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Biological Management of Bacterial Leaf Blight of Rice (*Xanthomonas oryzae* pv. *oryzae*) through Rice Rhizosphere Antagonist Actinobacteria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Rice (*Oryza sativa* L.) is a staple food crop for more than two billion people around the world. Bacterial leaf blight (BLB) caused by *Xanthomonas oryzae pv. oryzae (Xoo)* (Ishimiya) Swings et al. is one of the most destructive diseases of rice in all the major rice-growing. Use of antibiotics is the common practice for the management of BLB, but it has detrimental effect on human health and environment. Hence other eco-friendly strategies like biological control and host plant resistance (HPR) should be effectively exploited for BLB management. An attempt was made to manage the disease using actinobacteria isolated from rice rhizosphere. Among sixteen actinobacterial isolates,

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six were selected for secondary screening based on disc diffusion assay. Out of six isolates AUDT-502 produced maximum zone of inhibition (1.81 cm) followed by AUDT-508 (1.62 cm) and were evaluated under glasshouse condition. AUDT-502 (*Streptomyces rimosus*) appeared as the most promising bio agent which was able to inhibit the pathogen (Xoo) both in *in vitro* and *in vivo* condition. AUDT-502 increased the plant growth parameters like number of tillers (5.73), plant height (56.85 cm), root length (34.33cm) and dry root weight (3.90g) and also reduced the leaf blight lesion length and per cent disease index (4.43 per cent) significantly when compared to other treatments. The current study thus showed that there was a good correlation between *in vitro* inhibition of the pathogen and the *in vivo* control of the disease and AUDT-502 (*Streptomyces rimosus*) appears to be a hopeful bioagent for the management of bacterial leaf blight of rice.

Keywords: Rice; bacterial leaf blight; Xanthomonas oryzae pv. oryzae; in vitro; in vivo; rhizosphere; actinobacteria; Streptomyces rimosus.

ABBREVIATIONS

Xoo : Xanthomonas oryzae pv. oryzae
BLB : Bacterial leaf blight
HPR : Host plant resistance
TSA : Tryptone soy agar
SCA : Starch casein agar
COC : Copper-oxy-chloride
Rdna : Deoxyribonucleic acid

1. INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most consumed food crops in the world, which is grown on an area of 164.19 million hectares and produces 505.4 million tons (milled basis) of food annually [1]. Millions of people throughout the world rely on rice, the queen of grains, for their daily sustenance. For more than two billion people worldwide, it is a staple food crop [2]. Increased rice production is necessary to feed the growing populations in Asia and other parts of the world [3]. Although rice output and productivity have improved significantly over the past five decades, a significant amount of rice production is lost each year due to biotic (such as diseases and insect pests) and abiotic (such as high or low temperatures, drought, and salinity) stress factors. Blast, bacterial leaf blight, sheath blight, false smut, sheath rot, stem rot, and rice tungro diseases are some of the key diseases that have a substantial effect on rice output. One of the most damaging diseases of rice is bacterial leaf blight (BLB), which is brought on by *Xanthomonas oryzae pv. oryzae (Xoo)* (Ishimiya) Swings et al. It affects all the major rice-growing countries. Coastal regions of Andhra Pradesh, Assam, Bihar, Chhattisgarh, Eastern and Western Uttar Pradesh, Haryana, Karnataka, Punjab, Tamil Nadu, Tripura, and West Bengal are among the Indian states where this disease is endemic [4]. The extent of yield loss due to bacterial leaf blight is greatly influenced by varieties of the crop season, crop stages at which they are infected and the disease severity depends on environmental conditions and the amount of nitrogen fertilizers used. The major BLB outbreak struck Haryana, Punjab, the plains of Uttarakhand and Western Uttar Pradesh in 1979 and 1980 [5], the Palakkad region of Kerala [6], and some areas of Andhra Pradesh in 2010 and 2013 [7]. Over the past ten years, the disease has expanded rapidly throughout numerous rice-growing areas that were not previously thought to be endemic [8]. Use of antibiotics is the common practice for the management of BLB, but it has detrimental effect on human health and environment. Biocontrol agents and plant growth promoting microbes have emerged as promising tools for the management of plant diseases and in sustainable crop production [9]. Therefore, additional environmentally friendly techniques such as biological control and host plant resistance (HPR) strategies should be successfully used for BLB management. Throughout evolution, actinobacteria have been the most productive and resilient types of microbes. They are considered to be among the first living organisms on Earth. In the agricultural industry, actinobacteria, in particular *Streptomyces*, have demonstrated their capacity to create biofertilizers by providing nutrients to plants for improved development, boosting yield, regulating abiotic and biotic stress, and fending off phytopathogen attack [10].

2. MATERIALS AND METHODS

2.1 Bacterial Leaf Blight (BLB) of Rice

An intensive roving survey was conducted during *kharif* 2019 in different rice growing areas of Karnataka (Belagavi, Davanagere, Dharwad,

Haveri, Koppal, Shivamogga and Uttar Kannada districts) to assess the severity of rice bacterial leaf blight and gather samples of leaves exhibiting the typical symptoms of BLB. For isolation, naturally infected leaf samples that displayed typical bacterial leaf blight symptoms were used (Fig. 1). Wherever possible, samples were managed to be collected from various varieties. The highly virulent isolate KXo-26 was used in this study against susceptible variety BPT 5204.

2.2 Screening of Actinobacteria for Antagonism against *Xanthomonas oryzae pv. Oryzae*

The actinobacterial isolates were collected from the Department of Biotechnology, College of Agriculture, University of Agricultural Sciences, Dharwad (Table 1) which were isolated from the rhizosphere of the rice from Agricultural Research Station (Mugad), Dharwad [11]. The isolates were maintained on the starch casein agar medium (SCA). The plates were incubated at 28^0C for 5-7 days. All the isolates were stored in 15 per cent glycerol stocks in cryo vials and maintained for further studies.

2.3 Primary Screening of Actinobacteria for Antibacterial Activity against *Xoo*

Primary screening was performed by streak plate method on tryptone Soy Agar (TSA) media. The 16 actinobacterial isolates were inoculated in straight line on TSA plates and incubated for 5 days. The test organism (*Xoo*) strain streaked on the same plate in the opposite side. The plates were incubated at 30^0C for 48 hours. The plates were examined for the zone of inhibition of the test organism.

2.4 Secondary Screening of Antibacterial Activity Using Disc Diffusion Method

Extraction of antimicrobial compounds: The selected antagonistic actinobacterial isolates (AUDT-502, AUDT-508, AUDT-518, AUDT-528, AUDT-531 and AUDT-532) were inoculated separately into 100 ml of actinobacteria isolation broth (composition: glycerol: 5.0 g, sodium propionate: 4.0 g, sodium caseinate: 2.0 g, K2HPO4 : 0.5 g, asparagine: 0.1 g, MgSO4·7H2O: 0.1 g, FeSO4·7H2O: 1.0 mg, water: 1000 ml and pH: 8.0) and incubated in orbital shaker at 28°C and 190 rpm for 7 days. To extract the antimicrobial compounds, the cultures were filtered then centrifuged at 6000 rpm, 10 minutes. The supernatant was transferred aseptically into a screw capped bottle and used for further assay.

The antimicrobial activities of those extracts were tested against test organism *Xanthomonas oryzae* pv. *oryzae* by using agar disc diffusion method as described by Kirby-Bauer with some modification (1966). To prepare the test plates, *Xoo* isolate was cultured in nutrient broth to a concentration of 10^8 CFU/ml. The 100 ml of TSA agar was autoclaved and cooled down to 45 to 50°C and then mixed with 1 ml of the *Xoo* cultures. This mixture was poured into Petridishes and used as the test plates. Sterile paper discs of 6 mm diameter were introduced on the upper layer of the seeded agar plate. 100 µl of each actinobacterial extracts were loaded on the discs. The plates were incubated at 28 ± 1°C for 2 to 4 days and the inhibitory effect of the actinomycete isolates on *Xoo* growth was evaluated by measuring inhibition zones (cm).

Evaluation of actinobacteria for the management of BLB under glasshouse condition: The present glasshouse study was conducted during *summer* 2020 with 15 different treatments and 3 replication. Based on the results of secondary screening, two most promising antagonistic actinobacterial isolates (AUDT-502 and AUDT-508) were selected for glasshouse studies (Table 2). The earthen pots were filled with the autoclaved sterile soil and FYM mixtures. Before planting, each pot was applied with 2.4, 1.2 and 1.2 g of N, P and K respectively as per the package of practices (100:50:50 NPK/ha) for rice in the form of urea, single super phosphate and murate of potash. The seeds of susceptible rice variety, BPT-5204 were used. The rice seeds were surface sterilised with 1 per cent sodium hypochlorite solution and subsequently rinsed in sterile distilled water for two to three times to get rid of traces of sodium hypochlorite. Finally, seeds were air dried and used for further studies. Five plants were maintained in each pot.

Seed (BPT-5204) treatment details: For the treatment with pathogen (*Xoo*), The nutrient broth (250 ml flask with 100 ml broth) was inoculated with KXo-26 *Xoo* (Virulent Strain) isolate and incubated at 28 ^{0}C for two days. The surface sterilised seeds (BPT-5204) were treated with the pathogen inoculum with 10^8 cfu/ml. Foliar treatment with pathogen was done using Leaf clipping method (Kauffman et al. 1973) to

infect the plants of 45 days rice plants. The seeds were treated with actinobacteria isolates (AUDT-502 and AUDT-508). The actinobacterial isolates were grown in the SCA broth in conical flasks (250 ml) containing 100ml broth separately. The inoculated flasks were incubated at 28°C in shaker incubator for 5 days with shaking (150 rpm). The broth (10^8 cfu/ml) was used to treat the seeds by soaking for 6 hours. The actinobacteria inoculated broth (10^8 cfu/ml) as mentioned above was used for foliar spraying for respective treatments. For treating with *Pseudomonas fluorescens,* the talc formulation of *Pseudomona fluorescens* obtained from Institute of Organic Farming (IOF), Dharwad was used in this study as a reference treatment. A concentration of 0.5 per cent was used for both seed treatment and foliar spray at 10^8 cfu/ml. Seed treatment and foliar spray was done with 0.3 g agrimycin-100 and 0.12 g copper oxy chloride (COC) in a litre of water. This was also used as reference treatment.

2.5 Statistical Analysis

The statistical analysis of experiment was carried out as per the procedure given by Panse and Sukhatme [12]. Per cent data were transformed in to arc sine and square root values and were analyzed statistically. The data obtained in the present investigations for various parameters were subjected to ANOVA for a completely randomized design both under *in vitro* and *in vivo*.

3. RESULTS

3.1 *In vitro* Evaluation of Actinobacteria against *Xanthomonas oryzae* pv. *oryzae*

Primary screening: Among the sixteen actinobacterial isolates, six isolates *viz*., AUDT-502, AUDT-508, AUDT-518, AUDT-528, AUDT-531 and AUDT-532 were found effective against *Xoo* during primary screening (Fig. 2a and 2b). These six isolates were subjected to the secondary screening.

Secondary screening: The results of the secondary screening showed that, the crude extract two actinobacterial isolates (AUDT-502 and AUDT-508) exhibited higher antibacterial activity. The inhibition zone produced by the activity of AUDT-502 (1.81 cm) was significantly more than other isolates and was followed by AUDT-508 (1.59 cm), AUDT-531 (1.18) and AUDT-518 (1.07) (Table 3). Based on the results of the secondary screening (Fig. 2c), the two actinobacterial isolates *viz*., AUDT-502 and AUDT-508 were used for the evaluation against *Xoo* under glasshouse condition.

Evaluation of actinobacteria against BLB under glasshouse condition: The two actinobacteral isolates *viz*., AUDT-502 and AUDT-508 were evaluated under glasshouse condition against *Xoo* along with *Pseudomonas fluorescens* and Agrimycin 100 (recommended check for BLB) as reference treatments. The data on disease and plant growth parameters *viz*., lesion length, per cent disease index, no. of tillers, plant height, root length and dry root weight are presented in Tables 4 and 5.

The mean lesion length ranged from 0.00 to 11.7 cm (Table 4). The study revealed that the seed treatment (S.T.) of AUDT-502 and AUDT-508 showed mean lesion length of 5.8 and 6.4 cm at 15 days after inoculation with *Xoo.* Whereas in case of *Pseudomonas fluorescens* and Agrimycin 100, the seed treated plants showed 6.4 and 4.47 cm. The foliar treated plants with AUDT-502, AUDT-508, *Pseudomonas fluorescens* and Agrimycin 100 showed mean lesion length of 6.06, 8.24, 7.13 and 4.84 cm respectively. The mean lesion length of combined treatment (both seed treatment and foliar spray) of AUDT-502, AUDT-508, *Pseudomonas fluorescens* and Agrimycin 100 was 4.43, 6.2, 5.3 and 4.26 cm respectively. The highest mean lesion length was recorded from the pathogen treated control (11.70 cm). The untreated control and only bioagent treated control (ST+ FS with AUDT-502) showed no disease (Fig. 3). Among all the treatments, the combined treatment with AUDT-502 was statistically on par with agrimycn 100 in the presence of pathogen.

Per cent disease index: The severity of the disease was assessed using Wheeler's formula of per cent disease index (1969). The top five leaves were considered for the disease severity analysis. The mean per cent disease index of combined treatments of AUDT-502 and AUDT-508 was 4.43 and 6.2 respectively (Table 4). The maximum mean per cent disease severity (66.28 %) was observed in the pathogen treated control followed by the foliar sprayed plants with *Pseudomonas fluorescens* (49.98 %) and AUDT-508 (49.41 %). The minimum disease severity was obtained from combined treatment of

agrimycin-100 (27.96 %) followed by 35.86 per cent in AUDT-502 (ST + FS). The treatment with agrimycin 100 (ST + FS) was statistically superior over other treatments. The untreated control and only bioagent treated control showed no disease.

Plant height: All the bioagents enhanced the plant height when compared to the pathogen treated control when recorded at 60 days after sowing (Table 4). The highest plant height (56.85 cm) was noticed in bio agent treated control in the absence of pathogen (ST + FS with AUDT-502). This was followed by combined treatment of AUDT-502 (55.53 cm) and Agrimycin-100 (54.19 cm). The plant height of seed treated plants with AUDT-502, AUDT-508, *Pseudomonas fluorescens* and Agrimycin 100 was 46.06, 45.17, 44.63 and 48.95 cm respectively. The foliar sprayed plants with AUDT-502, AUDT-508, *Pseudomonas fluorescens* and agrimycin 100 showed plant height of 44.08, 44.96, 42.52 and 46.6 cm respectively. The plant height of combined treatment, AUDT-508, *Pseudomonas fluorescens* and Agrimycin 100 was 46.48 cm, 46.76 cm and 52.33 cm respectively (Fig. 4). The minimum plant height was noticed in pathogen treated control (36.46 cm).

Number of tillers per plant: The results depicted that the bio control agents increased the number of tillers per plant significantly when compared to the pathogen treated control (Table 4). Application of actinobacteria as seed treatment and foliar spray (in the absence of pathogen) significantly increased the number of tillers (5.73) compared to other treatments (Table 5). This was followed by the combined treatment of AUDT-502 (4.82) and agrimycine-100 (4.66) in the presence of pathogen. The number of tillers in the combined treatments of AUDT-508 and *Psuedomonas fluorescens* was 4.27 and 4.00 respectively. The least number of tillers was observed in the pathogen treated control (1.66) which was both seed treated and leaf inoculated of *Xoo* (Fig. 4).

Root length: Significant differences for root length of rice plants was observed among the selected treatments (Table 5). The root length was maximum in case of actinobacteria treated control (34.33 cm) *i.e.*, in the absence of pathogen. The ST + FS with AUDT-502 and agrimycine 100 showed the mean root length of 31.7 and 31.87 cm respectively which were statistically on par to each other. The mean root length of foliar sprayed plants with AUDT-502 and combined treatment of *Pseudomonas fluorescens* was 24.67 and 23.13 cm respectively. The least root length was recorded in pathogen treated control (19.60 cm) followed by the *Pseudomonas* treated plants (23.13 cm) when compared to untreated control (28.32 cm) (Fig. 3g).

Dry root weight: The dry root weight of the rice plants was found to increase significantly with bioagents. Among all the treatments, ST + FS with AUDT-502 showed maximum dry root weight (3.70 g/plant) next to the only bioagent treated control (3.90 g/plant). This was followed by the combined treatment of agrimycine-100 (3.27 g/plant). The dry root weight of combined treated plants (ST+FS) with *Pseudomonas fluorescens* was 3.17 g/plant. The least dry root weight was recorded from the pathogen treated control (1.14g/plant) which was followed by the foliar treated actinobacteria (AUDT-508) rice plants with dry root weight of 2.26 g/ plant (Table 5).

4. DISCUSSION

Antibacterial principle of actinobacterial isolates against *Xanthomonas oryzae* pv. *oryzae* causing bacterial leaf blight of rice was studied (Jaivel et al. 2014). The compound isolated was used for inhibition or well diffusion zone assay against *Xoo* and it produced 13-19 mm, zone of inhibition. The actinobacteria AUDT-502 was found the most promising isolate which was able to inhibit the pathogen (*Xoo*) both in *in vitro* and *in vivo* condition. AUDT-502 was previously characterized based on 16S rDNA as *Streptomyces rimosus* [11]. This isolate increased the plant growth parameters and reduced the per cent disease index significantly when compared to other treatments. The actinobacteria acts as good bio agents by releasing beneficial secondary metabolites to the plants and also by root colonization. These released secondary metabolites acts as a reason for development of Induced Systemic Resistance (ISR) in the rice plants. The current study thus showed that there was a good correlation between *in vitro* inhibition of the pathogen and the *in vivo* control of the disease. Hop et al. (2014) used actinobacteria isolates for management to *Xoo* and revealed that actinobacteria VN08-A-12 not only reduced the lesion length of *Xoo* but also reduced yield loss related to *Xoo* in different rice varieties. Application of AUDT-502 (*Streptomyces rimosus*) enhanced the growth parameters *viz.*, with

increased plant height, root length and dry root weight.

It significantly reduced the disease as well as boosted the plant and root growth and hence, may be considered as an ideal biocontrol agent for the management of BLB of rice. Possible reasons for these multiple benefits due to actinobacteria 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). Secondary metabolites produced by *S. fimicarius* and *S. laurentii* can be attributed to the antibacterial activity against *Xoo* [9]. Actinobacteria can also induce systemic resistance against pathogens, but the mechanisms are still poorly described. In the absence of a pathogen, a mild defense response is elicited under jasmonic acid and salicylic acid signaling that involves pathogenesis-related proteins and secondary plant metabolites [13]. Considering antipathogenic and growth promotion characteristics showed by *Streptomyces rimosus* (AUDT502) it appears to be a hopeful bioagent for further study [14].

Table 1. Actinobacterial isolates used for evaluation against *Xanthomonas oryzae* pv. *oryzae*

Sl. No.	Isolate code No.
1	AUDT- 501
2	AUDT- 502
3	AUDT- 504
4	AUDT- 505
5	AUDT- 507
6	AUDT- 508
7	AUDT- 511
8	AUDT- 513
9	AUDT- 516
10	AUDT- 518
11	AUDT- 522
12	AUDT- 523
13	AUDT- 525
14	AUDT- 528
15	AUDT- 531
16	AUDT- 532

Table 2. Treatment details of the experiment under glasshouse condition

Treatments	Description
T_1	Untreated control (healthy)
T_2	Pathogen (*Xoo*) treated control (ST + FS) at 10^8 cfu/ml
T_3	Only bioagent treated control (Actinobacteria AUDT-502 ; ST + FS) at 10^8 cfu/ml
T_4	Seed treatment with actinobacteria AUDT-502 at 10^8 cfu/ml
T_5	Foliar spray with actinobacteria AUDT-502 at 10^8 cfu/ml
T_6	Seed treatment + foliar spray with actinobacteria AUDT-502 at 10^8 cfu/ml
T_7	Seed treatment with actinobacteria AUDT-508 at 10^8 cfu/ml
T_8	Foliar spray with actinobacteria AUDT-508 at 10^8 cfu/ml
T_9	Seed treatment + foliar spray with actinobacteria AUDT-508 at 10^8 cfu/ml
T_{10}	Seed treatment with *Pseudomonas fluorescens* (0.5%) at 10^8 cfu/ml
T_{11}	Foliar spray with *Pseudomonas fluorescens* (0.5%) at 10^8 cfu/ml
T_{12}	Seed treatment + foliar spray with *Pseudomonas fluorescens* (0.5%) at 10^8 cfu/ml
T_{13}	Seed treatment with agrimycin 100 + COC (0.3 g + 0.12 g/L)
T_{14}	Foliar spray with agrimycin 100 + COC (0.3g + 0.12 g/L)
T_{15}	Seed treatment + foliar spray with agrimycin 100 + COC (0.3g + 0.12 g/L)

ST: Seed treatment
FS: Foliar spray

Table 3. *In vitro* efficacy of actinobacterial isolates against *Xanthomonas oryzae* pv. *oryzae*

Sl. No.	Actinobacterial Isolates	Mean inhibition zone (cm)
1	AUDT-502	1.81 (1.67)$^{+}$
2	AUDT-508	1.59 (1.60)
3	AUDT-518	1.07 (1.43)
4	AUDT-528	0.89 (1.37)
5	AUDT-531	1.18 (1.47)
6	AUDT-532	0.99 (1.41)
7	Control	0.00 (1.00)
	C.D @ 1%	0.04
	S.Em. ±	0.01

** : Angular transformed values +: √X+1 transformed values*

Table 4. Evaluation of potent actinobacteria against bacterial leaf blight and plant growth parameters of rice under glasshouse condition

Treatments	Treatments	Lesion length (cm)	PDI	Plant height (cm)	No. of tillers per plant
		15 DAI	30 DAI	60DAS	60 DAS
T_1	Untreated control (healthy)	0.00 (1.00)+	0.00 (0.00)*	52.00 (46.12)*	4.03
T_2	Pathogen (*Xoo*) treated control (ST + FS) at 10^8 cfu/ml	11.70 (3.54)	66.28 (54.49)	31.30 (28.80)	1.66
T_3	Only bioagent treated control (Actinobacteria AUDT-502; ST + FS) 10^8 cfu/ml	0.00 (1.00)	0.00 (0.00)	56.85 (48.92)	5.73
T_4	Seed treatment with actinobacteria AUDT-502 at 10^8 cfu/ml	5.80 (2.60)	44.25 (41.68)	46.06 (42.72)	4.43
T_5	Foliar spray with actinobacteria AUDT-502 at 10^8 cfu/ml	6.06 (2.65)	47.40 (43.492)	44.08 (41.58)	3.40
T_6	Seed treatment + foliar spray with actinobacteria AUDT-502 at 10^8 cfu/ml	4.43 (2.33)	35.86 (36.75)	55.03 (47.86)	4.82
T_7	Seed treatment with actinobacteria A_2 at 10^8 cfu/ml	6.33 (2.70)	43.80 (41.41)	45.17 (42.20)	3.27
T_8	Foliar spray with actinobacteria AUDT-508 at 10^8 cfu/ml	8.24 (3.04)	49.41 (44.64)	44.96 (42.08)	3.51
T_9	Seed treatment + foliar spray with actinobacteria AUDT-508 at 10^8 cfu/ml	6.20 (2.68)	40.36 (39.42)	46.48 (42.96)	4.27

Treatments	Treatments	Lesion length (cm)	PDI	Plant height (cm)	No. of tillers per plant
		15 DAI	30 DAI	60DAS	60 DAS
T_{10}	Seed treatment with Pseudomonas fluorescens (0.5%) at 108 cfu/ml	6.40 (2.71)	43.35 (41.16)	44.63 (41.89)	3.67
T_{11}	Foliar spray with Pseudomonas fluorescens (0.5%) at 108 cfu/ml	7.13 (2.85)	49.98 (44.97)	42.53 (40.68)	3.13
T_{12}	Seed treatment + foliar spray with Pseudomonas fluorescens (0.5%) at 108 cfu/ml	5.30 (2.50)	40.90 (39.79)	46.76 (43.12)	4.00
T_{13}	Seed treatment with agrimycin 100 + COC (0.3 g + 0.12 g/L)	4.47 (2.33)	41.23 (39.93)	48.95 (44.37)	4.20
T_{14}	Foliar spray with agrimycin 100 + COC (0.3g + 0.12 g/L)	4.84 (2.41)	44.18 (41.64)	46.60 (43.02)	3.33
T_{15}	Seed treatment + foliar spray with agrimycin 100 + COC (0.3g + 0.12 g/L)	4.26 (2.29)	27.96 (31.89)	54.19 (47.38)	4.60
	C.D. @ 1%	0.19	2.55	2.99	0.14
	S.Em. ±	0.05	0.85	0.93	0.05
	C.V.	4.69	4.44	4.10	4.03

DAS: Days after sowing DAI: Days after inoculation PDI : Per cent Disease Index
**: Angular transformed values +: √X+1 transformed values*

Table 5. Effect of actinobacteria on root growth of rice plants under glasshouse condition

Sl. No.	Treatments	Root length (cm)	Dry root weight (g/plant)
		90 DAS	90 DAS
1	Untreated control (healthy)	28.32 (32.13)*	3.07
2	Pathogen (*Xoo*) treated control (ST + FS) at 10^8 cfu/ml	19.63 (26.19)	1.13
3	Only bioagent treated control (Actinobacteria AUDT-502; ST + FS) 10^8 cfu/ml	34.33 (35.85)	3.90
4	Foliar spray with actinobacteria AUDT-502 at 10^8 cfu/ml	24.67 (29.73)	2.26
5	Seed treatment + foliar spray with actinobacteria AUDT-502 at 10^8 cfu/ml	31.67 (34.22)	3.70
6	Seed treatment + foliar spray with Pseudomonas fluorescens (0.5%) at 108 cfu/ml	23.13 (28.73)	3.17
7	Seed treatment + foliar spray with agrimycin 100 + COC (0.3g + 0.12 g/L)	31.87 (34.35)	3.23
	C.D. @ 1%	1.94	0.16
	S.Em. ±	0.63	0.05
	C.V.	4.48	4.6

DAS: Days after sowing
**: Angular transformed values*

Fig. 1. Symptoms of bacterial leaf blight of rice
a) Wavy margins on leaf blade
b) Blighted leaves
c) Severely BLB infected field
d) Snake hood symptom

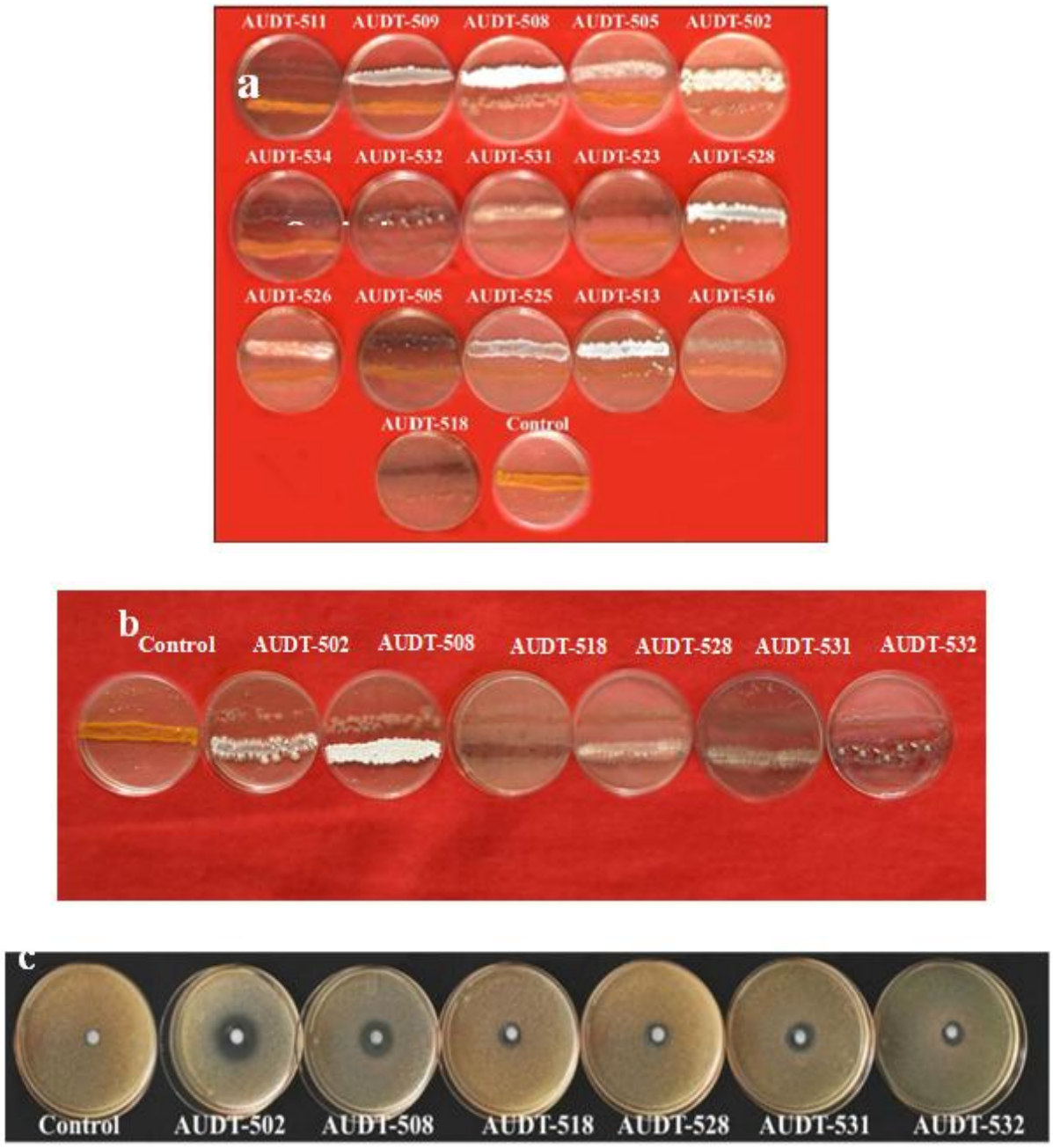

Fig. 2. *In vitro* efficacy of actinobacterial isolates against *Xanthomonas oryzae* pv. *oryzae* a) Primary screening of actinobacterial isolates against *Xanthomonas oryzae* pv. *oryzae.* b) Six potent actinobacterial isolates. c) Secondary screening of potent actinobacterial isolates using disc diffusion assay

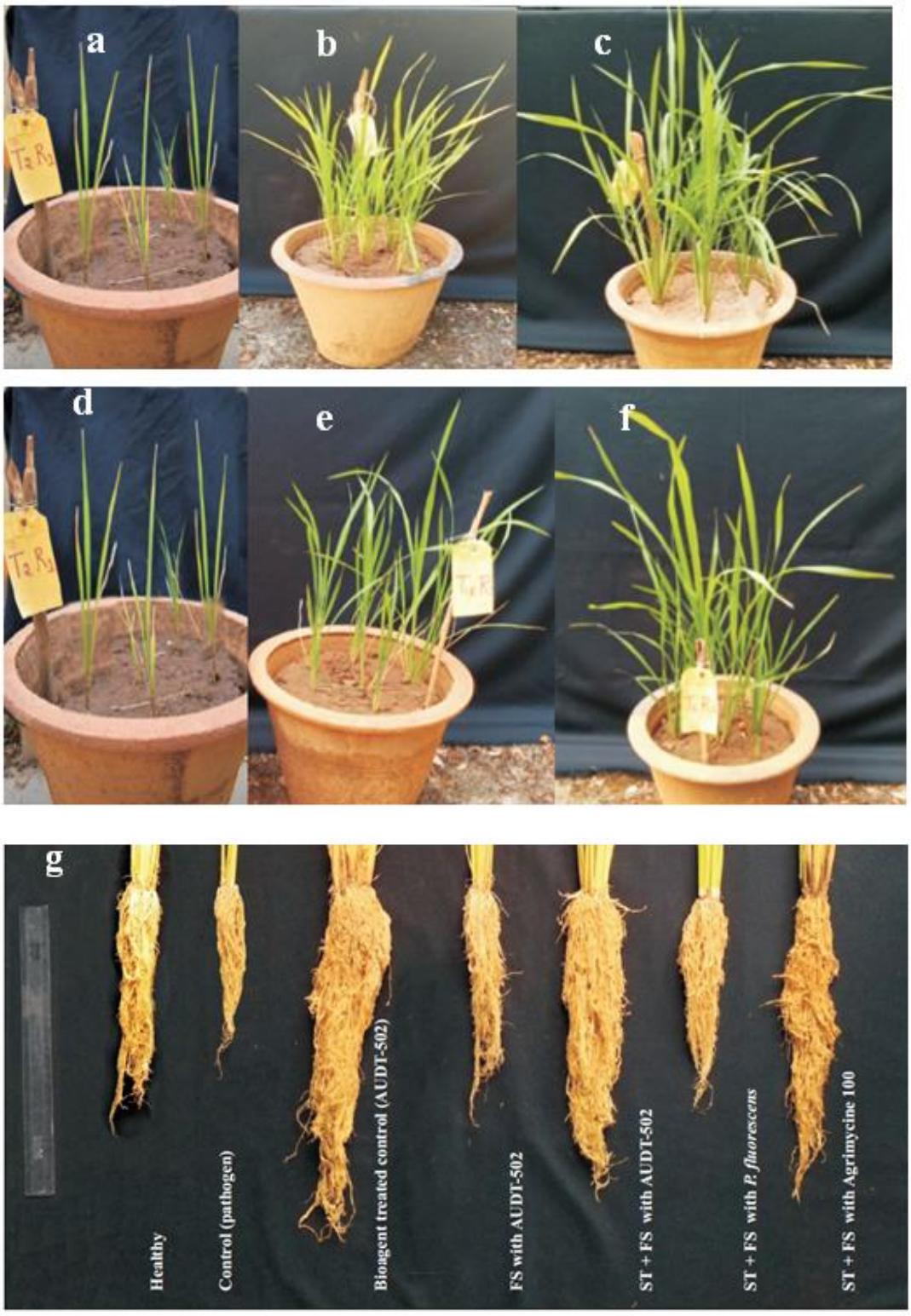

Fig. 3. *In vivo* efficacy of actinobacteria against bacterial leaf blight of rice. a) Control (pathogen). b) Healthy. c) Bioagent treated control (AUDT-502). d) Control. e) Seed treatment + foliar spray with AUDT-508. f) Seed treatment + foliar spray with AUDT-502. g) Effect of actinobacteria in presence of pathogen on root growth of rice

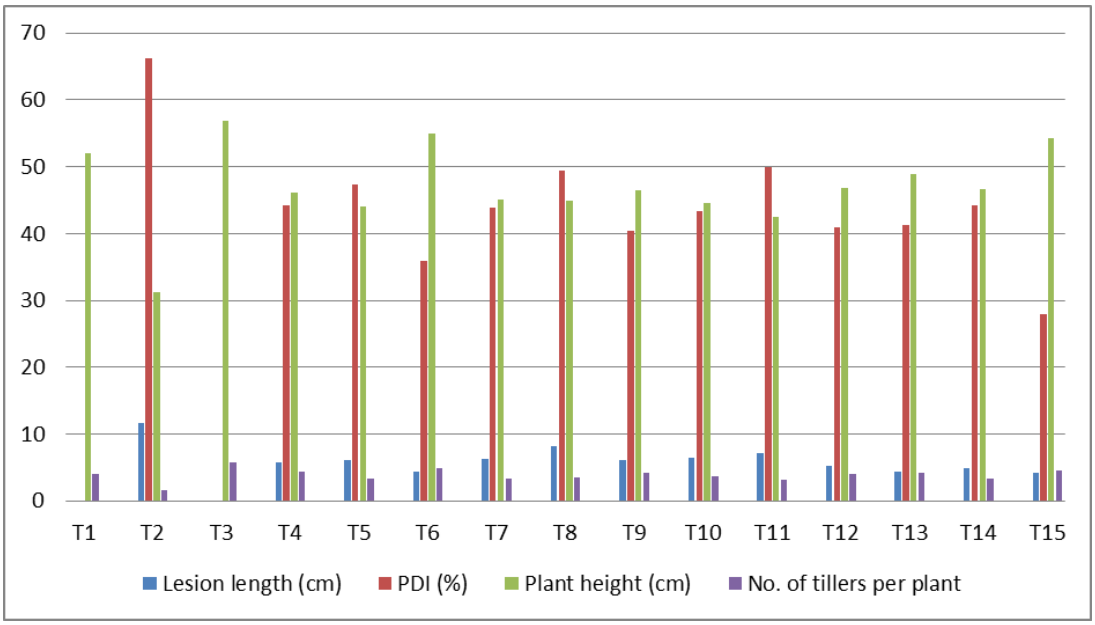

Fig. 4. Evaluation of potent actinobacteria against bacterial leaf blight and effect on plant growth parameters of rice under glasshouse condition

5. CONCLUSION

Evaluation of actinobacteria against BLB showed that, actinobacteria AUDT- 502 (*Streptomyces rimosus*) was found the most promising bio agent which was able to inhibit the pathogen (*Xoo*) both in *in vitro* and under glasshouse condition. Seed treatment and foliar spray of AUDT-502 (*Streptomyces rimosus*) at 10^8 CFU/ml was statistically superior over other treatments which increased the plant growth parameters and reduced the per cent disease index, however it was on par with seed treatment and foliar spray of Agrimycin-100 + COC (0.3 + 0.12 g/L).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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