Plant Archives Vol. 20 Supplement 1, 2020 pp. 1311-1315 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

BIOLOGICAL CONTROL OF SHEATH BLIGHT OF RICE CAUSED BY *RHIZOCTONIA SOLANI* KUHN USING MARINE ASSOCIATED *BACILLUS SUBTILIS*

T. Suthin Raj[1]*, A. Muthukumar[1], P. Renganathan[1], M. Charumathi[1] and H. Ann Suji[2]

[1*]Department of Plant Pathology, Faculty of Agriculture, Annamalai University, Chidambaram (Tamilnadu), India.
[2]Centre for Advance Studies in Marine Biology, Annamalai University, Chidambaram (Tamilnadu) India.

Abstract

The screening of biological control agents towards sheath blight in rice was developed and used to evaluate a panel of *Rhizoctonia solani* Kühn hyphae-associated bacteria for their ability to control sheath blight under *in vitro* and greenhouse conditions. Eventhough chemicals control the rice sheath blight disease, the use of continuous, inappropriate and non-discriminative chemicals is an agent to bring undesirable effect such as residual toxicity, development of resistance, ecological degradation, perilous to the well being of humans and animals and increases the expense for plant protection. In this scenario, the present work has been aimed to isolate antagonistic rhizobacteria from the least explored coastal sand dune ecosystem, characterize their biological control potential for the suppression of *R. solani* and evaluate them *in vitro* and green house study. The fungitoxic effect of 15 isolates of bacterial biocontrol agents from various seaweed, sea water and sediments were evaluated under *in vitro* conditions on growth of *Rhizoctonia solani*, which is one of the causal agents of sheath blight. *Bacillus subtilis* (Accession number MK370673) Bs-1 was the most successful, showing 60.20% discretion of colony growth with a minimum mean mycelial dry weight (120.75 mg/50m/broth) of the pathogen. The present study identified that the usefulness of bacterial biocontrol agents against fungal pathogens is due to larger levels and early accretion of phenolics and phytoalexins and the field study proved that, *R. solani* can be controlled by the usage of *Bacillus subtilis*.

***Key words*:** Seaweeds, *Rhizoctonia solani, Bacillus subtilis,* Antifungal compounds, Rice.

Introduction

Sheath blight of rice caused by a soil borne necrotrophic fungus *Rhizoctonia solani* Kuhn. It is regarded as one of the most widely distributed diseases of rice. Nowadays a major barrier to rice cultivation is the rice sheath blight disease by *Rhizoctonia solani.* (Savary *et al*., 2006). Rice sheath blight disease has been reported to cause approximately 50% yield reduction in test plots of susceptible cultivars. Depending upon the age of the plant, time of infection and severity, it causes yield loss to the extent of 5.9 to 69 percent (Venkat Rao *et al*., 1990). Sclerotia may be uneven to spherical and measure 4-5 mm in diameter, basidia and basidiospores are formed under normal conditions and measure 10-15×7-9 nm and 8-11×6.5 nm respectively. Roy (1993) indicated that *R. solani* inhabits organic matter in the soil as mycelium because of its plant pathogenic activity and its saprophytic nature. Srinivas *et al*., (2013) stated that, a total crop loss varies from 30 to 40 percent in prevalent areas and it extend to a total loss when it spreads to upper parts of the plant and panicles is seen because of rice sheath blight disease. This is usually encountered by the usage of fungicides with a wide range of activity that targets more than one disease. Presently, sheath blight disease management is done using systemic fungicides (Suthin Raj and John Christopher, 2008, Chahal *et al*., 2003, Suthin Raj *et al*., 2018) and the bacterial bio-control representatives similar to plant growth enhancing rhizobacteria (PGPR). Several marine bacteria isolated from coastal sea water were also found to have antagonistic activity against *R. solani* (Jayaprakashvel *et al*., 2005). However, the haphazard use of fungicides paves way to residual toxicity on the manufacture, development of chemicals resistance and also acts as an cause for environmental pollution and hence, there is an

Author for correspondence :

urgent need to develop alternative disease control procedures. Carling *et al*., (1990) and Suthin Raj *et al*., (2019) stated that a viable substitute to the use of chemical pesticide is felicitated by organic control of plant pathogens.

Materials and methods

Isolation, maintenance and identification of *R. solani*

The plants with representative signs of sheath blight disease were collected fresh from twenty traditional rice growing areas of Tamilnadu. The pathogens secluded from each of these localities formed one isolate of *R. solani*. The pathogen was isolated to potato dextrose agar (PDA) medium from diseased plants showing characteristic symptoms. The piece of the fruit with diseased symptoms was cut into small pieces, surface sterilized in 0.1% mercuricchloride solution for 30 seconds and then cleaned repeatedly with sterile distilled water and plated onto sterile PDA medium in 9 cm Petri dishes. The plates were incubated at room temperature (28±2°C) for five days and then checked for fungal growth. Rangaswami, (1972) had found the use of single spore isolation technique be used to obtain a clean culture.

Evaluation of antagonistic bacteria against *R.solani.*

Isolation of bacteria from seawater and sediments (Dhaarani et al., 2018): In the present study, Different serial dilutions such as 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} were prepared from the 10ml made-up samples of seawater and sediments and as well from 10ml of seawater samples. For each dilution, 100µl wash down was spread on to petri plates containing approximately 15 ml of 1.5% ZoBell marine agar. The plates were then incubated at 25±2°C and bacterial colonies with different morphology were selected up every 6h up to 4 days and marked on the fresh plates with ZoBell marine agar. Pure cultures of each isolates were long-established by subsequent restreaking. Then, they were chosen with unique codes and stored in glycerol suspension (glycerol/bacterial broth of 1:1 v/v) in Eppendorf tubes at -80°C for further analysis.

Talbe : List of bacterial biocontrol isolates with their NCBI Accession numbers

S.No	Bio inoculants	Accession Number
1	*Bacillus fusiformis*	MK368529
2	*Bacillus subtillis*	MK370673
3	*Bacillus amyloliquefaciens*	MK368811

PCR amplification of fungal ITS region from *Bacillus subtillis* isolates: The primers FIGS 1(Forward) and FIGS 2 (Reverse) were used to amplify the *Bacillus velezensis* species in different soil samples.

FIGS 1 - 5'- GTA AGC CGT CCT TCG CCT CG - 3'

FIGS 2 - 5'- GCC ATA CTA TTG AAT TTT GC - 3'

The cocktail for the amplification was prepared in 0.2 ml PCR tubes as detailed below:

DNA 25 ng/µl 2.00µl

dNTPs (2.5 mM) 2.00 µl

Forward Primer (30 picomole) 2.00 µl

Reverse Primer (30 picomole) 2.00 µl

10 × assay buffer 2.00 µl

Taq polymerase (3 units/µl) 0.40 µl

Magnesium chloride 2.00 µl

Sterile distilled H_2O 8.60 µl

Total 20.00 µl

Then the 0.2 ml PCR tubes were placed on to a thermocycler (Agilent technologies) and the thermal cycler was programmed as follows:

Profile 1: 94°C for 1 min Initial denaturation

Profile 2: 94°C for 1min Denaturation

Profile 3: 58°C for 1min Annealing

Profile 4: 72°C for 1 min Extension

Profile 5: 72°C for 5 min Final extension

Profile 6: 4°C for infinity to hold the samples until attended.

Profiles 2, 3 and 4 were programmed to run for 30 cycles. The amplified PCR products were run on 1.5% agarose gel in tris-borate buffer. The gel was stained with ethidium bromide, visualized on a UV-transilluminator and photographed in the gel documentation unit (Alpha Innotech Corp, USA).

Screening of marine bacterial isolates for antibiotic production: According to the morphological, Gram's discoloration and biochemical

Table : List of bacterium recorded from the samples of seawater and sediments.

S.No.	Place	Sea Water	Sediments
1	Samiyarpettai	*Bacillus cereus* *Aeromonas salmonicida* *Bacillus amyloliquefaciens*	*Lactobacillus fermenti*
2	Parangipettai	*Bacillus subtilis*	*Lysinibacillus fusiformis*
3	Cuddalore	*Aeromonas salmonicida*	*Aeromonas salmonicida*
4	Thondi	*Lactobacillus fermenti*	*Aeromonas hydrophila*
5	Rameshwaram	*Bacillus subtilis* *Aeromonas salmonicida*	*Bacillus subtilis* *Aeromonas hydrophila* *Aeromonas salmonicida*

Table 1: Evaluation of various isolates of *Bacillus subtilis* against *R. solani* by dual culture technique.

S. No.	Isolates	Linear growth (mm)		% Growth inhibition	Mycelial dry weight (mg/50m/broth)				
		Antagonist	*R. solani*		10%	20%	30%	40%	Mean
1	*Bacillus subtilis*	62.00	28.00	68.80[a]	208.00	162.00	84.00	29.00	120.75[a]
2	*Bacillus cereus*	60.80	29.20	68.55[b]	226.00	196.00	101.00	36.00	139.75[b]
3	*Aeromonas hydrophila*	58.60	31.40	65.11[b]	267.00	219.00	117.00	49.00	163.00[c]
4	*Aeromonas salmonicida*	55.50	35.50	61.66[c]	294.00	223.00	136.00	52.00	176.25[c]
5	*Lactobacillus fermenti*	51.70	38.30	57.40[d]	324.00	246.00	156.00	56.00	195.50[d]
6	Control			0.00[e]	480.00	480.00	480.00	480.00	480.00[e]

*Values in the column followed by common letters do not differ significantly by DMRT (P=0.05).

individuality described in the Bergey's manual, 26 strains having up to 5 bacterial category were enunciated from different samples like seaweed, sediment and seawater and were assessed for antibiotics production. Bacteria grown-up in the medium developing reserve zone around the discs were measured as antibiotic producers. Thus 3 bacteria from 16 strains were observed as antibiotic producers and they were then taken up for further viewing against plant pathogens.

Dual culture: *B. subtilis* was developed on nutrient agar (peptone-5g, meat extract-1g, yeast extract 2g, sodium chloride- 5g, pH 7.0) medium. An 8 mm vigorously growing PDA culture disc of the pathogen was set aside on PDA medium in a sterilized petri dish at one side, 1.5 cm away from the edge of the plate and incubated at a temperature of 28±2°C. After forty eight hrs, actively growing 48-h-old cultures of the respective experimental bacteria were individually noticed on to average at the contrary side of the plate, 1.5 cm away from the edge of the plate. And at room temperature of (28±2°C) the inoculated plates were incubated. Three replications were maintained for antagonist activity. Potato dextrose agar medium (PDA Medium) inoculated with the pathogen alone served as a control. After 8 days, the radial progress of the pathogen was seen and measured. The results were expressed as percent growth inhibition over control. The most effective isolates of *B. subtilis* were used for further study.

Mycelial dry weight: PDA was prepared in 250 ml Erlenmeyer flasks and autoclaved. Culture filtrates of *Bacillus subtilis* at 10 ml were added to 40 ml broth in flask so as to get a final concentration of 20 percent of the filtrate in broth. The broth was inoculated with 8mm culture disc of *R. solani* and incubated for 10 days at 28±1^{0}C. The control solution was the broth without the inclusion of filtrate. After the incubation period, on an earlier weighed filter paper, the mycelial mat was harvested and dried out at 105^{0}C for 12 h. in a hot air oven and was cooled in desiccators. The mycelial weight was documented as mg/50 ml/broth.

Evaluation of *Bacillus subtilis* for the management of *R.solani* under field conditions: The field trials were conducted at Shathankudi Village, Perambalur-District between December 2017 and March 2018 in a field with a history of rice sheath blight incidence. Trials were set in plots (33×13feet) laid out in a randomized block design. Thirty days old rice seedlings of var. ADT 36 were transplanted in cement carriages. *R. solani* was inoculated over the plant canopy by one gram rice hull/rice grain, placed on basal leaves and closed with polythene bags on the 20th day after transplanting. The below given treatment schedule was designed on the basis of the above phenomena. The cultivar ADT 36 was raised as per the Crop Production Guide (2017).

Treatment details

T_1: Application of *Bacillus subtilis* (seed treatment)

T_2: Application of *Bacillus subtilis* (prophylactic

Table 2: Effect of *Bacillus subtilis* (Marine environmental bacteria) on growth and yield attributes under greenhouse conditions.

Treatments	Mean Plant hight (cm)	Mean No. of productive tillers	Mean 1000 g weight	Straw yield (ton/ ha.)	Grain yield (g/ plant)
T_1 - Application of *Bacillus subtilis*(Seed treatment)	83.00[c]	12[e]	18[d]	5.42[c]	26[d]
T_2 - Application of *Bacillus subtilis*(prophylactic spray at 30, 50 and 70 DAT)	76.90[e]	10[f]	17[d]	4.10[c]	23e
T_3 - T_1 + T_2	95.47[a]	14[a]	26[a]	8.72[a]	35[a]
T_4- Seed treatment with mancozeb +spraying, 30 and 45 DAT	92.13[a]	14[c]	23[a]	8.10[a]	34[b]
T_5- Control	74.00[d]	10[f]	14[e]	4.55[f]	18[e]

*Values in the column followed by common letters do not differ significantly by DMRT (P=0.05).

Table 3: Effect of *Bacillus subtilis* (Marine environmental bacteria) on Sheath blight incidence under field condition

Treatments	Sheath blight incidence on 30th DAT	% Increase over control	Sheath blight incidence on 50th DAT	% Increase over control	Sheath blight incidence on 70th DAT	% Increase over control
T_1 - Application of *Bacillus subtilis* (Seed treatment)	3.7[b]	81	8.1[b]	79	11.7[d]	83
T_2 - Application of *Bacillus subtilis* (prophylactic spray at 30, 50 and 70 DAT)	3.1[b]	84	7.3[b]	81	10.9[d]	84
T_3 - $T_1 + T_2$	2.3[a]	88	6.6[a]	83	8.3[a]	88
T_4- Seed treatment with mancozeb +spraying, 30 and 45 DAT	2.6[a]	86	7.0[a]	82	10.2[b]	85
T_5- Control	7.5[e]		8.7[e]		9.2[c]	

*Values in the column followed by common letters do not differ significantly by DMRT (P=0.05).

spray at 20, 40 and 60 DAT)

T_3: $T_1 + T_2$

T_4: Seed treatment with Hexaconazole + spraying 50 and 75 DAT

T_5: Control.

Disease Incidence

The evaluation of sheath blight damage for rice plant was visualized on their 30th, 50th and 70th days after transplantation. The strength of sheath blight was calculated as per cent disease index (PDI) grade chart given by Ravinder Reddy (1982) and using the formula given by McKinney (1923) as described earlier.

Plant Growth Parameters

Growth parameters *viz*., plant height, number of productive tillers, 1000 g weight, straw yield and grain yield were analyzed for the plants.

Experimental design and data analysis

The experiments were conducted by completely randomized design (CRD) with three replications. The significant difference, if any, among the means were compared by the Duncan's multiple range test (DMRT). Whenever necessary, the data were distorted before statistical analysis following appropriate methods.

Results

Effect of Marine bacteria against *R.solani*

The results of the screening of five isolates of bacteria against *R. solani* on PDA plates are given in table 1. Among the *Bacillus* sp isolates *B. subtilis* Bs-1 was found to be the most effective against the test pathogen showing 60.20 percent reservation of colony growth and minimum mean mycelial growth of pathogen (120.75). It was further followed by isolated *B. cereus* showing 68.55 percent reservation and minimum mean mycelial growth (139.75) which were statistical on par with each other. A minimum growth inhibition (57.40) and minimum mycelial growth of the pathogen was found by the usage of the isolate *Lactobacillus ferment.* All the isolates significantly minimized the mycelial growth of the pathogen over the control.

Mycelial Growth

The mycelial growth of the pathogen was experimented against *B. subtilis* at 10, 20, 30 and 40 percent concentrations. Among them, *B. subtilis* isolated was significantly plummeting the growth of mycelium at 208, 162, 84 and 29 mg/50ml broth respectively. It was followed by *B. cereus* isolated with 226, 196, 101 and 36 mg/50ml broth. All the isolates significantly reduced the mycelia growth of the pathogen over the control (Table 1). Hence the superior isolate, *B. subtilis* was used for the then studies.

Effect of *B. subtilis* on incidence of sheath blight under field conditions

From the results (Table 2) it can be identified that the use of *B. subtilis* (seed treatment + prophylactic spray at 20, 40 and 60 DAT) (T_3) significantly has minimised the incidence of sheath blight at 30, 50 and 70 days after transplanting as compared to the other forms of treatments. This was followed by the treatment of Mancozeb (seed treatment + prophylactic spraying 30 and 45 DAT) (T_4).

Effect of *B. subtilis* and mancozeb on growth and yield of *R. solani* under field condition

Table 3 pictures that all treatments have significantly improved the fruit yield and growth, as compared to the control. From the various groupings that was assessed it was found that, seed treatment + prophylactic spraying 30, 50 and 70 DAT with *B. subtilis* (T_3) considerably enhanced the mean plant height (95.47 cm), mean number

of productive tillers (14 nos), mean 1000g weight (26g), straw yield (8.72 ton/ha) and grain yield (35 g/plant), in comparison to all other methods followed by spraying mancozeb (seed treatment + prophylactic spraying at 30 and 45 DAT) (T_4).

Discussion

From various regions of Tamilnadu, ten isolates of *B. subtilis* were isolated and tested their efficiency against *R.solani*. In the present study, it was found that among the ten isolates, for *B. subtilis* Bs-1 maximum kept the growth of *R. solani* in dual plating technique. A same sort of result was found in the studies of Vivekananthan *et al*., (2004) and Abarna *et al*., (2019). They have stated that, isolate Bs-1 has powerfully inhibited the growth of *R. solani* in laboratory circumstances and field situations. This may be because of *B. subtilis* isolates production of a collection of antifungal antibiotics such as 2, 4-diacetylphloglucinol, oligomycin, phenazine, pyoluteorin, pyrolnitrin and pyocyanin (Gupta *et al*., 2001, Suthin Raj *et al*., 2014). Hofte and Bakker, 2007 and Reddy *et al*., 2008 had earlier stated that, antifungal compounds like HCN, salicyclic acid and 2-hydroxyl phenazine produced by bacterial biocontrol agents has suppressed the plant pathogenic fungi.

References

Abarna, T., A.H. Abdul Raheem, R. Abhishek Livingstone, K. Abichandra, A. Abikannan and T. Suthin Ra. (2019). Bio efficacy of *Bacillus subtilis* and Plant extracts against *Colletotrichum capsici* (Syd.) Butler and Bisby under *in vitro* condition. *Journal of Applied Science and Computations.,* **6(4):** 2110- 2123.

Carling, D.E., D.J. Helm and R.H. Leiner (1990). *In vitro* sensitivity of *Rhizoctonia solani* and other multinucleate and binucleate *Rhizoctonia* to selected fungicides. *Plant Dis*., **74:** 860-863.

Chahal, K.S., S.S. Sokhi and G.S. Rattan (2003). Investigations on sheath blight of rice in Punjab. *Indian Phytopathology.,* **56:** 22-26.

Dhaarani, S., V. Dhanasekaran, R. Dharshini, S. Enitha, P. Nishanthi, M. Jayapriya and T. Suthin Raj **(**2018). Antimicrobial activity of bacteria associated with seawater against rice sheath blight caused by *Rhizoctonia solani* Kuhn. *International Journal of Innovations in Agricultural Sciences.,* **2 (1):**100-105.

Gupta, S.K., P.P. Gupta and C.D. Kaushik (2001). Changes in leaf peroxidase, polyphenol oxidase, catalase and total phenols due to *Alternaria* leaf blight in *Brassica* species. *Indian J. Mycol. Plant Pathol.,* **25:** 175-180.

Hofte, M. and P.A.H.M. Bakker (2007). Competition for iron and induced systemic resistance by siderophore of plant growth promoting rhizobacteria. *Soil Biology.,* **12:**121-133.

Jayaraj, J., J. Norrie and Z.K. Punja (2011). Commercial extract from the brown seaweed *Ascophyllum nodosum* reduces fungal diseases in greenhouse cucumber. *Journal of Applied Phycology.,* **23:** 353.

McKinney, H.H. (1923). A new system of growing grading plant diseases. *J. Agric. Res.,* **26:** 195-218.

Rangaswamy, G. (1972). *Diseases of Crop Plants in India*. Prentice Hall of India Pvt. Ltd., New Delhi, 504.

Ravinder Reddy, M. (1982). Evaluation of fungicides against major diseases of chilli. *M.Sc. (Ag.), Thesis*, Tamil Nadu Agric. Univ., Coimbatore, India, 64.

Reddy, B.P., K.R.N. Reddy, B.P. Subba Roa and K.S. Roa (2008). Efficacy of antimicrobial metabolites of *Pseudomonads fluorescens* against rice fungal pathogens. *Curr. Trends Biotechnol. Pharmacy.,* **2:** 178-182.

Roy, A.K. (1993). Sheath blight of rice in India. *Indian Phytopathology.,* **46:** 197-205.

Savary, S., P.S. Teng, L. Willocquat, F.W.J. Nutter (2006). Quantification and modeling of crop losses: A review of purposes. *Annual Review of Phytopathology.,* **44:** 89-112.

Srinivas, P., Ratan, Prakash Patel and G. Bindu Madhavi (2013). Review on Banded leaf and sheath blight of rice caused by *Rhizoctonia solani* Kuhn. *International Journal of Applied Biology and Pharmaceutical Technology.,* **61(2):** 80-97.

Sutha S.P., M. Venkatesan K. Arunkumar (2011). Endobiotic bacteria in some seaweeds of Thondi coastal region in Palk Bay Tamil Nadu India. *J. Mar. Biol. Ass. India*., **53:** 251-256.

Suthin Raj, T., D. John Christopher and H. Ann Suji (2014). Efficacy of *Pseudomonas fluorescen*s on control of chilli fruit rot caused by *Colletotrichum capsici. African journal of Microbiological Research.,* **8(29):**2772-2777.

Suthin Raj, T. and D. John Christopher (2008). Effect of bio-control agents and fungicides against Chilli fruit rot (*Colletotrichum capsici*(s) Butler and Bisby) and effect on seed quality of chilli. *Green farming.,* **1(8):** 20-22.

Suthin Raj, T., A. Muthukumar, N. Muthukumaran, G.B. Sudhagar Rao and H. Ann Suji (2019). Induction of defence enzymes activities in rice plant treated by seaweed algae against Rhizoctonia solani Kuhn causing sheath blight of rice. *Journal of Pharmacognosy and Phytochemistry.,* **SP2:** 210-218.

Suthin Raj, T., S. Vignesh, A. Arumuka pravin and H. Ann Suji (2018). Bio chemical characterization of brown seaweed, Identification of antifungal activity associated with mangrove leaf and its efficacy on management of rice sheath blight caused by *Rhizoctonia solani* (Kuhn). *International Journal of Tropical Agriculture.,* **36(2):** 449-461.

Vivekananthan, R., M. Ravi, A. Ramanathan and R. Samiyappan (2004). Lytic enzymes induced by *Pseudomonas fluorescens* and other biocontrol organisms mediate defense against the anthracnose pathogen in mango. *World J. Microbiol. Biotechnol.,* **20:** 235-244.