Journal of Biological Control, 35(3): 130-136, 2021, DOI: 10.18311/jbc/2021/28034

Research Article

Evaluation of rice associated *Bacillus* spp. against sheath blight and bacterial blight of rice

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ABSTRACT: Bacterial antagonist, *Bacillus* spp. cultures were isolated from different places of Kuttanad region. Three effective endophytic *Bacillus* strains viz., B 15, B 17 and B 33 were tested in the separate field experiments against sheath blight and bacterial blight diseases of rice during *Kharif* 2017, *Rabi* 2018-19, *Kharif* 2019 and *Kharif* 2020. In the field study, the *Bacillus* cultures were treated as standard bioagent application methods of seed (10 g/kg), soil (1 kg/acre) and foliar (20 g/litre of water) spraying against the major diseases in rice. The rice associated native *Bacillus* cultures B 15, B 17 and B 33 were found equally effective for the sheath blight and bacterial blight diseases management. All the three native *Bacillus* species can be used as potential biopesticides against rice sheath blight and bacterial diseases in Kuttanad region.

KEY WORDS: *Bacillus* spp., bacterial blight, rice, sheath blight

(Article chronicle: Received: 26-06-2021; Revised: 18-09-2021; Accepted: 21-09-2021)

INTRODUCTION

Sheath blight of rice (*Oryza sativa* L.), caused by *Rhizoctonia solani* – AGA1-IA is a serious disease in Kuttanad region of Kerala. The disease can cause yield losses ranging from 5.2 to 50% depending on environmental conditions, crop stages at which the disease appears (Rajan 1987; Sharma and Teng, 1996). Application of nitrogen fertilizers in high doses (Roy, 1978) close planting (Kannaiyan and Prasad, 1983) and high relative humidity (Dath 1990) favour the disease development. The disease can be effectively controlled by systemic fungicides but these chemicals are hazardous to the environment and human health. Many workers have used antagonistic bacteria for the management of sheath blight disease (Mew and Rosales, 1986; Gnanamanickam *et al*., 1992; Krishnamoorthy and Gnanamanickam, 1997).

Bacterial Leaf Blight (BLB) disease caused by *Xanthomonas oryzae pv. oryzae* is the second important threat for rice production in tropical and temperate countries due to its high epidemic potential. All growth stages of rice are susceptible to BLB and the yield loss due to the disease ranges between 20 and 30%. Severe infection can result in losses up to 80%, based on the crop stage, degree of susceptibility and environmental conditions (Ou, 1985). Rice is a crop which gets drastically affected by climatic changes. Gnanamanickam *et al*. (1999) reported the occurrence of bacterial ooze from infected leaves in warm and humid climate, which contributed to the spread of the disease. The rice crop was vitiated during the three consecutive flash floods which submerged the Kuttanad region in Kerala during 2018, 2019 and 2020 with hectares of cultivated lands damaged. Bacterial leaf blight disease, one of the most common diseases of rice in Kerala has substantially increased in its incidence and severity during flood period resulting in major crop losses. The disease is controlled by spraying high doses of bactericides. The possibility of development of resistance in pathogens, by continuous exposure to these bactericides can result in frequent outbreak of this disease. Therefore, biological control appears to be a viable alternative. Endophytes are beneficial micro organisms that live either a part or whole of their life time inside the host without causing any apparent disease. They occupy the same niche inside host plants as that of pathogens and hence can be possibly utilized as good candidates for the management of plant pathogens. Yousefi *et al.* (2018) reported the antagonistic potential of endophytes isolated from rice against BLB. Four endophytes

viz., Enterobacter spp., *Bacillus* spp*., B. subtilis* and *Pseudomonas putida* exhibited good control of disease with 37.25, 34.25, 33.25 and 28.5% reduction of BLB incidence over control. Endophytes are emerging tools for plant disease management with additional benefits of plant growth promotion and enhanced tolerance to adverse environmental conditions. In this context, *Bacillus* spp. may represent an ecofriendly strategy for managing *Rhizoctonia solani* and *Xanthomonas oryzae* pathogen in rice. The present study was undertaken to test the efficacy of efficient endophytic *Bacillus* spp. isolated from Kuttanad rice in the management of sheath blight and bacterial blight diseases in the region.

MATERIALS AND METHODS

Survey was conducted at different locations of Kuttanad for the plant sample collection for isolation of sheath blight and bacterial blight pathogens and antagonist organism. The endophytic *Bacillus* spp. were isolated from healthy plant leaves using Tryptic soya agar medium containing pancreatic digest of casein 15.0 g, pancreatic digest of soybean meal 5.0 g, NaCl 5.0 g, agar 15.0 g, distilled water 1000 ml, pH 7.3 (Araujo *et al.,* 2002). The efficacy of antagonist *Bacillus* was tested against pathogens by dual culture technique. The antagonist bacteria were identified by gram staining method.

Gram staining

A thinly spread air-dried bacterial film was fixed on a clean glass slide by a light flame. The specimen was treated with 0.5% aqueous crystal violet for 30 seconds and afterwards washed with running tap water for one minute, rinsed in water and decolorized with 95% ethanol. The specimen was again rinsed with tap water and counter-stained with safranin for approximately 10 seconds. It was eventually washed with water and observed under microscope at 10x and 40x magnifications.

Biochemical characterization

The basic biochemical tests were performed for further confirmatory test. Various biochemical methods like catalase tests, gelatin liquefaction, starch hydrolysis and hydrogen sulphide production have been used for characterizing *Bacillus* isolates. Rapid identification of potentially and economically viable bioagents is possible through various methods of biochemical characterization. (Weller *et al.,* 2002).

Catalase tests

1 ml of bacterial culture was taken in a clean test tube and added 0.5 ml of 3% hydrogen peroxide for testing the rapid elaboration of oxygen bubbles occurs. Immediate liberation of air bubbles indicates that the organism is catalase positive. (Welton *et al.,* 1972).

Gelatin liquefaction

20 ml of a sterile gelatin agar medium was poured into sterile petriplates and allowed to solidify. Each isolate was inoculated into the medium as a single streak for the zone of hydrolysis observed. The plates were then incubated in an inverted position at 37ºC for 24 hours. Then the plates were flooded with mercuric chloride solution. (Hilderbrand *et al*., 1992).

Starch hydrolysis test

Sterile starch agar plates were prepared and a single streak of the bacterial culture was plated. The isolates were incubated at 30ºC for three days and then flooded with Lugol's iodine solution for clear zone development. (Nan *et al.,* 2019)

Hydrogen sulphide production

SIM agar medium tubes were stab inoculated by test isolates and incubated for 24-48 hours at 37ºC. Tubes were then observed for the presence of black coloration along the line of stab inoculation indicating hydrogen sulphide production (Clarke *et al*., 1953).

Three efficient endophytic strains viz., B 15, B 17 and B 33 were found effective against sheath blight and bacterial blight pathogen under in vitro conditions of dual culture technique. It was promoted for mass multiplication in the talc formulations for in vivo study purpose. Talc based formulations were prepared for B 15, B 17 and B 33 strains following the method described by Nandakumar *et al.* (2001) and used for field experiments. The field experiments were conducted consecutively for four seasons during *Kharif* 2017, *Rabi* 2018-19, *Kharif* 2019 and *Kharif* 2020 at Rice Research Station, Moncompu using the above individual strains of *Bacillus* spp. against the two major diseases of rice viz., sheath blight and bacterial blight diseases as separate experiment. Each treatments included seed treatment (10 g/kg of seed), soil application (1 kg/acre at 35 DAS) and foliar application (2% at 55 DAS). The systemic fungicide Hexaconazole 5 EC (0.2%) and the bactericide Streptocycline were included as standard checks for comparative analysis. *Pseudomonas fluorescens* (P 1*)* obtained from College of Agriculture, Vellayani was also used as standard in the field experiments. The experiment was laid out in a Randomized Complete Block Design (RBD) with six treatments and four replications. MO 16 (Uma) was used as test variety. Pregerminated seeds were used for direct sowing in plot sizes of 5x2 m^2. Fertilizer was applied as per Package of Practices (POP), Kerala Agricultural University (90:45:45 NPK kg/ha). The sheath blight pathogen was multiplied on autoclaved paddy straw and artificially applied at the base of the crop during tillering stage. The bacterial blight pathogen was

multiplied in liquid nutrient broth and artificially inoculated by leaf clip method at maximum tillering stage. Three sampling units of 1 m^2 area were fixed in each plot at random.

Observations on sheath blight and bacterial blight severity were recorded just before and 20 days after foliar application of the above treatments. Degree of severity was graded based on height of the plant portions affected by the sheath blight disease and expressed as percentage of the total area as per the SES scale of rice - IRRI (2014). Bacterial blight severity was measured based on the percentage of leaf area affected by the pathogen and graded as 0-9 SES scale system for calculation of Percent Disease Index (PDI). Grain yield from each plot was recorded and expressed in kg/ha at 14% moisture. Data on percentages were transformed and analysis of variance was performed with transformed values. Significance among mean treatments was determined according to Duncan's multiple range tests (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

The present study was conducted at different locations of agro ecological regions in Kuttanad like Lower Kuttanad, Upper Kuttanad and Karilands. Three effective strains o*f* *Bacillus* spp. (B 15, B 17 and B 33) were isolated for the mass production of biocontrol agents against sheath blight and bacterial blight pathogen and their efficacy to control sheath blight and bacterial blight were confirmed by dual culture technique. The purple color rod shaped gram positive *Bacillus* bacterium was observed under microscope in the gram staining technique (Figure 1).

Immediate liberation of air bubbles were observed in the three *Bacillus* spp. and confirmed its catalase positive nature (Figure 2). The clear zone of hydrolysis was noticed in the medium of gelatin agar under *in vitro* condition (Figure 3).

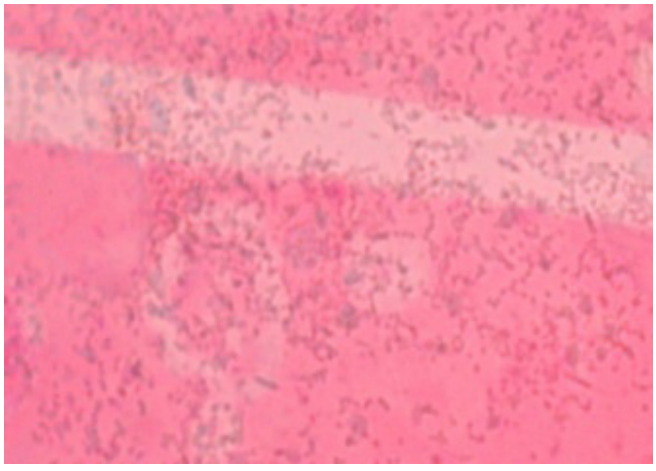

Fig. 1. Gram staining *Bacillus* spp.

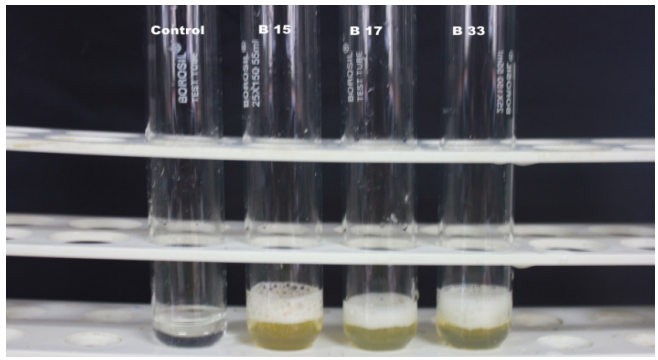

Fig. 2. Catalase test

Fig. 3. Gelatin liquefaction

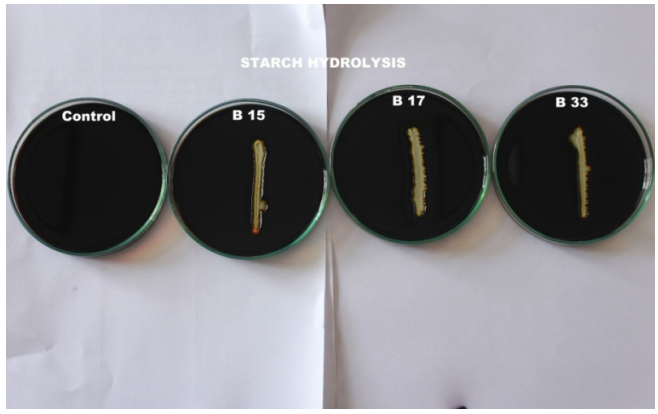

Fig. 4. Starch hydrolysis

In the starch hydrolysis test, a clear zone around the organism indicates positive result and confirmed *Bacillus* spp. (Figure 4). The hydrogen sulphide production studies of *Bacillus* spp. proved that the presence of black coloration along the line of stab inoculation indicating hydrogen sulphide production. The biochemical tests indicated that the three efficient isolates were confirmed as gram positive and positive response for catalase, gelatin liquefaction, starch hydrolysis and hydrogen sulphide production tests (Table 1).

The pooled results of sheath blight management trial revealed that the plots treated with *Bacillus* B 17 formulation

Table 1. Biochemical tests of *Bacillus* spp.

***Bacillus* cultures**	**Catalase test**	**Gelatin liquefaction**	**Starch hydrolysis test**	**Hydrogen sulphide production**
B 15	+	+	+	+
B 17	+	+	+	+
B 33	+	+	+	+

recorded lower sheath blight severity (24.73 %) (Table 2). This was followed by standard check fungicide Hexaconazole 5 EC (25.74%), B 15 (26.06%), B 33 (27.06%) and standard *Pseudomonas fluorescens* P 1 (28.04%). The maximum yield was obtained from Hexaconazole 5 EC (5345 kg ha^{-1}) and was on par with B 17 (5173 kg ha^{-1}) and B 15 (5136 kg ha^{-1}). The control plot recorded lowest yield of 3599 kg ha^{-1} (Table 3).

Table 2. Influence of endophytic *Bacillus* spp. on sheath blight disease (%) during *Kharif* 2017*, Rabi* 2018-19, *Kharif* 2019 and *Kharif* 2020 (pooled data of four seasons)

Treatments	**Dose/l**	**Sheath blight severity (%)**				
		***Kharif* 2017**	***Rabi* 2018-19**	***Kharif* 2019**	***Kharif* 2020**	**Mean**
B 15	Seed-10 g/kg Soil 1 kg/acre Foliar 20 g/l	9.0 (17.47)	17.78 (24.88)	31.39 (34.02)	24.72 (29.80)	20.72 (27.06)
B 17	Seed-10 g/kg Soil 1 kg/acre Foliar 20 g/l	7.9 (16.32)	15.83 (23.42)	28.61 (32.33)	18.05 (25.10)	17.59 (24.73)
B 33	Seed-10 g/kg Soil 1 kg/acre Foliar 20 g/l	4.6 (12.42)	14.16 (22.06)	33.75 (35.49)	24.72 (29.80)	19.30 (26.06)
PI	Seed-10 g/kg Soil 1 kg/acre Foliar 20 g/l	3.8 (11.36)	18.61 (25.55)	34.86 (36.15)	31.39 (34.02)	22.16 (28.04)
Hexaconazole	2 ml/l	4.4 (12.22)	18.89 (25.70)	15.97 (23.50)	36.11 (36.93)	18.84 (25.70)
Control	-	13.1 (21.22)	38.89 (38.53)	55.00 (47.87)	68.89 (56.07)	43.97 (41.50)
LSD @5% (*P* = 0.05)		**3.33**	**4.98**	**4.37**	**6.58**	
CV (%)		**9.72**	**10.73**	**8.05**	**12.13**	

*Figures given in parentheses are arcsine transformed values

Table 3. Influence of endophytic *Bacillus* spp. on grain yield (kg/ha) of sheath blight experiment during *Kharif* 2017, *Rabi* 2018-19*, Kharif* 2019 and *Kharif* 2020 (pooled data of four seasons)

Treatments	**Dose/l**	**Grain yield (kg/ha)**				
		***Kharif* 2017**	***Rabi* 2018-19**	***Kharif* 2019**	***Kharif* 2020**	**Mean**
B 15	Seed-10 g/kg Soil 1 kg/acre Foliar 20 g/l	3924	7029	2891	6700	5136
B 17	Seed-10 g/kg Soil 1 kg/acre Foliar 20 g/l	4246	6792	2805	6850	5173
B 33	Seed-10 g/kg Soil 1 kg/acre Foliar 20 g/l	3709	6697	2875	6250	4883
PI	Seed-10 g/kg Soil 1 kg/acre Foliar 20 g/l	4408	5771	3625	5500	4826
Hexaconazole	2 ml/l	4085	6959	3913	6425	5345
Control	-	3227.5	3868	2250	5050	3599
LSD @5% (*P*=0.05)		**NS**	**205.47**	**951.60**	**NS**	
CV (%)		**19.518**	**22.07**	**20.49**	**18.81**	

Table 4. Influence of endophytic *Bacillus* spp. on bacterial blight disease (%) during *Kharif* 2017*, Rabi* 2018-19*, Kharif* 2019 and *Kharif* 2020 (pooled data of four seasons)

Treatments	Dose/l	Bacterial blight severity (%)				
		***Kharif* 2017**	***Rabi* 2018-19**	***Kharif* 2019**	***Kharif* 2020**	**Mean**
B 15	Seed-10 g/kg Soil 1 kg/acre Foliar 20 g/l	4.53 (12.25)	17.78 (24.88)	27.77 (31.76)	36.66 (37.23)	21.68 (27.69)
B 17	Seed-10 g/kg Soil 1 kg/acre Foliar 20 g/l	4.28 (11.97)	15.83 (23.42)	25.69 (30.40)	35.83 (36.75)	20.40 (26.85)
B 33	Seed-10 g/kg Soil 1 kg/acre Foliar 20 g/l	5.37 (13.44)	13.33 (21.39)	24.44 (29.60)	30.27 (33.34)	18.35 (25.33)
PI	Seed-10 g/kg Soil 1 kg/acre Foliar 20 g/l	5.25 (13.18)	20.28 (26.71)	16.52 (23.97)	36.66 (39.00)	19.67 (26.28)
Streptocycline	0.2 g/l	4.76 (12.66)	17.78 (24.88)	14.30 (22.22)	39.16 (38.70)	19.00 (25.84)
Control	-	18.81 (25.70)	38.89 (38.53)	56.25 (48.56)	62.50 (52.24)	44.11 (41.61)
LSD @5% (*P* = 0.05)		**2.12**	**5.17**	**6.80**	**3.62**	
CV (%)		**6.30**	**11.17**	**13.47**	**6.22**	

*Figures given in parentheses are arcsine transformed values

The use of *Bacillus* and *Pseudomonas* species for the control of *Scleroium rolfsii* and soil borne fungal pathogen under greenhouse conditions were reported by Kishore *et al.* (2005). Hena Jamali *et al.* (2020) confirmed that *Bacillus subtilis* (strain RH5) significantly increased plant growth and triggered resistance in rice plants through the production of defense-related antioxidant enzymes. The strain RH5 exhibited significant antagonistic activity (84.41%) against the rice sheath blight pathogen *R. solani* in the green house study.

The four seasons pooled data of bacterial blight experiment showed that the *Bacillus* B 33 culture recorded lowest bacterial blight severity (25.33%) followed by Streptocycline (25.84%), standard P 1(26.28%), B 17 (26.85%) and B 15 (27.69%) (Table 4). The B 33 culture treated plots gave maximum yield of 6477 kg ha^{-1} and was on par with bactericide Streptocycline (6177 kg ha^{-1}), B 17 (5876 kg ha^{-1}) and B 15 (5712 kg ha^{-1}). The control plot recorded with lowest yield of 4501 kg ha^{-1} (Table 4). Rice plants treated with *B. subtilis* UiTMB1 before being inoculated with BLB pathogen showed less severe disease symptoms with low disease severity index of 3.43 compared with untreated control of 8.4 (DSI). Besides controlling the disease, *B. subtilis* UiTMB1 also enhanced plant height, chlorophyll content, number of tillers and biomass of rice plants (Ku Asmah and Sapak, 2020). In the current study, the biocontrol agent B 33 showed maximum reduction of bacterial blight and was on par with yield of bactericide treated plots followed by other *Bacillus* cultures B 17 and B 15. The similar kind of study with Rice Associated *Bacillus spp.* was reported against sheath blight and bacterial panicle blight by Shrestha *et al.* (2016). They reported five selected rice associated *Bacillus spp*. showing highest antimicrobial activities and also significantly suppressed the disease development of sheath blight and bacterial panicle blight in a field condition, suggesting that they can be potential biological control agents for these rice diseases. The results of the present study are also similar to the earlier observations that the rice associated *Bacillus* cultures B 15, B 17 and B 33 were found to be effective in reducing sheath blight and bacterial blight diseases of rice.

CONCLUSION

Based on the above results, it can be concluded that the bioformulation of *Bacillus* cultures viz., B 15, B 17 and B 33 can be applied individually as seed treatment (10 g/kg) + soil (1 kg/acre) + foliar (20 g/litre of water) application for the effective control of sheath blight and bacterial blight diseases of rice in Kuttanad.

ACKNOWLEDGEMENTS

Authors would like to acknowledge the Director of Research, Kerala Agricultural University and Professor and

Table 5. Influence of endophytic *Bacillus* spp. on grain yield (kg/ha) of bacterial blight experiment during *Kharif* 2017, *Rabi* 2018-19, *Kharif* 2019 and *Kharif* 2020 (pooled data of four seasons)

Treatments	Dose/l	Grain yield (kg/ha)				
		***Kharif* 2017**	***Rabi* 2018-19**	***Kharif* 2019**	***Kharif* 2020**	**Mean**
B 15	Seed-10 g/kg Soil 1 kg/acre Foliar 20 g/l	5050	8075	4558	5163	5712
B 17	Seed-10 g/kg Soil 1 kg/acre Foliar 20 g/l	5395	7600	5031	5478	5876
B 33	Seed-10 g/kg Soil 1 kg/acre Foliar 20 g/l	5913	8095	5398	6501	6477
PI	Seed-10 g/kg Soil 1 kg/acre Foliar 20 g/l	5150	7095	4193	5268	5426
Streptocycline	0.2 g/l	5695	7955	5145	5913	6177
Control	-	4703	6023	3949	3330	4501
LSD @5% (*P* = 0.05)		**590.64**	**NS**	**718.25**	**1749.13**	
CV (%)		**7.37**	**14.85**	**10.12**	**22.01**	

Head, Rice Research Station, Moncompu for facilities and encouragement. The authors also acknowledge the funding agency of 13th Finance Commission Grand-in-Aid Scheme.

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