J. Mycopathol. Res. **42**(2) : 177-180 (2004)

Characterisation of rhizobacteria antagonistic to bacterial blight of rice from rhizosphere of basmati rice

CHATURBHUJ MEENA AND JAYASHREE GOPALAKRISHNAN
Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi 110012

Two rhizobacteria, RRb-11 (Delhi isolate) and RRb-103 (Almora isolate) were isolated from rhizosphere of basmati rice. Both were found to be antagonistic to *Xanthomonas oryzae* pv. *oryzae (Xoo).* The isolates were characterized biochemically and the isolate from Almora was tentatively identified as non-fluorescent *Pseudomonas* while Delhi isolate was identified to be fluorescent *Pseudomonas.* RRb-11 produced both siderophore and hydrocyanic acid unlike RRb-103, which only produced siderophore. RRb-11 showed better antagonistic activity than RRb-103 and has been identified as potential biocontrol agent.

Key words : rhizobacteria, rice, Siderophores, *Xanthomonas*

INTRODUCTION

Pusa Basmati 1 is an export quality basmati rice recommended for growing in the Northern plains of India. However, like other basmati rice, Pusa Basmati 1 is also highly susceptible to bacterial blight of rice caused by *Xanthomonas oryzae* pv. *oryzae (Xoo).* Screening of several isolates of *Xoo* from Northern parts of India showed that the Kaul isolate (from Kaul in Haryana) is the most virulent against all basmati rice varieties tested *ie.* Pusa Basmati I, Pusa Sughand 2 and Pusa Sughand 3. Chemical and biological control are essential for combating destructive bacterial diseases. The most oftenly used chemicals for bacterial diseases are antibiotics like streptomycin and oxytetracycline. Antibiotics remain active on plant surface only for short periods. Hence they may require several applications during the growing season. Bacteria often become resistant to the antibiotics and cause pollution. It is therefore, important to, use alternative management strategies such as biological control. Though Plant Growth Promoting Rhizobacteria (PGPR) antagonistic to pathogens of rice have been reported from other parts of India a need was perceived for isolating a strain from the basmati rice growing area of North (where the current variety is grown) and such a strain would be more competitive and probably a better colonizer. With this is mind, the present investigation were initiated to isolate bacteria antgonistic to *Xoo* from the rhizosphere of basmati rice and evaluate them for their plant growth promoting effects and identify those which could provide effective biocontrol.

MATERIALS AND METHODS

Isolation of rhizobacteria

The rhizobacteria were isolated using dilution plate method (Mondal, 1998) and christened as RRb1 and RRb2 and so on.

In vitro antagonism studies

In vitro studies were made by dual culture technique using each of RRb's against the Kaul isolate *Xoo*. The diameter of zone of inhibition indicated the degree of antagonism.

Morphological character and gram reaction

Morphological character, flagellation as well as gram reaction were studied following Laboratory Manual of Plant Bacteriology (Anonymous, 1995).

Colony character and fluorescence

Colony characters were studied on nutrient sucrose agar (Sucrose-5 g ; Yeast extract-4 g ; Peptone-4 g ; Beef extract-2 g ; Agar-20 g ; Distilled water-1000 ml) plates. For fluorescence studies King's B and King's A medium were prepared according to King *et al.* (1954).

Biochemical characters

Gelatin liquefaction : Nutrient agar medium with 0.4% gelatin (w/v) was poured into sterile petriplates and was allowed to solidify. The plates were spot inoculated separately with the test bacterium and three replicates were kept for each isolate. The plate were incubated at 20°C for 3 days, after which the plate surface was flooded with 5-10 ml of acidified mercuric chloride solution (Hg Cl2, 12 g ; conc. HCl, 16 ml ; distilled water, 80 ml) : positive reaction for gelatin hydrolysis was indicated by clearing zone surrounding the bacterial growth.

Catalase production : A loopful bacterial culture from a 24 hr slant was taken and placed into a drop of 20-volume hydrogen peroxide on a clean glass plate. Production of bubbles indicate a positive result.

H_2S gas production : Production of Hydrogen Sulphide gas was tested by filter paper (Whatman no. 42) strip impregnated with lead acetate solution. The strip was dried and autoclaved. The autoclaved strip was then kept inside the slants inoculated with each isolate. One end of the strip was held by the cotton plug while other end was free and hanging. The inoculated slants along with strip were kept for incubation at 28°C for 3 days.

Levan production : To test the levan production NSA medium containing 5 % sucrose was used. The medium was sterilized by steaming for three successive days for 30 minutes and poured into petriplates. The plates were streaked with dilute suspension of the culture and incubated at 28°C for 3 days. Production of large, white and mucoid colonies indicated the levan production.

Arginine dihydrolase test : 5 ml of Thornley's medium 2A (Thornley, 1960) was dispensed in test tube and 24 hr old culture was stab inoculated. The medium was covered with sterile paraffin oil and incubated at 28°C for 72 hr. Three replications were kept for each isolate. A change of color from pale pink to deep red indicate positive activity.

Oxidase test : A filter paper saturated with 1 % freshly prepared tetramethyl-para-phenylenediamino-dihydrocholoride was streaked with 24 hr old culture. For positive reaction a red or purple colouration is expected within 10 seconds.

RESULTS AND DISCUSSION

The bacterium exhibited typical *Pseudomonas* morphology of being rod-shaped and measuring 0.6 × 1-1.2 mm. It had a polar flagella and the bacterium was gram-negative. The colonies were creamy white in color, smooth, circular and raised, measuring 1-3 mm in diameter in the case of RRb-11, while the colonies were irregular white in color and 2-3 mm diameter in case of RRb-103. Both the isolates, when characterized biochemically, were found to be catalase positive, utilized citrate, hydrolyzed arginine and produced H_2S. RRb-11 was flourescent and produced pigments, but not RRb-103. Both the isolates were found negative for levan production. The isolate RRb-103 (from Almora) was unable to produce yellowish green diffusible pigment on King's B or bluish green diffusible pigment on King's A medium and was unable to liquefy gelatin. Therefore, it was tentatively identified as non-fluorescent *Pseudomonas*. On the other hand, RRb-11 produced yellowish green diffusible fluorescent pigment of King's B medium but not blue green diffusible pigments on King's A medium suggesting that this isolate did not belong to *Pseudomonas aeruginosa,* which is only able to produce blue green diffusible pigments on King's A medium. RRb-11 was found to be gelatin liquefier and was tentatively identified

as *Pseudomonas fluorescens.*

Table 1 : Antagonistic properties of two rhizobacteria

Rice rhizobacteria	Replications					Mean*
	R1	R2	R3	R4	R5	
RRb-11 (Delhi)	19	25	17	19	20	20
RRb-103 (Almora)	13	15	12	16	14	14

* Average of 5 plate

Table 2 : Morphological, cultural and biochemical character of RRb's (Delhi and Almora) isolates

Character	Morphological, cultural and boichemical characters	
	RRb-11 (Delhi isolate)	RRb-103 (Almora isolate)
I Morphology		
Shape	Rod	Rod
Gram reaction	Gram negative	Gram negative
II Cultural		
Colony	Circular, 1-3 mm in dia. Shiny slightly raised, creamy white	Irregular 2-3 mm in dia. White in Color
Growth in single stroke inoculation of NA plate	Echinulated	Echinulated
Yellowish green diffusible pigment of King's B	+	–
Bluish green diffusible on King's A medium	–	–
Oxygen requirement	Aerobic	Aerobic
III Biochemical		
Gelatin liquefaction	+	–
Arginine hydrolyses	+	+
Oxidase test	+	+
H_2S production	+	+
Levan production	–	–
Citrate utilization	+	+
Catalase production	+	+
HCN production	+	–
Siderophore	+ (15 mm zone)	+ (5 mm zone)

\+ : Positive test

– : Negative test

The mechanism of biocontrol by rhizosphere microorganism has often been correlated with production of antibiotic, HCN and siderophores etc (Jagadeesh and Kulkarni, 2003). Appearance of an orange coloured zone after 48 hr of incubation on chrome azurols–(CAS) agar indicated that the isolated strains of *Pseudomonas* were able to chelate Fe^{3+} from CAS medium. RRb-11 and RRb-103 produced 15 mm and 5 mm zone respectively on CAS agar (Table 2). Similar observation for siderophore production has been demonstrated by several workers (Schwyn and Nieland, 1987 ; Mondal 1998 ; Gupta *et al.*, 2002 ; Jagadeesh and Kulkarni, 2003 ; Kavitha *et al.,* 2003). In the case of HCN test a change in colour of filter paper from yellow to reddish brown confirmed production of strong HCN in RRb-11 (Table 2) but not in RRb-103. HCN production from *Pseudomonas* has been reported by Bakker and Schippers (1998) ; Mondal (1998) ; Anith *et al.,* (1999) and Kavitha *et al.*, (2003). RRb-11 was found to be more antagonistic to *Xoo* than RRb-103 (Table 1) and this biocontrol activity may be due to the HCN production. The above studies have been identified as RRb-11 as a potential biocontrol agent for bacterial blight of rice and the bacterium has been characterized to be fluorescent *Pseudomonas.*

ACKNOWLEDGEMENT

The first author acknowledges the J. R. F. of U.G.C. for these studies. The authors also sincerely thank Head, Division of Plant Pathology, IARI for guidance and support.

REFERENCES

Anonymous (1995). *Laboratory manual of Plant Bacteriology,* Division of Plant Pathology, IARI, New Delhi.

Anith, K. N. ; Tilak, K. B. V. R. and Manmohan, T. P. (1999). Analysis of mutation affecting antifungal properties of fluorescent *Pseudomonas* sp. during the cotton *Rhizoctonia* interaction. *Indian Phytopathol.* **52** : 366-369.

Bakker, A. W. and Schippers, B. (1987). Microbial cyanide production in rhizosphere in relation to potato yield reduction and *Pseudomonas* spp. mediated plant growth stimulation. *Soil Microbial. Biochem.* **19** : 451-457.

Gupta, C. P. ; Dubey, R. C. and Maheshwari, D. K. (2002). Plant growth enchancement and suppressing of *Macrophomina phaseolina* causing chareoal rot of peanut by fluorescent *Pseudomonas. Biol. Fertil. Soils,* **35** : 399-405.

Jagadeesh, K. S. and Kulkarni, J. H. (2003). Mechanism of biocontrol in the rhizobacteria of tomato, antagonistic to the *Ralstonia solanacearum.* In *Abstract and Short Paper* (compiled and edited by Reddy, M. S. ; Anandaraj, M. ; Eapen, S. J. ; Sarma, Y. R. and Kloepper, J. W.) : 6th *International Workshop on Plant Growth Promoting Rhizobacteria.* 5-10 October 2003, Calicut, Kerala, India, pp 475-480.

Kavitha, K. ; Nakkeeran, S. ; Chandrasekar, G. ; Fernando, W. G. D. ; Mathiyazhagan, S. ; Renukhadevi, P. and Krishanamoorthy, A. S. (2003). Role of antifungal antibiotic, siderophore and IAA production in biocontrol of *Pythium aphanidermatum* inciting damping off in tomato by *Pseudomonas chlororaphis* and *Bacillus subtilis*. In *Abstract and Short Paper* (complied and edited by Reddy, M. S. ; Anandaraj, M. ; Eapen, S. J. ; Sharma, Y. R. and Kloepper, J. W.) 6th *International Workshop on Plant Growth Promoting Rhizibacteria*. 5-10 October 2003. Calicut, Kerala, India, pp 493-497.

King, E. O. ; Ward, M. K. and Raney, D. E. (1954). Two simple media for the demostration of pyocyanine and fluorescin. *J. Lab. Clin. Med.* **44** : 301-307.

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

Mondal, K. K. (1998). *Secondary Metabolite of Bacteria Associated with Cotton Rhizosphere and their Role in Bacterial blight Disease of Cotton.* Ph. D. Thesis. IARI, New Delhi.

Schwyn, B. and Neiland, J. B. (1987) Universal chemical assay for the detection and determination of siderophore. *Anal. Biochem.* **160** : 47-56.

Thornley, M. J. (1960). The differentiation of *Pseudomonas* from gram-negative bacteria on the basis of arginine metabolism. *J. Appl. Bacteriol.* **1** : 37-52.

(Accepted for publication June 10, 2004)