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SCREENING OF BACTERIAL ENDOPHYTES FROM RICE LEAF SHEATH AGAINST A VIRULENT ISOLATE OF *Rhizoctonia solani*

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ABSTRACT

The research was conducted to identify the bacterial endophytes from rice leaf sheath against Rhizoctonia solani that caused sheath blight disease of rice. This study was done in the Mycology and Advanced Nematology Laboratory of the Department of Plant Pathology, Sher-e-Bangla Agricultural University. Twelve R. solani isolates were collected from six upazilas of Bangladesh. Among twelve isolates, RSRPS11 showed the highest relative lesion height (30.66%) on BRRI Dhan 34 in pot. The highest disease severity (20.45%) was also recorded for isolate RSRPS11. Eight bacterial endophytes were isolated from healthy leaf sheaths of rice from these six areas. Endophytic bacteria showed creamy, milky white, creamy yellow, orange yellow, and pale white colonies in color. Colony size variation was also observed. At 7 days of dual culture with R. solani, five isolates showed no inhibition, and Iso-4 produced the highest (51.11 %) inhibition zone. These strains of R. solani and the potential bacterial endophyte could be used in future research of sheath blight disease management.

Keywords: bacteria, blight, endophytes, inhibition, sheath

INTRODUCTION

A staple food for about 50% of the world's population is rice (Adenle *et al.,* 2019). According to Bangladesh Rice Knowledge Bank (2019), the current rice yield of 2.74 t ha^{-1} needs to be enhanced to 3.74 t ha^{-1}. However, various biotic and abiotic factors contribute to significant variations in rice productivity across different areas and seasons, with diseases being the main factor causing a decline in rice yield. There are over 60 diseases that affect rice crops annually, with sheath blight, bacterial leaf blight, stem rot, and blast being some of the most significant ones in different regions of the world that grow rice (Latif *et al.*, 2011). One of the most destructive epidemic diseases of rice is sheath blight, which is caused by *Rhizoctonia solani* (Li *et al.,* 2012). It can reduce rice yield by up to 50% globally (Zheng *et al.,* 2013). Aus, Aman, and Boro - Sheath blight is becoming a more significant issue for rice farming in Bangladesh for all three seasons (Miah *et al.,* 1985; BRRI, 2018).

Managing sheath blight is challenging due to the survival of *R. solani* in soil as sclerotia and within rice plant tissues as mycelia (Jayaprakashvel *et al*., 2012). For over three decades in Asian countries, the primary approach to controlling sheath blight in rice has been the application of fungicides (Zheng *et al.,* 2013). However, this method has various drawbacks, including the development of pathogen resistance, phytotoxicity, adverse effects on human health, environmental pollution, high costs, and biodiversity loss (Groth *et al*., 1990; Sehajpal *et al.,* 2009). Therefore, alternate approaches to managing diseases are required that offer efficient control without having detrimental effects on the ecosystem. The use of beneficial bacteria and fungi as biological control agents to prevent plant disease is an environmentally acceptable alternative to the commonly employed chemical fungicides. (Nagorska *et al.,* 2007).

A variety of microbial communities play an important role in the vigor and health of plants. They are found both outwardly as epiphytes and inside as endophytes in plant tissues and organs (Hardoim *et al*., 2015). To identify possible endophytes for biocontrol agents and plant growth promotion, it is essential to investigate the variety of bacterial endophytes in landraces and rice types growing in this area. Therefore, the goal of the current study was to identify potential isolates with plant-beneficial features, such as antagonistic activities against *R. solani.* Endophytic bacteria can colonize and cause antagonism at the infection site, which is why researchers are currently working on using them as effective biocontrol agents (Hardoim *et al*. 2008). The study found that bacterial endophytes can effectively control sheath blight concurrently. Based on the aforementioned facts, the current study aimed to isolate *R. solani* causing sheath blight of rice, conduct pathogenic variation of *R. solani,* and screening potential endophytes against sheath blight of rice.

MATERIALS AND METHODS

Collection of samples

Twelve healthy and sheath blight-affected samples of rice plants at the reproductive stage were collected from six different rice growing areas of Bangladesh, including the SAU campus in Dhaka, Ghatail, Sirajganj, Kalihati, Patuakhali, and Pirgacha.

Isolation and identification of the pathogen

Infected rice sheaths were thoroughly inspected and tested within 48 hours of sample collection. Using the tissue planting procedure, the fungus was removed from the rice plants' affected areas (Bashar *et al*., 2010). Sheath blight-affected rice sheaths and culms were cut into tiny (5–6 mm) pieces with sterile scissors. Following a two-minute wash in sterile water, these parts were surface cleaned for two to three minutes using 2% Chlorox. After rinsing the sheath pieces for two to three minutes with sterile water, they were dried on sterile tissue paper. Potato dextrose agar (PDA) medium was made for the purpose of isolating the fungus. The ingredients of PDA were as follows, according to Ricker and Ricker (1936). On PDA plates, the dried materials were cultured for seven days at room temperature (25±2°C). By analyzing the physical and cultural characteristics such as mycelial growth, colony appearance, sclerotia formation, and size of sclerotia, and comparing them with pertinent literature, the pathogen responsible for rice sheath blight was identified.

Designation of collected isolates of *Rhizoctonia solani*

According to their source and location, the isolated samples were labeled. An isolate identified as RSTGM01 implies *R. Solani* (RS); the isolate was obtained from the district of Tangail (T), Upazila Ghatail (G), Village Mirpur (M), and the 01 indicates the collection number following the method designed by Aminuzzaman *et al.* (2010).

Pathogenicity study

To identify a virulent pathogen, a pathogenicity study was conducted. Healthy, uniform seedlings of BRRI Dhan 34 were chosen for the pot experiment 25 days after they were sown. Seedlings were inoculated with *R. solani* at 38 days following transplantation (Kumar *et al.,* 2012). To inoculate the rice plants, immature mycelial sclerotia were put under the leaf sheath. Aluminum foil was quickly used to cover the infected sheath. Three days later, the aluminum foil was removed when the usual lesions showed up. Seven days following inoculation, the lesion length on the sheath of the inoculated plants was measured (Park *et al.,* 2008).

Evaluation of rice sheath blight disease severity

Disease severity was used to measure the disease.
Agrios (2005) used the following formula to calculate the severity of the disease:

$$\% \text{ Disease severity} = \frac{\text{Total lesion height}}{\text{Total sheath height}} \times 100$$

The following formula from Sharma and Teng (1990) was used to determine RLH:

$$\% \text{ Relative Lesion Height} = \frac{\text{Maximum height at which lesion appears}}{} \times 100$$

Using a standard evaluation system (SES), disease severity and relative lesion height were calculated using a disease scale (0–9) (IRRI, 2013). Isolates were grouped into four categories according to their disease reaction: very virulent (8–9), moderately virulent (4–7.9), less virulent (1-3.9), and avirulent (0). Table 1 describes in detail for sheath blight of rice disease.

Table 1. Sheath blight of rice disease score using the Standard Evaluation System (SES), (IRRI 2013).

Disease score	Description
0	No infection
1	lesion limited to the less than 20% of the plant height
3	Spread of lesion up to 21-30% of plant height
5	Spread of lesion up to 31-45% of plant height
7	Spread of lesion up to 46-65% of plant height
9	Spread of lesion more than 65% of the plant height

Isolation of endophytes

A number of healthy samples collected during disease sample collection from the same rice field of different region of Bangladesh. A stock solution was made from the healthy sheaths. Nutrient Agar (NA) media was used for bacterial isolation. After incubation, bacterial colonies were visible after 24 hours of growth. The colonies could be seen and counted because of the high dilution. Eight bacterial isolates were found from these samples marked as ISO-1, ISO-2, ISO-3, ISO-4, ISO-5, ISO-6, ISO-7 and ISO-8. Then pure culture was prepared following the streaking method. To establish pure cultures and perform morphological analysis, a total of 8 isolates were streaked from all the samples. The colony morphological characteristics were studied and revealing various distinct colony features.

In-vitro* evaluation of endophytes against *Rhizoctonia solani

The dual culture method was implemented as per Azadeh *et al.* (2010). On a PDA medium plate, a single sclerotium of a virulent pathogenic fungus was positioned on one side of a petri plate. In the case of bacteria, the isolates were spaced 15 mm from the center and streaked aseptically parallel to the pathogenic fungus. The control trial was conducted without applying any bacteria to the plate (Plate 1).

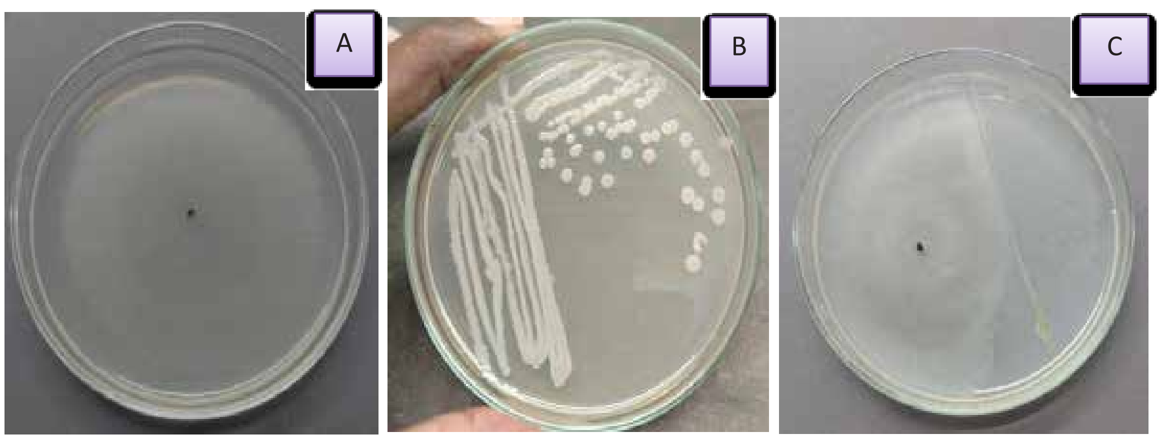

Plate 1. Dual culture of bacterial isolates and *R. solani*; **A.** Pure culture of *R. solani,* **B.** Pure culture of bacterial isolate, **C.** Dual culture

The antagonistic impact was measured by measuring the length of the free growing zone between the isolated bacteria and the tested fungi on all testing plates, which were incubated at 28ºC for 5-7 days. The inhibitory zone, expressed to the closest millimeter, was measured between the two cultures. The following formula was used to calculate the growth inhibition percentage:

$$\%\ \text{Inhibition of growth} = \frac{R - R1}{R} \times 100$$

R = The fungal growth in the absence of hostile bacteria, from the colony's center to the plate's center.
R1 = The fungal growth is directed towards the hostile bacteria from the colony's center.

Analysis of data

Using the Statistix 10 software, the data were statistically examined. For *in-vitro* research, Complete Randomized Design (CRD) with three replications was used to collect the data for the various parameters examined in this study.

RESULTS AND DISCUSSION

Pathogenic variation of *R. solani* on the development of sheath blight disease of rice in pots

The development of the sheath blight disease was observed to be statistically different in response to the inoculation of rice variety BRRI Dhan 34 with *R. solani.* On various days after transplantation (DAT), relative lesion height and disease severity were recorded. The isolates were divided into three groups: avirulant, less virulent, and moderately. According to the pathogenicity perspective, three characteristics are important. Initially, isolates have the potential to cause a wide range of diseases. In addition, the isolates' host range may extend from extremely narrow to very large, and they can be avirulent or highly virulent (Saxena, 1997). A correlation was found by Tu (1967) and Akai *et al.* (1960) between aerial mycelium growth and virulence pattern. In contrast to strains that were demonstrated to be less virulent due to their low mycelium growth, the latter investigation revealed that strains with less aerial mycelium were more virulent.

In this study, RSRPS11, which had less mycelium, was more virulent. RSDSS06 and RSRPS12 isolates were found to have high mycelial growth but were less virulent. The study conducted by Basu *et al.* (2004) revealed that there was no discernible relationship between an isolate's mycelial development and its pathogenicity towards its host.

Effect of *R. solani* isolates on the height of the lesion in infected sheath

After 10 days of inoculation, the relative lesion height percentage was assessed. Isolate RSRPS11 showed the highest relative lesion height (RLH). No lesion was found in the case of isolates RSTGM02, RSTKH04, RSDSS07, and RSSSJ10. The RLH was statistically lowest in RSDSS05 (17.59 %), RSDSS06 (17.95%), and RSRPS12 (18.71%) than others. Different isolates showed different disease reactions after 10 days of inoculation. Isolates RSTGM02, RSTKH04, RSDSS07 and RSSSJ10 were avirulent. All of the other isolates were less virulent (Table 2).

Table 2. Relative lesion height and disease reaction of sheath blight of rice based on the disease scale at 10 days

Isolates	Relative Lesion Height (%)	Disease Scale	Disease Reaction
RSTGM01	24.59	3	less virulent
RSTGM02	0	0	avirulent
RSTGM03	21.42	3	less virulent
RSTKH04	0	0	avirulent
RSDSS05	17.59	1	less virulent
RSDSS06	17.95	1	less virulent
RSDSS07	0	0	avirulent
RSPGP08	26.57	3	less virulent
RSSSJ09	21.59	3	less virulent
RSSSJ10	0	0	avirulent
RSRPS11	30.67	3	less virulent
RSRPS12	18.71	1	less virulent

Effect of *R. solani* isolates on severity of sheath blight disease

After 10 days of inoculation, the highest disease severity was recorded from RSRPS11 (20.45 %). No severity in RSTGM02, RSTKH04, RSDSS07 and RSSSJ10 isolates and were significantly lowest as compared to others. RSTGM03, RSSSJ09 showed 18.07% and 15.3% disease severity, respectively. The lowest severity was identified 2.77% in RSPGP08 isolate. In case of disease severity, disease rating scale was found 0-3 scale. Different isolates showed different disease reaction after 10 days of inoculation. Isolates RSTGM02, RSTKH04, RSDSS07 and RSSSJ10 were avirulent. All of other isolates were less virulent (Table 3).

Table 3. Disease severity and disease reaction of sheath blight of rice based on the disease scale at 10 days

Isolates	Disease severity (%)	Disease Scale	Disease Reaction
RSTGM01	6.53	1	less virulent
RSTGM02	0	0	avirulent
RSTGM03	18.07	1	less virulent
RSTKH04	0	0	avirulent
RSDSS05	5.24	1	less virulent
RSDSS06	6.81	1	less virulent
RSDSS07	0	0	avirulent
RSPGP08	2.77	1	less virulent
RSSSJ09	15.30	1	less virulent
RSSSJ10	0	0	avirulent
RSRPS11	20.45	3	less virulent
RSRPS12	4.74	1	less virulent

Isolation and cultural characteristics of bacterial endophytes

By utilizing the dilution plate approach as outlined in the materials and methods, eight bacterial isolates were obtained from samples of healthy rice plants and purified on NA media. The names ISO-1, ISO-2, ISO-3, ISO-4, ISO-5, ISO-6, ISO-7, and ISO-8 were assigned to the bacterial isolates, in that sequence (Plate 2). Several bacteria's morphologies were examined based on how they appeared (Color, size, shape, size, elevation, texture, and margin) on the nutrient agar plate (Table 4). According to Aneja *et al.* (2015), *Bacillus cereus* and *Escherichia coli* generate spherical, white complete colonies that are, respectively, large and small in size, flat and elevated in elevation, and dry or wet in texture. *Pseudomonas aeruginosa* is characterized by round, smooth, greenish-yellow colonies with convex elevations and entire borders. (Ningthoujam and Shovarani, 2008). Round, small, smooth, and raised colonies of pale yellow and yellow colors are produced by *Burkholderia* and *Xanthomonas,* respectively (Kim *et al.,* 2021).

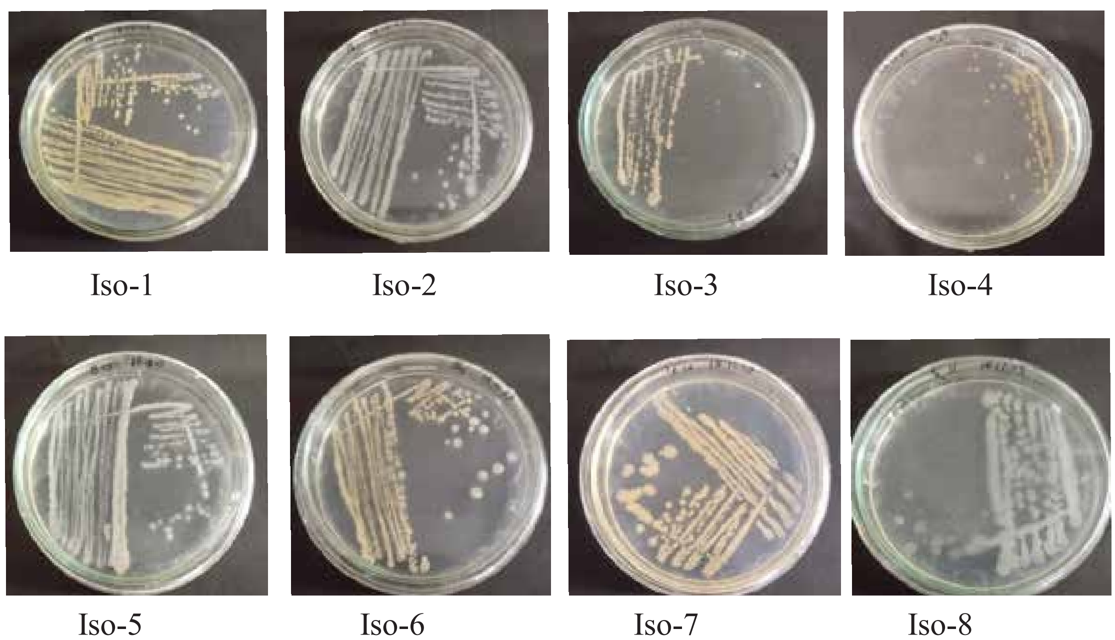

Plate 2. Pure culture of different bacterial isolates

In this research, ISO-1, ISO-6 and ISO-7 was found creamy in color, ISO-2 and ISO-5 were milky white, ISO- 3 was creamy yellow and ISO-8 was pale white in color. ISO-2, ISO-3, ISO-4, and ISO-7 were round in shape. ISO-6 and ISO-8 were irregular. ISO-1, ISO-3, ISO-4, ISO-6 and ISO-8 were found raised elevation, ISO-2, ISO-5 and ISO-7 were flat. In case of texture, ISO-1, ISO-3, ISO-4, and ISO-6 were moist, ISO-2, ISO-5, and ISO-7 were dry, and ISO-8 was velvet in texture.

Table 4. Cultural characters of the isolated bacterial endophytes

Isolates	Color	Colony size	Colony shape	Elevation	Texture	Margin
ISO-1	Cream	Small to medium	Round to oval	Raised	Moist	Smooth
ISO-2	Milky White	Medium	Round	Flat	Dry	Sandy
ISO-3	Creamy yellow, Butter	small	Round	Raised	Moist	Smooth
ISO-4	Orange yellow	Small	Round	Raised	Moist	Smooth
ISO-5	Milky White	Small to medium	Round to irregular	Flate	Dry	Sandy
ISO-6	Cream	Large	Irregular	Raised	Moist	Smooth
ISO-7	Cream	Large	Round	Flate	Dry	Smooth
ISO-8	Pale white, water	Large	Irregular	Raised	Velvet	Smooth

***In-vitro* evaluation of endophytes against sheath blight disease of rice**

A study of the antagonistic effect of bacterial isolates and their antibiosis was shown at 7 days (Table 5). The isolates of ISO-3, ISO-5, ISO-6, ISO-7, and ISO-8 were found to be unable to prevent the development of *R. solani* in dual culture techniques. The largest zone of inhibition (51.11%) was observed in case of ISO-4 while ISO-1 showed the lowest inhibition zone (0.24%), followed by ISO-2 (0.17%). Nagendran *et. al.* (2013) reported that the endophytic *Bacillus* isolates displayed more than 30% inhibition against *R. solani in vitro* condition. *Penicillium chrysogenum*, *Streptomyces* sp., and *Chaetomium globosum* were all shown to be antagonistic toward test pathogens (Naik *et al.,* 2009). They found that the maximum percent suppression was seen by *C. globosum* against *Nigrospora oryzae* (64.29%), R. *solani* (62.13%), *Alteraria alternata* (62.15%), and *Phoma sorghina* (61.11%). *M. phaseolina* growth (79.81%) was significantly inhibited by *P. chrysogenum*. All investigated strains exhibited antagonistic pathogen activity against *R. solani*, according to Mao *et al.,* (2016).

Table 5. Antibiosis of bacterial isolates with their antagonistic effect

Bacterial Isolates	Pathogen Growth in dual culture (Diameter in mm)	% Inhibition of mycelium growth (mm) of *R. solani*
ISO-1	34	0.24
ISO-2	37	0.17
ISO-3	45	0
ISO-4	22	51.11
ISO-5	45	0
ISO-6	45	0
ISO-7	45	0
ISO-8	45	0
*Control (F)	45	-

CONCLUSION

This research was conducted to identify potential endophytes that could protect rice against sheath blight. A total of twelve isolates of *Rhizoctonia solani* have been collected from six distinct regions in Bangladesh. In case of pathogenicity, isolate RSRPS11 showed the highest relative lesion height (RLH) and disease severity percentage in BRRI Dhan 34 in pot. In case of disease reaction, Isolate RSTGM02, RSTKH04, RSDSS07, and RSSSJ10 were avirulent, whereas the other seven isolates showed less virulence. Among eight (8) potential bacterial endophytes, Isolate-4 showed excellent performance and produced the highest inhibition zone against *R. solani in vitro* conditions at 7 days after inoculation. The results of this investigation showed that bacterial endophytes could effectively control rice sheath blight. The use of bacterial endophytes as a bio-fungicide has hence great potential for controlling this disease, with the added advantage of reducing the risk associated with the application of fungicides that pollute the environment.

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