# *In-vitro* Efficacy of Various Rhizobacterial Isolates Against *Rhizoctonia solani*, the Causal Agent of Rice Sheath Blight Disease

***K. Vijay Krishna Kumar*[*1],*** *M. S. Reddy[1], J. W. Kloepper[1], K. K. Lawrence[1], D. E. Groth[2], M. E. Miller[3] and Binghai Du[4]*

[1]Andhra Pradesh Rice Research Institute, Acharya NG Ranga Agricultural University, Maruteru, India and Currently, Dept. of Entomology and Plant Pathology, Auburn University, AL, USA,[2]Rice Research Station, LSU AgCenter, LA, USA and [3]Dept. of Biological Sciences, Auburn University, USA and [4]Dept. of Microbiology, College of Life Sciences, Shandong Agricultural University, No 61, Diazong Street, Taian, Shandong Province, China.

## Abstract

Sheath blight of rice caused by *Rhizoctonia solani* is an economically significant disease causing severe yield losses. Biological control of the disease using plant growth promoting rhizobacteria (PGPR) is a potential alternative to the presently available chemical control methods. The present study focuses on screening of 70 rhizobacterial isolates of *Baci-llus*, *Brevibacillus*, *Paenibacillus* and *Arthrobacter* spp for antagonistic activity against mycelial growth, sclerotial germination and sheath blight lesion development on leaf blades under *in-vitro* conditions. Dual culture studies revealed that the mycelial growth of *R. solani* was inhibited up to 83% by these PGPR and 10 strains were found to exhibit antagonism of over 70%. Superior strains were within the following species: *Bacillus subtilis*, *B. mycoides, B. vallismortis, B. sphaericus* and *P. macerans*. Two strains of *B. subtilis* and one strain of *P. macerans* and *P. polymyxa* completely inhibited the sclerotial germination of test pathogen *in-vitro*. The hyphal reduction of germinated slcerotia by other bacterial strains was up to 42%. Inhibition studies on sheath blight lesion development by PGPR on detached leaves revealed that 56 strains were effective with disease severities in the range of 2.9% to 97% as against control with 100% severity. Maximum inhibition of lesion development was noticed with two strains of *B. subtilis* and one strain of *B. atrophaeus* with disease severities of 2.9%, 39% and 32% respectively. Two *B. subtilis* strains that were effective in inhibiting mycelial growth, sclerotial germi-nation of *R. solani* and also on lesion development were further selected for greenhouse and field studies on plant growth promotion and sheath blight disease management.

## Introduction

Rice sheath blight caused by *Rhizoctonia solani* is a global production constraint incurring heavy economic losses. The disease manifests initially as water soaked lesions on leaf sheaths and leaf blades during tillering stage of the crop and progresses rapidly under favorable conditions. Grain losses to an extent of 40% are reported annually with the disease (Tan Wan Zhong *et al*., 2007). The pathogen survives in the soil and also in stubbles of previous season's crop and thus reinfests the current season rice crop (Kozaka, 1961). Genetic sources of resistance for this disease are not adequate and current management strategies mostly involve use of chemical fungicides. The adverse effects of chemical fungicides on environment and beneficial microflora are evident and so biological control of the disease is a promising and economic alternative in sheath blight disease management.

Among the biocontrol agents, plant growth promoting rhizobacteria (PGPR) are widely used in managing soil borne diseases of several field crops. The PGPR colonize on the root systems through seed bacterization and show antagonism against soil borne phytopathogens. Ability of these PGPR in plant growth promotion and protection against soil borne diseases further

depends on many factors like rhizosphere competence, persistence of bacteria on seeds and plant roots, root colonizing capacity as well as synthesis and release of various metabolites (Nautiyal, 1997). A successful bioagent against rice sheath blight should be able to control both the mycelial and sclerotial stage of sheath blight pathogen besides contributing to growth promotion and yields. Several bacterial strains were found to possess the ability to protect rice plants from diseases such as blast, sheath blight, sheath rot and stem rot diseases (Vasantha Devi *et al*., 1989). Of these, PGPR group offers an effective means of anta-gonism against sheath blight pathogen (Luo Jin Yan *et al*., 2005). Besides, these PGPR also contribute to enhanced growth of the seedlings, induction of systemic resistance against diseases and thereby in yield increase (Pathak *et al*., 2004). The present study is aimed at *in-vitro* screening of different PGPR strains for their antagonism against rice *R. solani* and identifying superior strains for their further use in greenhouse and field conditions for sheath blight management as an alternate or supplement to presently available chemical control methods.

## Materials and Methods

A multinucleate and virulent isolate of *R. solani* belonging to AG-1 IA anastomosis group was obtained from Plant Pathology Laboratory of LSU, AgCenter. The pathogen was originally isolated from rice sheath blight infected fields at LSU AgCenter. The culture was grown and maintained on PDA at 28±1ºC for 7 days. The bacterial cultures (n=70) of genera *Bacillus* (n=47), *Paenibacillus* (n=9*)*, *Brevibacillus* (n=13) and one *Arthrobacter* were screened in the present study and they are maintained on Tryptic Soy Broth (70%) and 30% glycerol at -80ºC. Fresh cultures were prepared from the frozen stock on plates containing 10% TSM.

### *In-vitro* effect on mycelial growth of pathogen

The antagonistic properties of PGPR strains was tested against *R. solani* on 10% TSA (Tryptic Soy Agar) plates by adopting dual culture technique (Gupta *et al*., 2001). An agar block (5 mm diameter) of 5-day-old culture of test pathogen was placed in the centre of plates (85 mm diameter) containing 10% TSA. A loopful 24-h-old culture of PGPR strain was then streak inoculated at either sides of pathogen disc at a distance of 2 cm apart. The fungal pathogen culture inoculated centrally on TSA plates, but uninoculated by PGPR strain, served as control. Each treatment was replicated thrice and the inoculated plates were incubated at 25±1ºC for 5 days and inhibition of the colony growth of the test pathogen was measured.

### Efficacy of PGPR isolates on the germination of sclerotia of *R. solani in-vitro*

The antagonistic effect of PGPR on sclerotial germination of *R. solani* was determined by adopting the procedure of Kazempour (2004) with slight modifications. Ten-day old sclerotia of the pathogen that are produced on PDA are collected and surface sterilized in 2.5% sodium hypochlorite solution. The PGPR isolates were multiplied in Erlenmeyer flasks by adding a loopful of actively growing cultures in 50 ml of Tryptic Soy Broth (10% TSB) and incubating for 24 hrs on a rotary shaker at 175 rpm at room temperature (26±2ºC). Five sclerotia were later inoculated in flasks of each bacterial isolate and were placed for a period of 24 hrs on a rotary shaker at 175 rpm at room temperature. The sclerotia are then removed and subsequently placed onto PDA plates and incubated for 72 hrs at 26ºC and the germination and radial growth was measured. Controls were run separately for each bacterial isolate by replacing bacterial inoculum with 1 ml of sterile distilled water. Each experiment considering ten bacterial isolates were run in five replications and obser-vations on per cent sclerotial germination were recorded. The radial growth of mycelium from sclerotia is recorded and percent inhibition of rate of mycelial growth from sclerotia was determined by using the following formula.

$$\%1 = \frac{100\,(C - T)}{C}$$

where, I= inhibition of mycelial growth, C= mycelial growth of pathogen in the control plate (mm) and T= growth of pathogen in treatment (mm).

**Detached leaf bio-assay: effect of PGPR on sheath blight lesion development**

The antagonistic potential of live PGPR against rice sheath blight pathogen, *R. solani* was tested by detached leaf piece assay method as described by Guleria *et al* (2007) with slight modifications. Cell suspen-sions of PGPR were prepared at a concentration of 4 x $10^8$ cfu $ml^{-1}$ following the procedure that was described previously. Leaves used in this experiment were at second position from base of the culm. The live cultures were sprayed on 60-day-old, surface sterilized cut rice leaf pieces (8 cm) of variety "Cocodrie" (high yielding and highly susceptible to sheath blight disease) and then were inoculated with *R. solani* by placing one-week-old sclerotium at the centre of leaf. The leaves were placed in petri dishes (14 cm diameter) containing moistened filter papers and are supported by clean glass slides so as to check them from rolling inwards. Five leaves for each bacterial isolate were used and rice leaf bits sprayed with only sterile distilled water and inoculated with sclerotia of the pathogen served as control. The petri plates with leaves were incubated in a growth chamber (25 ± 1ºC, 16 h light) and observations on lesion length around sclerotium were recorded after 5 days of inoculation. The disease intensity was calculated by Highest Relative Lesion Height (HRLH) method where

$$\text{HRLH} = \frac{\text{Highest lesion height}}{\text{Leaf length}} \times 100$$

The disease severity as indicated by the area of leaf tissue affected by the pathogen was graded into five categories wherein

0= no symptoms; 1= 1-10%; 2=11-25%; 3=26-50% and 4= >50% leaf area affected.

**Statistical analysis**

The data are subjected to statistical analysis and treatment means are differentiated using SAS (PROC-ANOVA).

**Results and Discussion**

**Dual culture studies**

Among the 70 PGPR strains selected for their *in-vitro* efficacy against rice *R. solani*, 66 strains were found to inhibit the test pathogen up to 83.13%. Of these, 10 strains exhibited an inhibition of *R. solani* to an extent of 70% and above. These superior strains include three strains of *Bacillus subtilis*, two strains of *B. mycoides* and *P. macerans* and one strain of *B. vallismortis* and *B. sphaericus* with no significant differences among them. Antibiosis was the most commonly exhibited mode of antagonism as indicated by inhibition zones ranging from 3.67 mm to 5 mm by these superior PGPR except for strains ABU1627 (*B. mycoides*) and ABU1240 (*B. cereus*). The mycelial inhibition of *R. solani* was found to be 41.02% for *Bacillus* spp; 24.90% for *Brevibacillus* spp; 43.26% for *Paenibacillus* spp and 48.63% for *Arthrobacter* spp.

Antibiosis was not observed with one *A. viscosus* against *R. solani*. For 13 different strains of *Brevibacillus* belonging to *B. choshinensis, B. brevis* and *B. reuszeri* under study, the inhibition of test pathogen was up to 48.23% and the antibiosis was also negligible with these PGPR strains (a maximum of 1.67 mm inhibition zone).

**Studies on sclerotial germination**

Of all PGPR under study, only four strains completely inhibited sclerotial germination of *R. solani*. These included two strains of *B. subtilis* and one strain of *P. macerans* and *P. polymyxa*). Reduction in hyphal growth from sclerotial bodies for other PGPR strains ranged from 6.67% to 42.67%. The mean hyphal reduction for different PGPR genera were about 23.06% (*Bacillus* sp), 19.49% (*Brevibacillus* sp), 24.95% (*Paenibacillus* sp) and 25.33% (*Arthrobacter* sp) (Fig. 1).

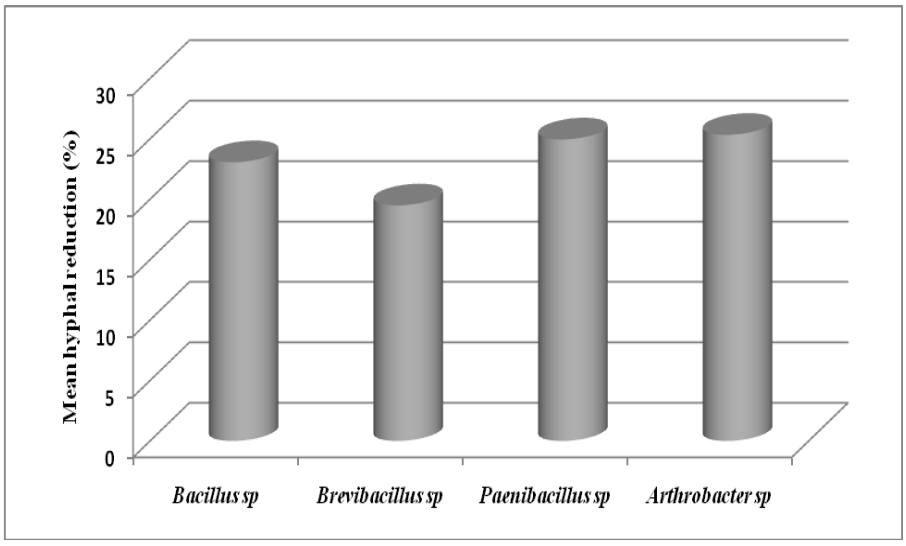


**Fig. 1.** Mean hyphal reduction of germinated sclerotia of rice *R. solani* by different PGPR under *in-vitro* conditions.

**Effect of PGPR on sheath blight lesion development**

On detached leaves, of the 70 PGPR, 56 strains were found to arrest the progress of sheath blight lesions to varying degrees. The disease severity in these effective PGPR treated leaves ranged from 2.92% to 97.08% as against control with 100% disease severity. The disease severity scale ranged from 1 to 4 for these PGPR. Among the strains, maximum inhibition of lesion development was noticed with one strain of *B. subtilis* (2.92% disease severity) (severity scale of 1) and one strain of *B. atrophaeus* (32.08% severity and a mean severity scale of 1.67). Overall, lesion development was 70.41% with *Bacillus* sp; 89.84% with *Brevibacillus* sp; 71.61% with *Paenibacillus* sp and 50% with *Arthrobacter* sp (Fig. 2).

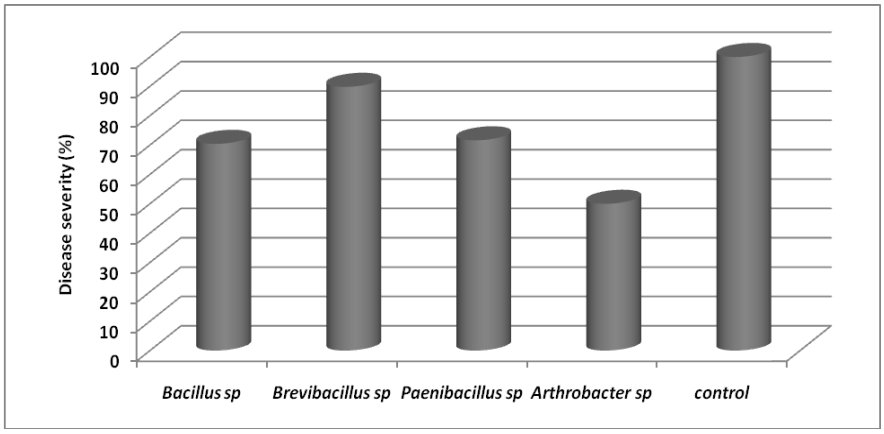


**Fig. 2.** *In-vitro* inhibition of rice sheath blight lesion development by PGPR on detached leaves.

Suppression of rice sheath blight pathogen, *R. solani* by non-fluorescent PGPR like *Bacillus* spp was previously attributed to the production of chitinase (Krishnaveni, 1991) and other antifungal metabolites. A large number of bacterial strains were found to possess the ability to protect rice plants from sheath blight disease (Vasantha Devi *et al*., 1989) and these were identified through dual plate assays. The exploitation of these biocontrol agents for management of sheath blight at field level in the long run is an exciting possibility.

Studies from our laboratory revealed that of different PGPR, *Bacillus subtilis* (AP301 and AP219) were found to be very effective in inhibiting mycelial growth, sclerotial germination of sheath blight pathogen, *R. solani* and arresting the sheath blight disease spread on detached rice leaves. These effective strains are now being screened for their effects on development of sheath blight disease in the greenhouse. Future studies will determine if the inhibition noted here results from production of antifungal compounds alone or also by production of chitinase.

## References


Biswas, A. 2006. Efficacy of biotos: a new botanical against sheath blight disease of rice. Environment and Ecology. MKK Publication 24S (Special 2): 484-485.

Guleria, S., Aggarwal, R., Thind, T. S., and Sharma, T. R. 2007. Morphological and pathological variability in rice isolates of *Rhizoctonia solani* and molec-ular analysis of their genetic variability. Journal of Phytopathology 155: 645-661.

Gupta, C.D., Dubey, R.C., Kang, S.C and Maheshwari, D.K. 2001. Antibiotic mediated necrotrophic effect of *Pseudomonas* GRC2 against two fungal plant pathogens. *Current Sci*. 81: 91-94.

Kazempour, M.N. 2004. Biological control of *Rhizoctonia solani*, the causal agent of rice sheath blight by antagonistic bacteria in greenhouse and field conditions. Plant Pathology Journal. 3(2): 88-96.

Kozaka, T. 1961. Ecological studies on sheath blight of rice plant caused by *Pellicularia sasakii* and its chemical control. Chugoku Agric. Res. 20:1-13.

Krishnaveni, M. 1991. Biological control of sheath blight disease of rice caused by *Rhizoctonia solani* Kuhn with *Pseudomonas fluorescens*. Masters Dissert-ation, University of Madras.

Luo JinYan., Xie GuanLin., Li Bin., Luo Yuan Chan., Zhao LiHan., Wang Xiao., Liu Bo., and Li Wen. 2005. Gram-positive bacteria associated with rice in China and their antagonists against the pathogens of sheath blight and bakanae disease in rice. Rice Science. 12(3): 213-218.

Mathivanan, N., Prabavathy, V.R., and Vijayanandraj, V. R. 2005. Application of talc formulations of *Pseudomonas fluorescens* Migula and *Trichoderma viride* Pers. ex S.F. Gray decrease the sheath blight disease and enhance the plant growth and yield in rice. Journal of Phytopathology 153(11/12): 697-701.

Nautiyal, C. S. 1997. Rhizospheric competence of *Pseudomonas* sp. NBRI 19926 and *Rhizobium* sp. NBRI 19513 involved in the suppression of chickpea (*Cicer arietinum* L.) pathogenic fungi. FEMS Microbiol. Ecol. 23: 145-158.

Pathak, A., Sharma, A., Johri, B.N., and Sharma, A.K. 2004. *Pseudomonas* strain $GRP_3$ induces systemic resistance to sheath blight in rice. International Rice Research Notes. 29 (1): 35-36.

Tan WanZhong., Zhang Wei., Ou ZengQi., Li ChengWen., Zhou GuanJun., Wang ZhiKun., and Yin Li. 2007. Analyses of temporal development and yield losses due to sheath blight of rice (*Rhizoctonia solani* AG1. 1A). Agricultural Sciences in China 6(9): 1074-1081.

Vasantha Devi, T., Malarvizhi, R., Sakthivel, N., and Gnanamanickam, S. S. 1989. Biological control of sheath blight of rice in India with antagonistic bacteria. Plant Soil 119: 325-330.