*Int.J.Curr.Microbiol.App.Sci* (2017) Special Issue-5: 124-128

*International Journal of Current Microbiology and Applied Sciences*
*ISSN: 2319-7706* **Special Issue-5 (October-2017) pp. 124-128**
Journal homepage: http://www.ijcmas.com

**Original Research Article**

# Biological Control of Blight of Rice Using RR8 Rhizosphere Bacteria

**Ch. Ramanamma* and M. Santoshkumari**

Department of Botany, Y.A. Govt Degree College (w), Chirala, A.P., India
**Corresponding author*

**A B S T R A C T**

Rice bacterial blight**,** is one of the most serious bacterial disease caused by the bacterium Xanthomonas *oryzae* pv. Oryzae thriving in warm and humid environment and it has been observed in rice-growing regions of Asia resulting in severe crop losses. Bacterial blight first becomes evident as water-soaked streaks that spread from the leaf tips and margins, becoming larger and eventually releasing a milky ooze that dries into yellow droplets. Characteristic grayish white lesions then appear on the leaves, signaling the late stages of infection. Infected seedlings usually are killed by bacterial blight within two to three weeks of being infected adult plants may survive, though rice yield and quality are diminished. Methods of controlling rice bacterial blight are limited in effectiveness. Chemical control has been largely ineffective in minimizing bacterial blight. Biological control methods, which rely on the use of bacterial antagonists of pathogens (disease-causing organisms), can reduce bacterial blight. Several bacterial strains were isolated from different rhizospheres. Among these, strain RR8 exhibited strong antibacterial activity against the rice bacterial blight (BLB) pathogen *Xanthomonas oryzae* pv. oryzae and antifungal activity against *Fusarium oxysporium* (invitro studies) that causes wilt disease. The present study findings suggest that RR8 belongs to the *P. fluorescens* group and can serve as potential biocontrol of BLB. Biocontrol assumes a special significance in being an ecologically conscious, cost-effective alternative strategy for BLB management without the negative effect of synthetic chemicals that can cause environmental pollution and may induce pathogen resistance

**Keywords**

Bacterial blight, rhizobacterium, biocontrol, antagonism, *Pseudomonas fluorescens*-RR8, antibacterial activity

## Introduction

Rice is the most important cereal food crop in the world as rice remains the most necessary food to millions of people in asia and it has been stated by the FAO that rice is the traditional crop and traditional staple food in Asia. Considering the world total area under cultivation among all cereals, rice occupies the second position, the highest being under wheat followed by rice (135.5 million hectares).of its total area and production 90% is centered in south and south East Asia. Rice plants are affected by many diseases i.e, bacterial, fungal, viral, insects and nematodes (Kannaiyan) diminishing the yield and quality. Rice bacterial leaf blight is one of the most serious bacterial disease caused by *Xanthomonas oryzae* pv. *oryzae* thriving in warm Water soaked lesions move from tip downwards on the edges of leaves. Characteristic grayish white lesions then appear on the leaves, signaling the late stages of infection gradually symptoms turn into yellow and straw coloured stripes with

wavy margins. In early morning in humid areas yellowish, opaque, turbid drops of bacterial ooze may be seen. In Kresek (wilt) phase, leaves roll completely, droop and plants die completely.

Bacterial blight has been observed in rice-growing regions of Asia resulting in severe crop losses. Since methods of controlling rice bacterial blight are limited in effectiveness. Chemical control has been largely ineffective in minimizing bacterial blight. Biological control of bacterial leaf blight disease can play a vital role in integrated rice disease management

## Materials and Methods

A method was established for isolating and characterizing bacterial strains associated with rhizospheres of rice growing in various parts of India. One gram root soil was sampled from each of the rhizosphere sample and agitated in 100 mL of sterile distilled water for 30 min. The suspension was serially diluted and plated on King's B agar medium (King *et al.*, 1954).

The plates were incubated at 28 °C for 2 days and Single colonies exhibiting bluish green fluorescence were picked under UV light fluorescence (365 nm)) and further purified on the same medium. The selected bacterial strains were characterized by performing standard biochemical tests. Several bacterial strains were isolated from different rhizospheres. Among these, strain RR8 exhibited strong antibacterial activity against the rice bacterial blight (BLB) pathogen, *Xanthomonas oryzae* pv.oryzae and antifungal activity (invitro studies). *Acrocylindrium oryzae* (Sheath rot of Rice), Fusarium oxysporium (wilt rot of Rice) and curvularia species (Black kernel) were isolated from the rice leaves and tested with antagonistic bacterium RR8.

Isolate of X.o.pv.oryzae APX2 maintained on Wakimoto, potato semi synthetic agar medium(PSA) was used.Fifty five days old rice plants were sprayed with inoculum on both sides of fully expanded leaves of TN-1 with the help of an automiser connected to an air line compressor at 50 lb. X.o.pv.oryzae was isolated from inoculated plants lPeptone sucrose agar (PSA) was poured in 9.0 cm diameter Petri plates and after cooling, 100 µL (106 cfu/mL) of Xoo suspension were uniformly spread on the agar surface. Then a loop ful of RR8 inoculum was streak inoculated in the Petri plates and incubated at 28 °C. After 2 days the zone of growth inhibition of Xoo around the strain was measured for studying biological control using flourescent rhizosphere saprophytic bacterium RR 8.

Antagonistic affect of rhizosphere bacterium (RR8) on fungal growth: *Acrocylindrium oryzae* (Sheath rot of Rice), *Fusarium oxysporium* (wilt rot of Rice) and *Curvularia* species (Black kernel) were isolated from the rice leaves and tested with antagonistic bacterium RR8 For the detection of antagonism of RR8 bacteria against the fungal cultures, streak inoculation method was used. 20 ml of SM medium (contained g/l sucrose, 20; L-asparagine2; KH2PO4, 1; MgSo4, 0.5) was poured in to sterilized petri plates.

The plates were held in upside down position after the agar layer was set for one or two days at 28 o C so as to dry off the surface film of water. Then the RR8 rhizosphere bacterium was streak inoculated. After two days. The fungal cultures without rhizosphere bacteria were used for control. The plates after inoculation were incubated for one week at 28 0 c.

Treatment with RR8 was carried out on rice seeds. RR8 treated Fifty five days old rice

plants were sprayed with *Xanthomonas oryzae* pv. oryzae (Xoo) and the efficacy of induced systemic resistance activity was determined against bacterial blight pathogens.

## Results and Discussion

8 Isolates were isolated from the rhizosphere of TN1rice i.e. susceptible variety of bacterial leaf blight of rice disease. All the Eight isolates were screened in vitro for the antagonistic activity against *Xanthomonas oryzae* pv. Oryzae (Xoo). Among the eight tested rice rhizobacteria (RRb) isolates against Xoo, RR8isolate showed inhibition zone at the site of antagonistic growth of BLB and some fungal pathogen. In vivo it is observed that pretreatment of seeds with RR8 (*Pseudomonas fluroscens*) suspension resulted in reductions in bacterial leaf blight of rice. The seed treatment with RR8 also provided good protection of seed against seed borne pathogens resulting increased seed germination and seedling vigour of rice varieties as compare to control.

In the present investigation isolation and screening of different rice rhizobacteria isolates were isolated and antagonistic activity were tested against to bacterial leaf blight pathogen under in vitro condition and other rice fungal pathogens. The results indicated that among the microorganisms tested, *Pseudomonas fluorescens* (RR8)) was highly effective in checking the growth of the pathogen. The rhizosphere bacteria inhibited the growth of X.o.pv.oryzae APX2, *Acrocylindrium oryzae*, *Fusarium oxysporium* and *Curvularia* cultures showing the antagonizing ability of the RR8 rhizo bacterium.. Biocontrol assumes a special significance in being an ecologically conscious, cost-effective alternative strategy for BLB management without the negative effect of synthetic chemicals that can cause environmental pollution and may induce pathogen resistance. As an alternate to hazardous chemicals, antagonistic organisms are found effective in controlling the growth of the rice pathogens.

Antagonistic bacteria are considered ideal biological control agents for obvious reasons, like rapid growth, easy handling, and aggressive colonization of the rhizosphere (Weller 1988). Bacterial antagonists have been evaluated with various degrees of success for the suppression of rice diseases of fungal origin (Vasudevan *et al.*, 2002). The antagonistic fluorescent pseudomonas produces one or more metabolites, such as phenazine-1-carboxylic acid, DAPG, pyoluteorin, pyrrolnitrin (PRN), and oomycin. Among these, DAPG is a polyketide antibiotic with a broad spectrum of antimicrobial properties and is produced by fluorescent

The inhibition of Pseudomonas is carried out by the production of some siderophores like Pseudobactin, pyochelin, pyoveradine, ferribactin, ferrichrome, ferrioxamine, phytosiderophorous etc., antibiotics like phenazines, pyoluteorin, tropolone, pyocyanine,2,4- diacyetyl phloglucinol (DAPG), pyrrolntrin. Secondary metabolites like Hydrogencyanide, phenazine-1-carbosylic acid (PCA),Oomycin A, indole-3-acetic acid, chitinsae, β- 1,3 glucanase, laminariase. So by the production of many antibiotics, siderophores and other toxins *Pseudomonas fluroscences* acts as an effective and broad spectrum antagonistic spectrum. (Karthikeyan Arumug *et al.*, 2013) producing some plant growth promoting substances that mainly include Auxins, gibberellins and cytokinins. Hence it is collectively known as plant growth promoting Rhizobacteria (PGPR).

In the present study it is observed that pretreatment of seeds with RR8 (Pseudomonas fluroscens) suspension

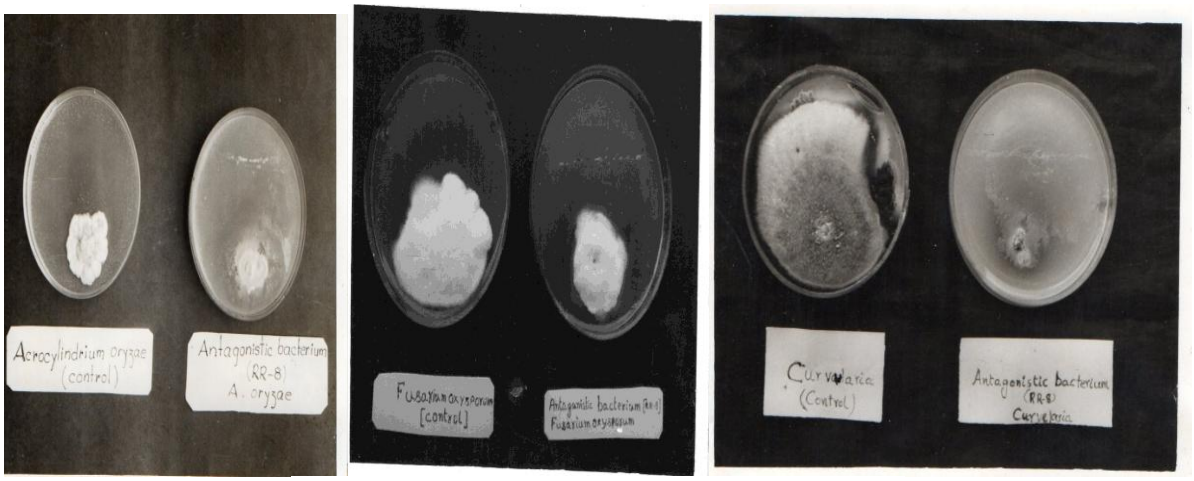
resulted in reductions in bacterial blight these results correlate with the results of the present study. Hence from this it's concluded that by the production of many antibiotics, siderophores and other toxins antagonistic *Pseudomonas fluroscences* (RR8) inhabit the rice pathogens like bacterial blight (BLB) pathogen *Xanthomonas oryzae* pv. Oryzae,. *Acrocylindrium oryzae* (Sheath rot of Rice), *Fusarium oxysporium* (wilt rot of Rice) and *Curvularia* species (Black kernel) effectively against the occurrence of Rice pathogens.

Control infected control pretreated RR8

X.o.pv.oryzae APX2

Infected

X.o.pv.oryzae APX2 Treated with RR8




Antagonistic effect of RR8 on fungal pathogens

## References

Karthikeyan, A., Parthasarathy R, Manikandan A Isolation of *Trichoderma viride* and *Pseudomonas fluorescens* organism from soil and their treatment against rice pathogens. J. Microbiol. Biotech. Res., 2013, 3 (6):77-81.

Krishna rao, N.S.R., and M.V.Naidu.1978. Invitro nutrition of *Xanthomonas oryzae* (uyeda and Ishiyama) Dowson, inducing paddy bacterial leaf blight Ind. Exptl.Biol.,16:133-135

Srinivasan, M.C., M. J. Thirumalachar, and M. K. Patel, 1959. Bacterial blight disease of rice. Current Science. 28: 469-470.

Vasantha Devi, T., Malarvizhi R, Sakthivel N, and Gnanamanikam S.S,.1989.Stains of fluorescent bacteria that were isolated from rice rhizhosphere of southern India and sowed antagonism towards *Rhizoctonia solani* were evaluated for biological control of rice sheath blight. Pant and soil, 199 (2): 325-330.