bacillus subtilis-B-7	59.4 (50.4)[bc]	58.4 (49.8)[a]

* Figures in the parentheses indicate the arc-sine transformed values.

** Figures in a column followed by the same letters are not significant by DMRT at 5 % level of significance.

ability to inhibit the growth of *F. moniliforme*. For fluorescent pseudomonads, the growth inhibition was, in general, more in PAF medium than in PDA medium (Table 1). This may be due to the fact that, PAF is comparatively rich and preferred medium for fluorescent pseudomonads and thus enhances the production of secondary metabolites by fluorescent pseudomonads. Whereas the fungal growth inhibition by bacilli was more or less equal in both PAF and PDA. Among the *Pseudomonas fluorescens* isolates, PF-2 was most effective, followed by PF-13 and PF-9 in reducing the growth of *F. moniliforme*. Among the *Bacillus subtilis* isolates, B-7 (59.4% in PAF and 58.4% in PDA) and B-44 (57.7% in PAF and 58.4% in PDA) were most effective in reducing the growth of *F. moniliforme* (Table 1).

In the experiments on lytic enzymes and antimicrobial compounds involved in suppression of fungal growth by antagonistic bacteria, we studied the production of lytic enzymes *viz.*, chitinase and b-1-3-glucanase, siderophore (microbial iron transport agent), salicylic acid and hydrogen cyanide. Among the nine antagonistic bacteria studied, PF-2 was found to produce maximum amount of chitinase and b-1-3-glucanase (Table 2). The isolate PF-2 was also most effective in suppressing the mycelial growth of *F. moniliforme* in PAF medium. High glucanase activity was also recorded in PF-6, B-44 and B-7. Production of chitinase and b-1-3-glucanase have been found involved in the suppression of *Fusarium solani* by *Pseudomonas stutzeri* (Lim *et al.*, 1991) and *Rhizoctonia solani* by *Pseudomonas fluorescens* (Velazhahan *et al.*, 1999). Many reports suggest the production of siderophore as one of the mechanisms of disease suppression by fluorescent pseudomonads (Leong, 1986). All the bacteria in our study produced siderophore (Table 2). However, the amount of siderophore produced varied among the isolates. Production of salicylic acid by antagonistic bacteria has been reported to be involved in the induction of plant defense response and disease suppression (Whipps, 2001). The isolate PF-7 produced maximum amount of salicylic acid. However, no correlation was found between

Table 2. Production of secondary metabolites by bacterial strains antagonistic to *F. moniliforme*

Bacterial strains	Chitinase Activity (nmol / min/ mg protein)	Glucanase Activity (nmol / min/ mg protein)	Siderophore Production (μ mol benzoic acid/ml)	Salicylic Acid (SA) Production (mg / ml)	HCN Production**	
					PAF	PAF + Glycine
Pseudomonas fluorescens -PF-2	2[a]	3.47[a]	0.09[e]	4.51[bc]	++	+++
Pseudomonas fluorescens -PF-6	0.39[bc]	1.97[b]	8.72[b]	4.13[bc]	++	+++
Pseudomonas fluorescens -PF-7	0.61[b]	2.3[ab]	6.55[bc]	14.25[a]	+	+
Pseudomonas fluorescens -PF-9	0.39[bc]	0.03[c]	2.65[cde]	2.45[cd]	+	+
Pseudomonas fluorescens -PF-13	0.61[b]	0.04[c]	5.15[bcd]	2.07[cd]	++	+++
Pseudomonas fluorescens -PF-C-1	0.28[bc]	0.06[c]	0.66[de]	5.9[b]	+	+
Bacillus subtilis-B-45	0.39[bc]	0.08[c]	5.3[bcd]	2.12[cd]	+	+
Bacillus subtilis-B-44	0.13[c]	2.33[ab]	19.76[a]	3.25[bcd]	+	+
Bacillus subtilis-B-7	0.66[b]	2.23[b]	8.19[b]	1.08[d]	+	+

* Figures in a column followed by the same letters are not significantly different from each other by DMRT at 5% level of significance.

** The intensity of developed brown colour due to HCN production was indicated by +, ++, +++, where + indicates light brown, ++ indicates brown, +++ indicates reddish brown, respectively.

disease suppression and high amount of salicylic acid production by isolate PF-7.

The production of volatile cyanide is very common among the rhizosphere microorganisms especially fluorescent pseudomonads (Bakker and Schippers, 1987; Dowling and O'Gara, 1994). Among the antagonistic bacteria tested, PF-2, PF-6 and PF-13 were found to be moderately cyanogenic while others were weak in cyanide production (Table 2). The production of volatile cyanide was more when the PAF medium was supplemented with glycine (4.4 g/litre). This may be due to the fact that glycine is the direct precursor of microbial cyanide production (Askeland and Morrison, 1983). The effect of volatile metabolites on the radial growth of *F. moniliforme* was done by growing the bacteria in two medium *viz.*, PAF and PAF supplemented with glycine. The growth suppression was more pronounced when the antagonists were grown on glycine supplemented medium (Table 3). This indicates that pronounced growth suppression on glycine amended PAF medium was probably due to greater production of cyanide. Volatile metabolites and hydrogen cyanide have been reported to play an important role in the biocontrol of plant diseases by antagonistic bacteria (Dowling and O'Gara, 1994). Voisard *et al.* (1989) reported that cyanide production by *Pseudomonas fluorescens* strain CHA0 played an important role in the suppression of black root-rot of tobacco caused by *Thielaviopsis basicola*.

Under greenhouse condition all the bacteria except PF-7 significantly reduced the percentage of bakanae-infected seedlings. The bacterium B-44 (*Bacillus subtilis)* was most effective in reducing the disease incidence (Table 4). Among the *Pseudomonas fluorescens* isolates, PF-9 was most effective followed by PF-13 and PF-6 (Table 4). The data also revealed that many antagonistic bacteria were superior to the check fungicide carbendazim. The present data reveal that the antagonistic bacteria tested could successfully reduce the bakanae disease. Suppression of plant diseases by antagonistic bacteria has been reported by many workers (Reddy *et al.*, 2005). Our study demonstrates that *Bacillus subtilis* isolate B-44 and

Table 3. Inhibition of fungal growth by the volatiles produced by the antagonistic bacteria

Bacterial strains	Percent Inhibtion of fungal growth by volatiles	
	PDA+PAF	PDA+PAF-G
Pseudomonas fluorescens -PF-2	22.6 (28.3)^{a}	39.6 (38.8)^{a}
Pseudomonas fluorescens -PF-6	6.8 (14.1)^{bc}	22.8 (28.5)^{bc}
Pseudomonas fluorescens -PF7	6.3 (11.2)^{c}	2.1 (13.3)^{c}
Pseudomonas fluorescens -PF-9	16.1 (23.6)^{ab}	46.5 (43.0)^{a}
Pseudomonas fluorescens -PF-13	1.8 (11.5)^{c}	25.9 (30.6)^{b}
Pseudomonas fluorescens -PF-C-1	2.7 (15.4)^{bc}	0.0 (0.0)^{f}
Bacillus subtilis-B-45	15.1 (22.7)^{ab}	10.1 (18.5)^{de}
Bacillus subtilis-B-44	12.2 (20.3)^{abc}	15.9 (23.5)^{cd}
Bacillus subtilis-B-7	12.2 (20.2)^{abc}	25.3 (30.1)^{b}

* Figures in the parentheses indicate transformed values

* Figures in a column followed by the same letters are not significantly different from each other by DMRT at 5% level of significance.

Table 4. Effect of seed bacterization with antagonistic bacteria on the reduction of bakanae infected seedlings of rice in glass house

Bacterial strains	Percent reduction of bakanae disease
Pseudomonas fluorescens -PF-2	46.9 (42.2)^{abc}
Pseudomonas fluorescens -PF-6	54.5 (47.6)^{abc}
Pseudomonas fluorescens -PF-7	0.8 (3.1)^{d}
Pseudomonas fluorescens -PF-9	76.3 (65.9)^{ab}
Pseudomonas fluorescens -PF-13	64.7 (53.7)^{abc}
Pseudomonas fluorescens -PF-C-1	33.3 (34.9)^{bcd}
Bacillus subtilis-B-45	33.9 (35.6)^{bcd}
Bacillus subtilis-B-44	86.7 (76.9)^{a}
Bacillus subtilis-B-7	18.9 (25.5)^{cd}
Carbendazim (2 g/Kg)	32.9 (33.6)^{bcd}

* Figures in the parentheses indicate transformed values

** Figures in a column followed by the same letters are not significantly different from each other by DMRT at 5% level of significance

Pseudomonas fluorescens isolates like PF-9, PF-13 and PF-6 can be considered as an important input in the integrated management of the bakanae disease of rice.

REFERENCES

Ahamed, S. and Srivastava, M. 2002. Evaluation of fungal and bacterial antagonists against *Fusarium moniliforme* causing foot rot disease of rice. *Annals of Agricultural Research*, **23**: 319-321.

Askeland, R. A. and Morrison, S. M. 1983. Cyanide production by *Pseudomonas fluorescens* and *Pseudomonas aeruginosa*. *Applied and Environmental Microbiology*, **45**: 1802-1807.

Bakker, A. W. and Schippers, B. 1987. Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomonas* spp. mediated plant growth stimulation. *Soil Biology and Biochemistry*, **19**: 249-256.

Dhingra, O. D. and Sinclair, J. B. 1985. *Basic Plant Pathology Methods*. CRC Press, Boca Raton, Florida, USA. 355 pp.

Dowling, D. N. and O'Gara, F. 1994. Metabolites of *Pseudomonas* involved in the biocontrol of plant disease. *TIBTECH*, **12**: 133-141.

King, E. O., Ward, M. K. and Raney, D. E. 1954. Two simple media for the demonstration of pyocyanin and fluorescein. *Journal of Laboratory and Clinical Medicine*, **44**: 301-307.

Krishnaveni, D., Laha, G. S. and Muralidharan, K. 2002. Evaluation of rice cultivars against bakanae disease. pp. 113-115. In: B. Sarath Babu, K. S. Varaprasad, K. Anitha, R. D. V. J. Prasad Rao, S. K. Chakravarty and P. S. Chandurkar (Eds.), *Resources Management in Plant Protection*, Volume II. Plant Protection Association of India, Hyderabad, India.

Leong, J. 1986. Siderophores: their biochemistry and possible role in the biocontrol of plant pathogens. *Annual Review of Phytopathology*, **24**: 187-209.

Lim, H., Kim, Y. and Kim, S. 1991. *Pseudomonas stutzeri* YLP-1 genetic transformation and antifungal mechanisms against *Fusarium solani*, an agent of plant root-rot. *Applied and Environmental Microbiology*, **57**: 510-516.

Lorek, H. 1948. Production of hydrocyanic acid by bacteria. *Physiologia Plantarum*, **1**: 142-146.

Lowry, O. H., Rosebrough, N. J., Fan, A. L. and Randall, R. J. 1951. Protein measurement with Folin-phenol reagent. *Journal of Biological Chemistry*, **193**: 265-275.

Meyer, J. M., Azelvandre, P. and Georges, C. 1992. Iron metabolism in *Pseudomonas*: Salicylic acid, a siderophore of *Pseudomonas fluorescens* CHA0. *Biofactors*, **4**: 23-27.

Nelson, N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. *Journal of Biological Chemistry*, **152**: 375-380.

Ogawa, K. 1988. Damage by "Bakanae" disease and its control. *Japan Pesticide Information*, **52**: 13-15.

Ou, S. H. 1985. *Rice Diseases* (second edition). Commonwealth Mycological Institute, UK. 380pp.

Reddy, C. S., Laha, G. S. and Muralidharan, K. 2005. Interactions between rhizophere microorganisms and plant pathogens, pp. 163-218. In: K. G. Mukherji, K. V. B. R. Tilak, S. M. Reddy, L. V. Gangwane, P. Prakash, and I. K. Kunwar (Eds), *Frontiers in Plant Sciences*. I. K. International Pvt. Ltd., New Delhi.

Reeves, M., Pine, L., Neilands, J. B. and Bullows, A. 1983. Absence of siderophore activity in *Legionella* sp. grown in iron deficient media. *Journal of Bacteriology*, **154**: 324-329.

Rosales, A. M. and Mew, T. W. 1997. Suppression of *Fusarium moniliforme* in rice by rice-associated antagonistic bacteria. *Plant Disease*, **81**: 49-52.

Schaad, N. 1988. *Laboratory guide for the identification of plant pathogenic bacteria (second edition)*. American Phytopathological Society, Minnesota, USA. 158pp.

Singh, R. and Sunder, S. 1997. Foot rot and bakanae of rice: retrospects and prospects. *International Journal of Tropical Plant Diseases*, **15**: 153-176.

Stolp, H. and Gadkari, D. 1981. Non-pathogenic members of the genus *Pseudomonas*, pp.719-741. In: M. P. Starr, H. Stolp, H. G. Truper, A. Balows and H. G. Schlegel (Eds), *The Prokaryotes: A Handbook on Habits, Isolation and Identification of Bacteria*. Vol.1, Springer-Verlag, Berlin.

Sunder, S. and Satyavir. 1998. Vegetative compatibility, biosynthesis of GA and virulence of *Fusarium moniliforme* isolates from bakanae disease of rice. *Plant Pathology*, **47**: 767-772.

Velazhahan, R. R., Samiyappan, P. and Vidhyasekaran, P. 1999. Relationship between antagonistic activities of *Pseudomonas fluorescens* strains against *Rhizoctonia solani* and their production of lytic enzymes. *Journal of Plant Disease and Protection*, **106**: 244-250.

Voisard, C., Keel, C., Hass, G. and Defago, G. 1989. Cyanide production by *Pseudomonas fluorescens* suppress black root rot of tobacco under gnotobiotic conditions. *EMBO* Journal, **8**: 351-358.

Whipps, J. M. 2001. Microbial interactions and biocontrol in the rhizosphere. *Journal of Experimental Botany*, **52**: 487-511.

(Received: 30.01.2006; Revised: 04.12.2006; Accepted: 15.12.2006)