Available online at www.jpsscientificpublications.com
Volume – 2; Issue - 1; Year – 2018; Page: 100 – 105
ISSN: 2456-7353
DOI: 10.22192/ijias.2018.2.1.2

International Journal of Innovations in Agricultural Sciences (IJIAS)

ANTIMICROBIAL ACTIVITY OF BACTERIA ASSOCIATED WITH SEAWATER AGAINST RICE SHEATH BLIGHT CAUSED BY *Rhizoctonia solani* Kuhn

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Abstract

Sheath blight caused by *Rhizoctonia solani* Kuhn [*Thanatephorus cucumeris* (Frank) Donk] occurs throughout the temperate and tropical rice growing regions. Although, chemicals control the rice sheath blight disease, the use of continuous, inappropriate and non-discriminative use of chemicals is known to cause undesirable effect such as residual toxicity, development of resistance, environmental pollution, health hazards to humans and animals and increased the cost of expenditure for plant protection. The fungitoxic effects of 12 isolates of bacterial biocontrol agents from various sea water and sediments were evaluated under *in vitro* conditions on growth of *Rhizoctonia solani*, the causal agent of sheath blight. *Bacillus subtilis* Bs-1 was the most effective, showing 70 % inhibition of colony growth with a minimum mean mycelial dry weight (121.75 mg/50m/broth) of the pathogen. The aim of this research work was to study the use of biocontrol agents as an alternative to fungicide in the control of sheath blight of rice. The present study revealed that the efficacy of bacterial biocontrol agents against fungal pathogens may be due to higher levels and early accumulation of phenolics and phytoalexins, and the field study proved that, *Rhizoctonia solani* can be controlled by the application of *Bacillus subtilis*.

Key words: Seawater, Antagonistic bacteria, Sheath blight and Antimicrobial activity.

1. Introduction

Rhizoctonia solani Kuhn is the causal agent of rice sheath blight which has become a major constraint to rice production during the last two decades (Savary *et al*., 2000). The intensification of rice cropping systems with the development of new short stature, high tillering, high yielding varieties, high plant density and an increase in nitrogen fertilization (Gangopadhay and Chakrabarthi, 1982; Ou, 1985) has seen the "emergence of *Rhizoctonia solani* as an economically important rice pathogen". This pathogen can survive in soil for many years by producing small (1 - 3 mm diameter) irregular shaped, brown to black sclerotia in soil and on plant tissues. The ability of *Rhizoctonia solani* to produce sclerotia with a thick outer layer allows them to float and survive in water. *Rhizoctonia solani* also survives as mycelium by colonizing soil organic matter as a saprophyte, particularly as a result of plant pathogenic activity (Roy, 1993).

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***Received*:** 10.01.2018; ***Revised*:** 30.01.2018;
***Accepted*:** 18.02.2018.

IJIAS ISSN: 2456-7353

The sclerotia present in the soil and/or on plant tissue germinate to produce vegetative threads (hyphae) of the fungus that can attack wide range of food and fibre crops. However, loss due to rice sheath blight disease generally vary from 30 to 40 per cent in endemic areas and when the disease spreads to upper parts of the plant and panicles, a total crop loss was observed (Srinivas *et al*., 2013; Hane graff and Suthin Raj, 2018). The use of fungicides with a broad spectrum of activity against more than one disease is common in rice. Presently, sheath blight disease management is mainly achieved through systemic fungicides (Suthin Raj *et al*., 2016) and the bacterial bio-control agents like plant growth promoting rhizobacteria (PGPR) offer a promising means of controlling plant diseases (Mew and Rosales, 1992; Suthin raj *et al.,* 2014). In order to survive some bacteria synthesis compounds show an inhibitory effect on the growth and attachment of co-occurring bacterial species competing for the same niche (Meusnier *et al*., 2001). However, few studies have been conducted on bacteria of seawater and sediments (Meusnier *et al*., 2001). Even though studies on isolation of bacteria from marine environment have been conducted in India, they are in connection with soil, sediments and fauna (Sivakumar *et al*., 2007). As a tropical country, India has vast coastal waters that experience wide fluctuation of climate such conditions is expected to contain bacteria with a wide array of chemical compounds.

2. Materials and Methods

Isolation, maintenance and identification of *Rhizoctonia solani*

Diseased chilli fruits showing typical symptoms of anthracnose disease were collected fresh from 20 conventional rice growing areas of Tamilnadu. The pathogens isolated from each of these localities formed one isolate of *Rhizoctonia solani*. The pathogen was isolated onto Potato dextrose agar (PDA) medium from diseased specimens showing typical symptoms. The infected portion of the fruit was cut into small pieces, surface sterilized in 0.1 % mercuric chloride solution for 30 seconds and then washed in repeated changes of sterile distilled water and plated onto sterile PDA medium in 9 cm petridishes. The plates were incubated at room temperature (28 ±2 °C) for five days and then observed for fungal growth. Pure cultures were obtained using the single spore isolation technique (Rangaswami, 1958). Identification of the isolates was done using the method given by confirmed by comparing them with pure culture by obtained from the ITCC, IARI, New Delhi and the purified isolates were maintained on PDA slants.

Evaluation of antagonistic bacteria against *Rhizoctonia solani*

Isolation of bacteria from seawater and sediments (Sutha *et al*., 2011)

In the present investigation, seawaters (Table A) were collected along the coast of Parangipettai, Cuddalore and Pichavaram. Sediment sample weighing 1.0 g was extracted in sterile water using an orbital shaker for 30 min and the volume was made up to 10 ml. Different serial dilutions such as, 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} were prepared from the 10 ml of made up samples (sediments) as well as from the 10 ml of seawater samples. For each dilution, 100 µl swab 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 picked up every 6 hrs upto 4 days and streaked on the fresh plates containing ZoBell marine agar. Pure culture of each isolates was confirmed by subsequent restreaking. Then, they were designated with unique codes and stored in glycerol suspension (glycerol/bacterial broth of 1:1 v/v) in Eppendorf tubes at -80 °C for further investigation**.** The following bacterial species were identified by biochemical studies in Center for Advance Studies in Marine Biology, Annamalai University, Chidambaram, Tamil Nadu, India.

Table – 1: List of bacterium recorded from the samples of seawater and sediments

S. No	Place	Bacterium	
		Seawater	Sediments
1	Pichavaram	*Bacillus subtilis* *Aeromonas salmonicida*	*Bacillus subtilis* *Aeromonas hydrophila*
2	Cuddalore	*Bacillus cereus* *Bacillus subtilis*	*Lactobacillus fermenti*
3	Parangipettai	*Aeromonas hydrophila* *Aeromonas salmonicida* *Lactobacillus fermenti Bacillus subtilis*	*Aeromonas hydrophila* *Aeromonas salmonicida*

Screening of marine bacterial isolates for antibiotic production (Ann Suji *et al*., 2014)

According to the morphological, Gram's staining and biochemical characteristics described in the Bergey's manual, 12 strains belonging to 5 bacterial category were enumerated from various samples like seaweed, sediment and seawater and were evaluated for antibiotics production. Bacteria grown in the medium developing inhibition zone around the discs were considered as antibiotic producers. Thus 3 bacteria from 6 strains were found as antibiotic producers and they were then taken up for further screening against plant pathogens.

Dual culture

Bacillus subtilis was grown on nutrient agar (Peptone – 5 g, Meat extract – 1 g, Yeast extract – 2 g, Sodium chloride - 5 g, pH 7.0) medium. A 8 mm actively growing PDA culture disc of the pathogen was placed on PDA medium in sterilized petridish at one side, 1.5 cm away from the edge of the plate, and incubated at room temperature (28 ± 2 °C). After forty eight hrs, actively growing 48 hrs old cultures of the respective test bacteria were separately streaked on to medium at the opposite side of the plate, 1.5 cm away from the edge of the plate. The inoculated plates were incubated at room temperature (28 ± 2 °C). Three replications were maintained for antagonist activity. Potato dextrose agar medium inoculated with the pathogen alone served as control. After 8 days, the radial growth of the pathogen was measured. The results were expressed as per cent growth inhibition over control. The most effective isolates of *Bacillus subtilis* were used for further study. Three replications were carried out for each treatment.

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 per cent of the filtrate in broth. The flask was inoculated with 8 mm culture disc of *Rhizoctonia solani* and incubated at 28 ± 1 °C for 10 days. Broth without any filtrate served as the control. Three replications were maintained. After the incubation period, the mycelial mat was harvested on a previously weighed filter paper and dried at 105 °C for 12 hrs in a Hot air oven, cooled in desiccators and the mycelial weight was recorded and expressed as mg/50 ml/broth.

Experimental design and data analysis

The experiments were conducted using 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 transformed before statistical analysis following appropriate methods.

3. Results and Discussion

Effect of Marine bacteria against *Rhizoctonia solani*

The results of the screening of five isolates ofbacteriaagainst *Rhizoctonia solani* on PDA plates are presented in Table - 1. Among the *Bacillus* sp. isolates *Bacillus subtilis* Bs-1 appeared to be the most effective against the test pathogen showing 70 per cent inhibition of colony growth and minimum mean mycelial growth of pathogen (121.75). It was followed by isolated *Bacillus cereus* showing 67.50 per cent inhibition and minimum mean mycelial growth (138.75) which where statistical on par with each other. The isolate *Lactobacillus fermenti* gave minimum growth inhibition (58.40) and minimum mycelial growth of the pathogen. All the isolates significantly reduced the mycelial growth of the pathogen over the control.

Mycelial Growth

The mycelial growth of the pathogen was tested against *Bacillus subtilis* at 10, 20, 30 and 40 per cent concentrations. Among them, *Bacillus subtilis* isolated was significantly reducing the growth of mycelium which recorded 207, 164, 84 and 30 mg/50 ml broth respectively. It was followed by *Bacillus cereus* isolated 2 with 228, 195, 100 and 36 mg/50 ml broth. All the isolates significantly reduced the mycelia growth of the pathogen over the control (Table - 1). Hence, the superior isolate *Bacillus subtilis* was used for further studies.

Table - 2: Evaluation of various isolates of *Bacillus subtilis* against *Rhizoctonia solani* by Dual culture technique

Isolates	Linear growth (mm)		% Growth inhibition	Mycelial dry weight (mg/50m/broth)				
	Antagonist	*R. solani*		10 %	20 %	30 %	40 %	Mean
Bacillus subtilis	63.00	27.00	70.00[a]	207	164	84	30	121.75[a]
Aeromonas hydrophila	58.40	31.60	65.10[b]	267	219	117	49	163.00[c]
Lactobacillus fermenti	51.70	38.30	578.40[d]	325	247	155	55	195.50[d]
Bacillus cereus	59.80	30.20	67.50[b]	228	195	100	36	138.75[b]
Aeromonas salmonicida	54.00	36.00	61.60[c]	294	223	136	52	176.25[c]
Control	0.00	90.00	0.00[e]	480	480	480	480	480.00[e]

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

Five bacterial biocontrol agents were isolated from seawater and sediments and their efficacy against *Rhizoctonia solani* was tested. The present study revealed that among the five isolates *Bacillus subtilis* Bs-1 was maximum inhibited the growth of *Rhizoctonia solani* in dual plating technique. A similar result was observed by Vivekananthan *et al*. (2004) and Anand *et al*. (2010). They reported that isolate Bs-1 was strongly inhibited the growth of *Rhizoctonia solani* in laboratory condition and field condition. This may be due to *Bacillus subtilis* isolate produce array of antifungal antibiotics such as 2, 4 - diacetylphloglucinol, oligomycin, phenazine, pyoluteorin, pyrolnitrin and pyocyanin (Gupta *et al*., 2001; Suthin raj, 2011). Similarly, antifungal compounds such as HCN, salicyclic acid and 2 - hydroxyl phenazine produced by bacterial biocontrol agents suppressed plant pathogenic fungi (Hofte and Bakker, 2007; Reddy *et al*., 2008; Suthin raj, 2011).

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DOI Number **DOI: 10.22192/ijias.2018.2.1.2**

How to Cite this Article:

S. Dhaarani, V. Dhanasekaran, R. Dharshini, S. Enthira, 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.

DOI: 10.22192/ijias.2018.2.1.2