

Isolation and screening of rice rhizobacteria against bacterial leaf blight of rice

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ABSTRACT

The bacterial leaf blight of rice caused by *Xanthomonas oryzae* pv. *oryzae* is the disease of great economic importance in Asia. Biological control of bacterial leaf blight disease can play a vital role in integrated rice disease management. In the present investigation isolation and screening of different rice rhizobacteria isolates were conducted and antagonistic activity were tested. Nineteen isolates were isolated from the rhizosphere of basmati rice i.e. susceptible variety of bacterial leaf blight of rice disease. All the nineteen isolates were screened in vitro for the antagonistic activity against *Xanthomonas oryzae* pv. *oryzae* (Xoo). Among the nineteen tested rice rhizobacteria (RRb) isolates against Xoo, three RRb isolates i.e. RRb 2, RRb 3 and RRb 4 showed inhibition zone at the site of antagonistic growth i.e. 0.767, 1.233 and 0.650 cm, respectively. The seed treatment with three effective isolates i.e. RRb2, RRb3 & RRb4, RRb3 and RRb4 provided good protection of seed against seed borne pathogens resulting increased seed germination and seedling vigour of rice varieties as compare to control.

Key words: rice, *Xanthomonas oryzae*, rhizosphere, bacteria, antagonistic activity

Bacterial leaf blight of rice caused by *Xanthomonas oryzae* pv. *oryzae* (Ishiyama) Swing *et al.* (1990) is the disease of great economic importance in Asia. In India, this disease is prevalent in almost all the paddy growing region in the state. It is considered to be a major constraint for low rice productivity in tropical Asia (Srinivasan *et al.*, 1959). The disease causes infection at nursery seedling, after transplanting and latter at booting or heading stage. Some of the chemicals are effective against this disease but they leave harmful residues in soil and plants. In recent years emphasis has been given on eco-friendly management practices. Therefore, present investigation was planned to manage the disease with the help of bio-agents which have bactericidal action.

Antagonistic activity of bioagents were judged against promising isolate of *Xanthomonas oryzae* pv. *oryzae* by agar well plate method. (Manav and Thind, 2002). Biological control of bacterial leaf blight disease can play a vital role in integrated rice disease

management (Singh *et al.*, 2010). In the present investigation, isolation and screening of different isolates of rice rhizobacteria were conducted and tested for the antagonistic activity against the BLB pathogen *Xanthomonas oryzae* pv. *oryzae*. There are also some reports that seed treatment with bio-agents influences the germination of seed and growth of seedling in several crops (Biswas *et al.* 2008). Therefore, the present study was undertaken to find out the activity of RRb isolates as seed treatment on seed germination seedling vigour of four rice varieties i.e. Pusa Basmati, BPT 5204, Rajendra Bhagwati and Hybrid Arize 6444.

Isolation of rice rhizobacteria (RRb) was made from rhizosphere of Basmati rice grown in experimental plot of bacterial leaf blight of rice at Rajendra Agricultural University, Pusa, Bihar.

Rhizosphere bacteria (RRb) were isolated by using nutrient agar medium. One gram of soil closely adhering to root zone were collected was suspended in 10 ml of sterile water and then it was vigorously shaken

for 15-20 minutes. Serial dilutions were done by transferring 1 ml of suspension to 9 ml sterile water. 0.1 ml suspension with 10^{-3} to 10^{-5} dilutions were spread on solidified nutrient agar medium and then plates were incubated at 28°C for 24 hours. The individual bacterial colonies were picked up and streaked on nutrient agar slants incubated at 28°C for 24 hours. Periodic sub culturing was done on the same medium. The isolated RRb isolates were tested for antagonistic effect against bacterial leaf blight of rice pathogen, *Xanthomonas oryzae* pv. *oryzae*.

For testing antagonistic activity, 10 ml *Xoo* suspension (10^7 CFU ml⁻¹) was mixed with 250 ml of melted cooled (40-45°C) nutrient sucrose agar (NSA) medium and poured into petriplates. The solidified petriplates were spot inoculated with 24 hour old culture of RRb isolates. After 2 days of incubation at 28°C, the plates were examined for antagonistic action indicated by the appearance of inhibition zone at the site of antagonistic growth and diameter of clear zone was measured. Control plates contained only *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) and were not inoculated by

Table 1. Selection of RRb isolates based on antagonistic properties

Rice Rhizobacteria (RRb)	Zone of inhibition in* (cm.) after 48 hr at 28°C.
RRb – 2	0.767
RRb – 3	1.233
RRb – 4	0.650
SE ±	0.057

* Average of three replications.

RRb. The seeds were soaked with isolated RRb isolates (*i.e.* effective against the BLB pathogen) overnight at room temperature and paper towel method was used to check the seed germination and seedling vigour of rice. Distilled water treated seeds served as control.

A total of 19 rhizobacteria were isolated from rhizosphere of Basmati rice using standard bacteriological techniques. The rhizobacterial isolates were designated as RRb-1 to RRb-19. All the isolates were screened in vitro for their bio control ability against *Xanthomonas oryzae* pv. *oryzae*.

Table 2. Effect of RRb isolates as seed treatment on seed germination and seedling vigour of rice.

RRb isolates		Rice varieties			
		Pusa Basmati	BPT 5204	Rajendra Bhagwati	Hybrid Arize 6444
RRb 2	Germn. %	70	76	72	96
	MSL	2.30	3.66	2.20	3.80
	MRL	3.60	3.20	5.10	2.84
	VI	413	521.36	525.60	637.44
	%Infected seed	3	2	4	0
RRb 3	Germn. %	82	90	76	98
	MSL	3.40	4.30	3.60	4.52
	MRL	4.10	4.00	4.70	3.60
	VI	615	747	630.80	795.76
	%Infected seed	1	0	2	1
RRb 4	Germn. %	84	86	80	100
	MSL	3.84	4.30	3.80	4.76
	MRL	3.66	4.10	4.80	3.90
	VI	630	722.40	688	866
	%Infected seed	0	0	4	0
Control	Germn. %	72	78	70	90
	MSL	3.20	4.00	3.30	3.30
	MRL	3.30	3.40	4.70	3.40
	VI	468	577.20	560	603
	%Infected seed	2	2	5	2

Germn. % : Germination percentage, MSL : Mean shoot length, MRL : Mean root length, VI : Vigour index

Out of 19 rhizobacteria isolated from rice rhizosphere, three RRb isolates namely RRb-2, RRb-3 and RRb-4 were found to be antagonistic towards *Xoo* when tested in the dual culture technique. The inhibition zone exhibited by RRb-2 (0.767 cm), RRb-3 (1.233 cm) and RRb-4 (0.650 cm) (Table 1). These three isolates were selected for further studies.

In *vitro* evaluation of *P. fluorescens* and *T. harzianum* was carried out by Singh *et al.* (2010) against *Xoo* by agar well plate method and dual culture method and it was revealed that *P. fluorescens* and *T. harzianum* were inhibitory to the growth of sheath blight. Kavidayal *et al.* (2006) also reported antibacterial activity. Padmaja and Jayaraman (2003) reported that culture filtrate of *P. fluorescens* and salicylic acid inhibited the growth of *Xoo*. The three effective isolates showed the inhibition zone against the BLB pathogen was tested for the seed germination and seedling vigour of four rice varieties (Pusa Basmati, BPT 5204, Rajendra Bhagwati and Hybrid Arize 6444) through seed treatment.

Seed treatment with RRb3 and RRb4 isolates was effective in increasing seed germination and seedling vigour of all four rice varieties studied (Table 2). The seed treatment with RRb2 isolates, was not effective to increase seed germination and seedling vigour of rice as compare to control.

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