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RESEARCH **P**APER

Identification and utilization of actinobacteria for biocontrol of rice sheath blight pathogen, *Rhizoctonia solani*

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Sheath blight of rice is an important soil-borne fungal disease caused by *Rhizoctonia solani*. Isolation and exploitation of bioagents associated with rice offers a great opportunity for sustainable management of rice diseases. Actinomycetes were isolated from rice rhizosphere and isolates were putatively selected based on characteristic colony morphology. 16S rDNA and ARDRA confirmed their identification as actinobacteria. Isolates belonged either to *Streptomyces* spp. or *Actinopolymorpha* spp. Under *in vitro* condition, isolates IABT-A1, IABT-A2, IABT-A3, IABT-A6, IABT-A7, IABT-A8 and IABT-A9 showed 98-100 per cent inhibition of *Rhizoctonia solani*, rice sheath blight pathogen. Further, five isolates were evaluated under glass house condition and the potent actinobacteria against rice sheath blight was identified. Among these isolates, IABT-A7 (*Actinopoymorpha* spp.) was most effective and application as seed treatment, soil and foliar spray was found most promising in terms of disease reduction and plant growth promotion. *Actinopoymorpha* spp. (IABT-A7) enhanced plant height, root length and root biomass in addition to reduced sheath blight infection in rice. This result indicates the role of actinobacteria isolate IABT-A7, as the probable stimulator of ISR (Induced Systemic Resistance) signaling pathway involved in plant disease resistance.

Key words : Rice, *Rhizoctonia solani*, Actinomycete, Biocontrol, Sheath blight

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INTRODUCTION

Rice (*Oryza sativa* L.) is the most widely cultivated food crop in the world with China and India being the major producers. Rice production is constantly challenged by several biotic stresses of which diseases like blast, sheath blight (ShB) and bacterial blight are important. ShB of rice is an important soil-borne fungal disease (*Rhizoctonia solani* Kuhn) causing up to 40 per cent of yield losses annually, especially when susceptible cultivars were grown (Kazempour, 2004). 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 favourable conditions. Grain losses to an extent of 40 per cent are reported annually with the disease (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). The disease manifests initially as water soaked lesions on sheaths of lower leaves near water line. As the disease advances the lesions expand and are bleached with a brown border. Under severe conditions plants may dry up and chaffiness of the panicle is seen.

Genetic sources of ShB resistance are not adequate, and present management strategies mostly involve use of chemical fungicides. The adverse effects of chemical fungicides on environment and beneficial microflora are evident and so an economic and viable alternative for ShB management is essential. Use of biocontrol agents in plant disease management is an ecologically-friendly and cost effective strategy which can be used in integration with other management tactics for sustained crop yields. A successful bioagent should not only be able to control or reduce the disease but also contribute to crop growth promotion and yield. Among different biocontrol agents, plant growth-promoting

rhizobacteria (PGPR) are widely used in managing soil borne diseases of several field crops.

PGPR group offers an effective means of antagonism against ShB pathogen (Luo *et al.*, 2005). Besides, they also contribute to enhanced seedling growth, induced systemic resistance against diseases and thereby yield increase (Pathak *et al.*, 2004). Rice plants and ecosystem are the natural habitat of many bacteria that are beneficial in pathogen control and in plant growth promotion (Chakraborty *et al.*, 2006). They also produce certain antibiotics and enzymes such as phenylalanine ammonia-lyase (PAL), peroxidases, and pathogenesis-related proteins (PR) in rice leaves when applied against ShB disease (Jayaraj *et al.*, 2004). Actinomycetes have been reported as an important biocontrol agents of phytopathogens especially, *Streptomyces* spp., as an attractive alternative for use in agriculture (Ara *et al.*, 2012). In this view, the present research was taken up to isolate actinomycetes from rice rhizhosphere, characterize them at molecular level and to evaluate against sheath blight pathogen *Rhizoctonia solani* under *in vitro* and *in vivo* condition.

RESEARCH METHODOLOGY

Present investigation was undertaken in the Department of Biotechnology, University of Agricultural Sciences, Dharwad, Karnataka, India during 2012-2013.

Sample collection and isolation of actinomycetes from rice rhizosphere :

Soil sample was collected from rhizosphere of rice crop from Rice Research Station (Mugad), Dharwad, Karnataka. The research station is located at 15º15´ North latitude and 74º 40´East longitude at an altitude of 697m above the mean sea level. For isolation of actinomycete, 10 g of soil was weighed and suspended in 100 ml of distilled water. Suspension culture of soil was serially diluted up to 10^{-3}. 100 µl of dilution each from 10^{-2} and 10^{-3} dilutions was spread on Starch Casein agar (SCA) medium containing cycloheximide (100 µg/ml) and streptomycin (50 µg/ml). The plates were incubated at 28^{0}C for 21 days (Shantikumar *et al.*, 2006). Colony selection was based on the colour, differences in morphology and rate of growth. Based on colony morphology putative actinomycete isolates were selected and subjected for molecular characterization.

Actinomycetes growth and DNA preparation :

Actinomycete isolates were grown in luria broth (LB) and incubated in shaker (200 rpm) for 4 days at 30°C. Total genomic DNA of the actinomycetes were extracted by versatile quick prep method for genomic DNA of Gram positive bacteria (Pospiech and Naumann, 1995).

16S rDNA gene specific PCR :

Polymerase chain reaction (PCR) of 16S rDNA gene was conducted and the primers used during the PCR amplification were primer 27f (5'-AGAGTTTGATCC TGGCTCAG-3') and primer 1492r (5'-ACGGCTACCTT GTTACGACTT-3') with the expected PCR amplified fragment size of 1.5 kb (Lane, 1991). For the specific amplification of 16S rDNA fragments of actinomycetes, the reaction mixture (50 µl) was as follows: 2 µl of template DNA, 1.25 mM $MgCl_2$, 1U Taq polymerase (Genei), 1mM of each deoxynucleoside triphosphates, 250 nM of each primer (27f and 1492r). PCR amplification was performed in a thermal cycler Eppendorf Master Cycler (5331) with a temperature programme : Initial denaturation at 95^{0}C for 5 min, followed by 35 cycles of denaturation at 94^{0}C for 1 min, annealing at 57.3^{0}C for 1 min, extension at 72^{0}C for 1 min 30 s and a final extension was done at 72^{0}C for 10 min. The amplification product were analysed by electrophoresis on 0.7 per cent agarose gel containing ethidium bromide (10 µg/ml) and visualized using UV transilluminator.

Restriction endonuclease digestions and analysis :

To identify the isolates at genus level, a rapid identification method was followed based upon amplified rDNA restriction analysis (ARDRA) of 16S rDNA. PCR amplified DNA was initially subjected to digestion with Sau3AI. Samples were electrophoresed on 1.5 per cent agarose gel and subsequent restriction based identification was done (Cook and Meyers, 2003).

Screening of actinomycetes against *R. solani* under *in vitro* condition :

Actinomycete isolate was streaked in the middle of the plate containing Soybean-Casein digest agar medium (SCBA). Agar plugs of mycelium (5 mm diameter) were cut from the edge of an actively growing pathogen colony on PDA with cork borer, and placed on either side after four days of actinomycetes inoculation. The experiment was replicated thrice with control as only pathogen. The plates were incubated for seven days at 25º±3 C. After incubation, the per cent inhibition was calculated by using following formula: Inhibition (%) = [(growth radius in untreated control- growth radius in treatment) ×100]/ growth radius in untreated control (Tachowisan *et al.*, 2003).

Evaluation of actinomycetes against *R. solani* under glasshouse condition :

The plastic pots having 4 kg capacity were filled with autoclaved sandy loamy soil and fertilizer was applied @ 100: 50: 50 NPK/ha. The seeds of susceptible rice variety, BPT-5204 (Samba Mahsuri) were surface sterilized and sown in pots. The pathogen was soil inoculated @ 4 per cent and

later inoculated to sheath of 4 week old plants in the form of agar plug of 4 days old culture covered with wet cotton which was properly bound with aluminium foil. The treatments are as follows:T_1: seed treatment of bioagent, T_2: soil application of bioagent, T_3: spraying of bioagent on leaves, T_4: combined (seed treatment +soil application +foliar spraying of bioagent) treatment, T_5: only pathogen, T_6: only bioagent, T_7: untreated control. For seed treatment, seeds were treated with actinobacteria (1.5×10^8 spores/ml) @ 1g of seed per 10 ml suspension along with 0.2 per cent carboxy methyl cellulose. For soil application, culture was multiplied on starch casein broth, population was adjusted to 1.5×10^8 spores/ml and soil drenched. Foliar spraying (1.5×10^8 spores/ml) was done at 45 days after sowing. The data on disease parameters, *i.e*, lesion length, number of dried leaves and plant growth characters *i.e.*, plant height, root length and dry weight of root were recorded and data were analysed statistically.

RESEARCH FINDINGS AND ANALYSIS

The findings of the present study as well as relevant discussion have been presented under the following heads :

Selective isolation of actinomycetes :

Actinomycetes colonies appeared after 20-30 days of incubation and their morphological characteristics were recorded. Isolates differed with varied colony morphology, pigmentation and texture (Table 1). Few isolates produced pigmentation like yellow colour (IABT-A3, IABT-A10), red colour (IABT-A1, IABT-A11). Some of them showed texture like powdery (IABT-A5, IABT-A6, IABT-A7), solid substratum (IABT-A4, IABT-A8, IABT-A9) and compact (IABT-A2). They were putatively identified as actinobacteria and pure culture was maintained.

PCR based identification of actinomycetes :

The isolates were subjected to 16S rDNA test of prokaryotes and ITS test of eukaryotes (18S rDNA region). PCR amplification was observed in all eleven isolates in 16S rDNA test with amplicon size of approximately 1.5kb (Fig. 1) but no amplification was seen in ITS test which confirmed that all isolated cultures were belonging to prokaryotes.

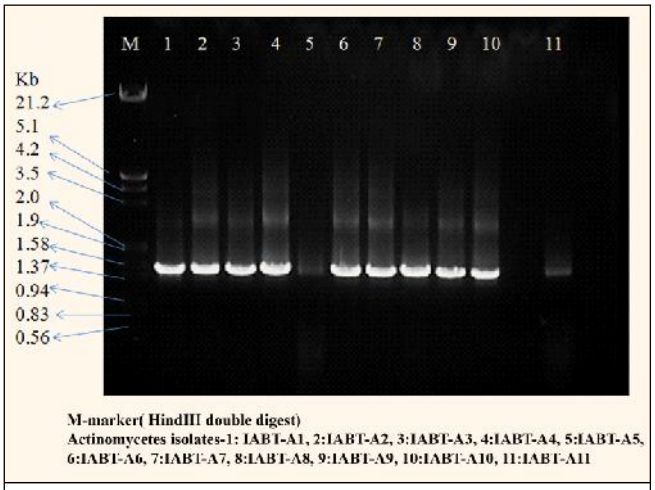

Fig. 1 : 16S rDNA amplification of actinomycete isolate

Characterization of actinomycete isolates at genus level :

Amplified rDNA restriction analysis (ARDRA) revealed that three isolates belonged to *Actinopolymorpha* spp. and remaining eight isolates were grouped under *Streptomyces* spp. (Table 2 and Fig. 2). Isolate IABT-A2, IABT-A6 and IABT-A7 belonged to *Actinopolymorpha* spp. and isolate

Table 1 : Morphological features of actinomycete isolates from rice rhizosphere and in vitro evaluation against *Rhizoctonia solani*

Sr. No.	Isolate	Colony characters			Per cent inhibition of mycelia growth of *R.solani*
		Morphology	Pigmentation	Texture	
1.	IABT-A1	White colour	Red colour	Metallic shiny colony	95.21 (77.34) c
2.	IABT-A2	White colour	White colour	Compact	100 (90) a
3.	IABT-A3	Black colour	Yellowish colour	Metallic shiny colony	100 (90) a
4.	IABT-A4	White colour	White colour	Solid substratum	84.07 (66.48) de
5.	IABT-A5	Black colour	White colour	Powdery	86.30 (68.27) d
6.	IABT-A6	Biscuit colour	White colour	Powdery	95.93 (78.44) bc
7.	IABT-A7	Dark chocolate colour	Grey colour	Powdery	100 (90) a
8.	IABT-A8	Biscuit colour	Whitish Biscuit colour	Solid substratum	100 (90) a
9.	IABT-A9	Light greenish colour	White colour	Solid substratum	96.67 (79.57) b
10.	IABT-A10	Brownish colour	Yellow colour	Metallic shiny colony	74.07 (59.39) f
11.	IABT-A11	White colour	Red colour	Solid substratum	82.22 (65.06) e
	Control				0.00
	S.E. ±				0.48
	C.D. (P = 0.01)				1.93
	C.V. (%)				1.18

* Figures in parentheses are arcsine transformed values

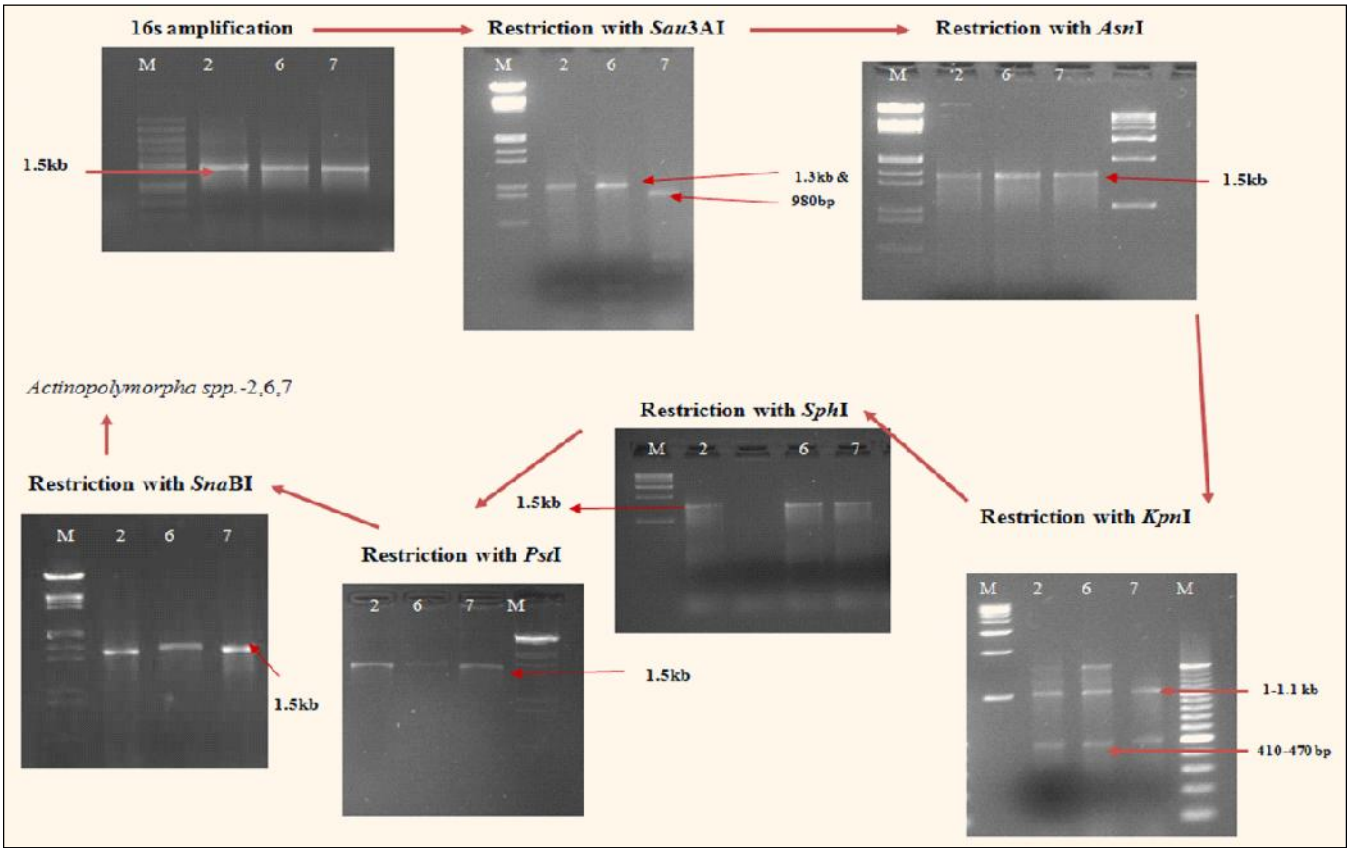

Fig. 2 : Identification of actinomycete isolates through rDNA restricion analysis(ARDRA)

Table 2 : ARDRA analysis of actinomycete isolates

Isolate Name	IABT-A1	IABT-A2	IABT-A3	IABT-A4	IABT-A5	IABT-A6	IABT-A7	IABT-A8	IABT-A9	IABT-A10	IABT-A11
Digestion with Sau3AI											
Results	=750bp	980-1350bp	=750bp	=750bp	=750bp	980-1350bp	980-1350bp	=750bp	=750bp	=750bp	=750bp
Branch	A	B	A	A	A	B	B	A	A	A	A
Digestion with AsnI											
Results	UC 1.5kb	UC 1.5kb	UC 1.5kb	UC 1.5kb	UC 1.5kb	UC 1.5kb	UC 1.5kb	UC 1.5kb	UC 1.5kb	UC 1.5kb	UC 1.5kb
Digestion with KpnI											
Results	410-470bp& 1-1.1kb	410-470& 1000-1100bp	410-470bp& 1-1.1kb	410-470bp& 1-1.1kb	410-470bp& 1-1.1kb			410-470bp& 1-1.1kb	410-470bp& 1-1.1kb	410-470bp& 1-1.1kb	410-470bp& 1-1.1kb
Digestion with SphI											
Results	UC 1.5kb	UC 1.5kb	UC 1.5kb	UC 1.5kb	UC 1.5kb	UC 1.5kb	UC 1.5kb	UC 1.5kb	UC 1.5kb	UC 1.5kb	UC 1.5kb
Cook's no.	A2.1.2	PstI UC 1.5kb SnaBI UC 1.5kb B1.2.1.1.1	A2.1.2	A2.1.2	A2.1.2	PstI UC 1.5kb SnaBI UC 1.5kb B1.2.1.1.1	PstI UC 1.5kb SnaBI UC 1.5kb B1.2.1.1.1	A2.1.2	A2.1.2	A2.1.2	A2.1.2
Species	Strepto.	Actinopolymorpha	Strepto.	Strepto.	Strepto.	Actinopolymorpha	Actinopolymorpha	Strepto.	Strepto.	Strepto.	Strepto.

UC-Uncut, Strepto.-Streptomyces spp.

IABT-A1, IABT-A3, IABT-A4, IABT-A5, IABT-A8, IABT-A9, IABT-A10 and IABT-A11 belonged to *Streptomyces* spp.

ARDRA has been shown to be useful in differentiating between bacterial species within a genus, for example, *Actinomadura*, *Gordonia*, *Nocardia*, *Rhodococcus*, *Saccharomonospora*, *Saccharopolyspora*, *Streptomyces* and *Tsukamurella*.

Antagonism of actinomycete isolates against *R. solani* under *in vitro* condition :

Eleven isolates of actinomycete were screened against *R. solani*. All the tested isolates were found promising with inhibition range of 71-100 per cent against the pathogen (Table 1). Actinomycete isolates IABT-A2, IABT-A7and IABT-A8 exhibited 100 per cent inhibition against *R. solani*. The next best isolates were IABT-A3, IABT-A1 and IABT-A9 with mean per cent inhibition of 99.62 per cent, 98.39 per cent and 98.27 per cent, respectively. The antifungal activity exhibited by actinomycete isolates might be due to siderophore production, antifungal compounds, secretion of hydrolytic enzymes such as chitinase, β-1,3 glucanase, chitosanase and proteases, which degrade the fungal cell wall or the secretion of antifungal compounds (Priya and Kalaichelvan, 2011). Based on per cent inhibition of the pathogen, five potent actinomycete isolates were selected for *in vivo* screening.

Evaluation of potent actinomycetes against *R. solani* under glasshouse condition :

Isolates IABT-A2, IABT-A7, IABT-A8, IABT-A1 and IABT-A6 were screened under *in vivo* condition and their effect on disease parameters and plant growth promotion was

Table 3 : Effect of actinomycete application on sheath blight and plant growth characters under glasshouse condition

Treatment no.	Treatments	sheath blight disease parameters				Plant growth parameters		
		Lesion length (cm)			No. of dried leaves per plant	Plant height (cm) 60 DAS	Root length (cm) 75 DAS	Dry wt. of root (g)
		40 DAS	60 DAS	Mean				
T_1	Seed treatment (IABT-A1)	0.60	0.82	0.71	4.00	46.77	24.10	1.21
T_2	Seed treatment (IABT-A2)	0.61	0.74	0.68	3.00	56.25	38.50	2.98
T_3	Seed treatment (IABT-A6)	0.37	0.55	0.46	3.00	68.89	36.17	2.38
T_4	Seed treatment (IABT-A7)	0.33	0.38	0.35	2.00	79.93	37.93	3.18
T_5	Seed treatment (IABT-A8)	0.79	0.77	0.78	3.33	49.82	35.50	1.88
Mean		0.54	0.65	0.59	3.06	60.33	34.44	2.32
T_6	Soil treatment (IABT-A1)	0.49	0.81	0.65	3.33	36.17	23.37	1.52
T_7	Soil treatment (IABT-A2)	0.39	0.70	0.55	3.33	52.15	30.60	2.22
T_8	Soil treatment (IABT-A6)	0.36	0.62	0.49	3.00	65.32	37.23	1.82
T_9	Soil treatment (IABT-A7)	0.31	0.51	0.41	2.67	75.31	38.50	1.81
T_{10}	Soil treatment (IABT-A8)	0.56	0.81	0.68	3.00	51.17	29.50	2.08
Mean		0.42	0.69	0.56	3.07	56.02	31.84	1.89
T_{11}	Foliar spray treatment (IABT-A1)	0.42	1.17	0.79	3.67	42.04	20.33	2.13
T_{12}	Foliar spray treatment (IABT-A2)	0.37	0.78	0.57	3.33	46.61	28.10	2.55
T_{13}	Foliar spray treatment (IABT-A6)	0.27	0.67	0.47	4.00	62.51	31.63	1.52
T_{14}	Foliar spray treatment (IABT-A7)	0.24	0.55	0.39	3.00	74.93	32.60	1.68
T_{15}	Foliar spray treatment (IABT-A8)	0.71	0.94	0.82	3.33	50.69	27.00	1.53
Mean		0.40	0.82	0.61	3.46	55.35	27.93	1.88
T_{16}	Combined treatment (IABT-A1)	0.33	0.73	0.53	3.00	50.73	30.43	1.90
T_{17}	Combined treatment (IABT-A2)	0.22	0.63	0.42	2.67	61.30	38.23	2.89
T_{18}	Combined treatment (IABT-A6)	0.17	0.41	0.29	2.00	71.25	37.57	3.59
T_{19}	Combined treatment (IABT-A7)	0.11	0.28	0.19	1.33	80.89	40.30	3.61
T_{20}	Combined treatment (IABT-A8)	0.44	0.68	0.56	2.00	52.40	34.40	2.45
Mean		0.27	0.48	0.37	2.2	63.31	36.18	2.88
T_{21}	Pathogen (treated Control)	3.63	4.33	3.98	7.67	38.59	28.13	1.88
T_{22}	Healthy (Untreated control)	0.00	0.00	0.00	0.00	39.70	29.43	1.96
	C.D. (P = 0.05)	0.10	0.08	0.38	1.18	0.99	0.09	
	S. E. ±	0.28	0.23	1.09	0.41	0.34	0.03	
	CV (%)	31.28	16.55	21	1.26	1.87	2.65	

recorded.

Effect on disease parameters: Lesion length and no. of dried leaves :

Among five actinomycete isolates, IABT-A7 was found as the best isolate in all the treatment methods studied with least lesion length and number of dried leaves compared to other isolates tested. However, all the isolates significantly reduced the disease compared to the control. In both soil treatment and foliar spray treatments, isolate IABT-A7 resulted least mean lesion length, 0.41 cm and 0.39 cm and number of dried leaves, 2.67leaves/plant and 3 leaves/plant, respectively. IABT-A7 when applied as seed treatment + soil treatment + foliar application significantly reduced sheath blight infection with least lesion length (mean 0.19 cm) and number of dried leaves (1.33 leaves/plant) (Table 3).

Effect on plant growth parameters :

Plant height :

All the five actinomycete isolates enhanced the plant height compared to control. The highest plant height (mean 80.37cm/plant) was recorded in the combine treatment of IABT-A7 isolate (Table 3). This was followed by the seed treatment of IABT-A7 (78.71cm/plant). At 60 DAS, combination methods resulted in the highest plant height in actinomycete isolate IABT-A7 (80.89 cm/plant).

Root length and dry weight of root :

Maximum mean root length (36.18 cm) was recorded in combined application of actinomycetes. However, seed treatment with actinomycetes also enhanced root length with mean 34.44 cm. Application of IABT-A7 as seed+soil+foliar spray increased the root length and dry weight of root to the maximum extent 40.30 cm and 3.61g/plant, respectively. Least root length was recorded in foliar application of IABT-A1 (20.33cm). Combined treatment of IABT-A6 also recorded 3.59g/plant. The next best treatments were seed treatment with IABT-A7 followed by seed treatment of IABT-A2 isolate with 3.18g/plant and 2.98g/ plant, respectively. Isolates IABT-A2 and IABT-A6 could enhance root length in all application methods, seed, soil and foliar application.

Application of actinomycetes enhanced the growth parameters as evident with increased plant height, root length and dry root weight. Effect of IABT-A7, identified as *Actinopolymorpha* spp., against *R.solani* was quite encouraging. It significantly reduced the disease as well as boosted the plant/ root growth and hence, may be considered as an ideal bioagent to manage sheath blight of rice. Direct promotion of plant growth by PGPR occurs when the plant is supplied with a compound that is synthesized by the bacteria, or when PGPR otherwise facilitates plant uptake of soil nutrients. Possibilities include siderophore synthesis, phytohormone synthesis, and solubilization of minerals to make them available for plant uptake and use (Gopalakrishnan *et al.*, 2011). Significant increase in plant growth parameters in the present study may be attributed to the production of plant growth regulators such as auxins, gibberellins, cytokinins and ethylene. Indole acetic acid promotes ethylene production by stimulating the enzyme in the ethylene biosynthetic pathway (Srividya *et al.*, 2012). Owing to antipathogenic and growth promotion characteristics, *Actinopolymorpha* spp., (IABT-A7) identified in this study appears to be a hopeful bioagent for further study.

LITERATURE CITED

Ara, I., Bukhari, N.A., Perveen, K. and Bakir, M.A. (2012). Antifungal activity of some actinomycetes isolated from Riyadh soil, Saudi Arabia: An evaluation for their ability to control *Alternaria* caused tomato blight in green house pot trial. *African J. Agric. Res.*, **7** (13) : 2042-2050.

Cook, A.E. and Meyers, P.R. (2003). Rapid identification of filamentous actinomycetes to the genus level using genus-specific 16S Rrna gene restriction fragment patterns. *Internat. J. Syste. & Evolutionary Microbiol.*, **53** (Pt 6) : 1907–1915.

Chakraborty, P., Chowdhury, A.K. and Chakraborty, B.N. (2006). Effectiveness of different methods of inoculation against sheath blight of rice. *Crop Res.*, **31** (3) : 481-482.

Gopalakrishnan, S., Kiran, K., Humayun, P., Vidya, M.S., Deepthi, K., Jacob, S., Vadlamudi, S., Alekhya, G. and Rupela, O. (2011). Biocontrol of charcoal-rot of sorghum by actinomycetes isolated from herbal vermicompost. *African J. Biotechnol.*, **10** (79) : 18142-18152.

Jayaraj, J., Yi, H., Liang, G.H., Muthukrishnan, S. and Velazhahan, R. (2004). Foliar application of *Bacillus subtilis* AUBS1 reduces sheath blight and triggers defense mechanisms in rice. *Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz*, **111** (2) : 115-125.

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 Pathol. J.*, **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.

Lane, D.J. (1991). 16S/23S rRNA sequencing. In: Stackebrandt, E., Goodfellow, M. (eds) *Nucleic acids techniques in bacterial systematics*, John Wiley & Sons, Chichester. pp. 115–147.

Luo, J. Y., Xie, G.L., Li, B., Luo, Y.C., Zhao, L.H., Wang, X., Liu, B. and 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 Sci.,* **12** (3) : 213-218.

Pathak, A., Sharma, A., Johsi, B.N. and Sharma, A.K. (2004). *Pseudomonas* strain GRP3 induces systemic resistance to sheath blight in rice. *Internat. Rice Res. Note*, **29** (1) : 35-36.

Pospiech, A. and Naumann, B. (1995). A versatile quick prep of genomic DNA from gram-positive bacteria. *Trends Genet.,* **11** (6) : 217-218.

Priya, C.S. and Kalaichelvan, P.T. (2011). Strategies for antagonistic activity of local actinomycete isolates against rice fungal pathogens. *Asian J. Exp. Biol. Sci.,* **2** (4) : 648-653.

Shantikumar, S., Baruah, I. and Bora, T.C. (2006). Actinomycetes of Lohtak habitat : Isolation and screening for antimicrobial activity. *Biotechnol.,* **5** (2) : 217-221.

Srividya, S., Adarshana, T., Bhat, D.V., Kajingailu, G. and Nilanjan, D. (2012). Streptomyces sp. 9p as effective biocontrol against chilli soilborne fungal phytopathogens. *European J. Expel Bio.,* **2** (1): 163-173.

Tachowisan, T., Peberdy, J.F. and Lumyong, S. (2003). Isolation of endophytic actinomycetes from selected plants and their antifungal activity. *World J. Microbiol. & Biotechnol.*, **19** (4) : 381-385.

Zhong, Tan Wan, 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). *Agric. Sci. China,* **6** (9): 1074-1081.

9th Year
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