

Original Research

# Biopriming Using Rhizospheric Actinobacteria- A Promising Tool in Paddy (*Oryza sativa*) Farming

Richa Raut<sup>1†</sup>, Pragati Abhyankar<sup>1</sup> and Sneha Babel<sup>2</sup>

<sup>1</sup>Department of Microbiology, Haribhai V. Desai College (Autonomous), Pune, Maharashtra, India

<sup>2</sup>Department of Mathematics and Statistics, Brihan Maharashtra College of Commerce (BMCC), Pune, Maharashtra, India

† Corresponding author: Richa Raut; [richaraut123@gmail.com](mailto:richaraut123@gmail.com)

## ORCID IDs of Authors-

1. Richa Raut- 0009-0000-5328-528X

2. Pragati Abhyankar- 0000-0002-2451-0430

3. Sneha Babel- 0009-0005-4691-2579

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**Abstract:** The germination of paddy seeds plays a critical role in the establishment of seedlings and their subsequent developmental processes. However, Impaired rice seed germination results from reduced levels of bioactive gibberellins (GAs), due to environmental stress. This inhibitory effect can be mitigated through hormone priming, nutrient priming, and osmo priming. These pretreatments stimulate the  $\alpha$ -amylase activity and initiate other developmental processes. Currently, new biopriming trends are emerging. Biopriming is the application of seed pre-treatment with diverse plant growth-promoting rhizospheric microbes. Reports show that actinobacteria are the least studied biopriming agents. In this study, around 43 actinomycete isolates were isolated from various rhizospheric soils cultivated with the rice variety, Indrayani. Data revealed a significant improvement in the germination process due to the biopriming of seeds using four multifunctional isolates. *Streptomyces ardesiacus* strain I7 marked the highest vigor index. *Streptomyces antibioticus* strain I4 showed significant antimicrobial activity against *Xanthomonas oryzae* subspecies

*oryzae* (Xoo). Colonization of actinomycetes at surface of paddy roots was observed using a scanning electron microscope. These actinomycete isolates can be further taken up for commercial consortium development.

## 1. INTRODUCTION

Rice, *Oryza sativa*, is the major staple food and the second most important cereal crop in the world's trade. (Adnan et al. 2018, Sharif et al. 2014) In India, monsoon rice (June to October) is primarily produced rather than post monsoon rice (November to March). The rate of global population growth suggests 60% improvement in agricultural production of rice. The ultimate solution to this problem is addressing the 'yield gap' in paddy farming. The yield gap is the difference between what is currently produced and the best possible yields of a crop. Agriculture faces two major challenges: rising food demand and maintaining good quality of food. Loss of aroma, taste and nutritional content are also major attributes, along with reduction in yield. To improve paddy farming and to make it more sustainable, farmers can focus on restoring the levels of the indigenous Plant Growth-Promoting Rhizobacteria (PGPR) in the soil. This is a natural alternative to the use of chemical fertilizers, which can harm the soil environment (Adnan et al. 2018).

Seed bio-priming constitutes a sophisticated method of biological treatment for seeds in which seeds are imbibed in water and then inoculated with beneficial microorganisms (Fiodor et al. 2023). Biopriming significantly enhances seed germination and seedling establishment under suboptimal conditions. It improves resistance against phyto-diseases, stress management, aroma production ultimately leading to improved crop yields and superior grain quality (Chakraborti et al. 2022, Chinachanta et al. 2021, Dhondge et al. 2021). An understanding of the PGP properties, natural inhabitants of soil and to apply them for enhancement of soil productivity is crucial for improving rice production efficiency and to gain best possible quality. Thus biopriming is the application of seed pre-treatment with diverse plant growth-promoting rhizospheric microbes, leading to plant immunity, enhancement of yield, and other agronomic traits such as grain size, aroma, and taste. Reports show that Actinobacteria are the least studied biopriming agents.

Actinomycetes constitute a distinct group of bacteria classified within the phylum Actinobacteria. Rhizospheric Actinobacteria are ubiquitous in the natural environment (Yadav et al., 2017). They exhibit a growing array of agricultural applications. Studies indicate their potential as microbiological inoculants through the exploration of mechanisms aimed at enhancing plant development, including biological nitrogen fixation, phosphate solubilization, production of siderophores (Hazem & Camele 2023) phytohormones (El-Tarabily and Sivasithamparam 2006) , other beneficial bio compounds such as antimicrobials possessing considerable potential for the regulation of phytopathogens (Kabdwal et al. 2023) and insect populations (Diab et al. 2024).

In India, Maharashtra is major rice producing state. It is grown in both coastal as well as in hilly region of Western ghats of Maharashtra. Currently rice production is facing many challenges, loss of aroma, moderate yield and threat of diseases. So the objective of our study was to isolate , identify actinomycete strains from rice rhizosphere and to evaluate their plant growth promoting abilities in order to apply them for enhancement of quality of paddy farming. Another aim of the research work was to develop an ecologically sustainable and holistic method of seed biopriming with a consortium of potent actinomycete strains for field application.

## **2. MATERIALS AND METHODS**

### **2.1 Isolation of indigenous rhizospheric Actinomycetes**

Actinomycetes were isolated from the samples collected from various paddy fields of rice variety Indrayani in Bhor and Mulshi tehsils of Pune districts. Sampling was carried out at three different stages of farming such as bulk soil, before sowing, rhizospheric soil , transplanted seedlings and harvesting. One gram of each soil sample was suspended in 9 mL of sterile physiological saline and was subjected to vortex. Upon mixing the samples were serially diluted up to  $10^{-5}$  dilutions, and aliquots from the  $10^{-4}$  and  $10^{-5}$  dilutions were spread plated (0.1 mL) on Starch casein agar (SCA) supplemented with cycloheximide (at a concentration of 50  $\mu\text{g/mL}$  of media) manufactured by Hi Media Laboratories, Mumbai, India, and subsequently incubated at  $28 \pm 2^\circ\text{C}$  for a period of

seven days. Distinct colonies with powdery and dry appearance were isolated and preserved on slants of International Streptomyces project 4 (ISP 4) medium.

## **2.2 Screening of isolates for Plant Growth Promoting (PGP) properties**

### **2.2.1 Ammonia production**

The isolates were inoculated into 5 ml of peptone water and subsequently incubated at a temperature of 28 °C for a duration of 7 to 12 days with agitation at 120 rpm. Following the incubation period, 0.5 ml of Nessler's reagent was added into each culture tube. The manifestation of a yellow to brown color serves as an indicator of a positive outcome for ammonia production (Cappuccino and Sherman, 1992). Ammonia producers were further screened for PGP features.

### **2.2.2 Production of Indole Acetic Acid**

Indole-3-acetic acid (IAA), an auxin production by selected isolates was studied by Salkowski's method. All isolates were grown in International Streptomyces project 2 (ISP 2) medium.

ISP2 medium with 0.2% Tryptophan at 28°C for 7 days. Cell-free supernatant was added with 2 ml of Salkowski's reagent (50 ml, 35% perchloric acid & 1 ml of 0.5M FeCl<sub>3</sub> solution). The tubes were incubated in the dark for 30 minutes at room temperature. Development of pink or red color indicates IAA production. Absorbance was recorded at 535 nm. (Ali et al. 2009, Anwar et al. 2016).

**2.2.3 Gibberellic acid (GA) production-** Actinomycete isolates were grown in ISP-2 medium. GA was extracted from cell-free extracts using ethyl acetate (1:1). The organic layer was evaporated, and dried extract was dissolved in 2 ml of ethanol before being mixed with 1 ml of 2,4-Dinitrophenyl Hydrazine and 5% KOH. Absorbance was recorded at 430 nm (Graham & Thomas 1961).

### **2.2.4 Siderophore production**

The ability of all PGPR isolates to produce siderophores was tested on Chrome Azurol S agar (CAS agar). After being spread out onto the CAS agar plate, each isolate was incubated for 4-5 days at room temperature. Orange halos surrounding the colonies indicated the presence of siderophore production. (Anwar et al. 2016).

### 2.2.5 Phosphate solubilization

The phosphate solubilization ability of isolates was examined using Pikovskaya's agar medium (Adnan et al. 2018). Each of the isolates was streaked on Pikovskaya's agar plate and incubated for 7 days at room temperature. The colonies showing clear halo zones surrounding it indicated phosphate solubilization.

### 2.2.6 Amylase production

Isolates were spot inoculated on starch agar and incubated for 7 days at 28 °C. After incubation, plates were flooded with iodine solution and kept undisturbed for 5-10 minutes. Later iodine solution was discarded from the plates and zone of clearance was noted (Ekka & Namdeo 2018).

Phosphate solubilisation, Siderophore production index and Amylase production index were calculated as follows:

$$\text{PSI/SPI/API} = \frac{\text{Colony diameter} + \text{Zone diameter}}{\text{Colony diameter}} \quad \dots(1)$$

### 2.2.7 Acetyl Pyrroline Production

Acetyl Pyrroline (2AP) is key volatile component responsible for Aroma in rice. Isolates were grown on the plates containing the ISP 4 agar medium supplemented with ornithine (4%) for 7 days. Analysis of aroma production was done by sniffing (Dhondge et al. 2021).

### 2.2.8 Putrescine production

Putrescine is produced due to ornithine decarboxylation. This increases the pH of the medium, changing the colour of the indicator from yellow to purple. Putrescine production was detected by using Moeller's decarboxylase broth supplemented with 1% ornithine. Yellow to Purple colour change was observed (Nassar et al. 2003).

### **2.2.9 Evaluation of Biocontrol properties**

The isolates were tested for antimicrobial activity against major rice pathogen *Xanthomonas oryzae* subspecies *oryzae* (Xoo) causing leaf blight of rice by giant colony technique. The pathogen was received from pathology department of Agriculture Research Station of Mahatma Phule Krishi Vidyapeeth, Lonavala, Maharashtra. Isolates were streaked on ISP 4 medium as a single thick band at the center of the plate and incubated for 5 days at 28 °C. After incubation, pathogen was streaked in triplicate, perpendicular to the giant colony. The plates were incubated for 48 hrs at room temperature. Inhibition of growth of test pathogen indicates biocontrol property. Secondary screening of antimicrobial producers was done by standard agar well diffusion assay explained by Balouiri et al. 2015. Cultures were grown in International Streptomyces project 2 (ISP 2) medium for 10 days. Cell free broth was obtained upon sonication and filtration. Standard streptomycin (200 µg/ml) and sterile ISP 2 medium were used as positive and negative control respectively.

### **2.3 Screening of isolates for seed germination ability**

#### **2.3.1 Seed germination bioassay**

Growth-promoting activity of isolates was evaluated by the modified 'ragdoll' method (Sreevidya et al. 2016). One gram of paddy seeds, purchased from a local market, was sterilized using 70% alcohol and was pre-soaked in water. They were further treated with spore suspension of individual actinomycetes  $10^{-7}$  CFU/ml for 24 hrs. and placed in one half of a wet paper towel, folded and rolled, and put in a big petri dish. Control seeds were treated with only sterile distilled water. The plates were sealed with parafilm and kept in incubator with light source at  $(28 \pm 2^\circ\text{C})$  for 10 days. At the end of incubation, root and shoot lengths of treated and control seedlings were measured. Germination percentage and vigor indices were calculated using the following formulae:

$$\text{Germination Percentage}(\%) = \frac{\text{No.of seeds germinated} \times 100}{\text{Total No.of seeds used}} \quad \dots(2)$$

$$\text{Vigor Index} = \text{Germination} (\%) \times \text{Average seedling length}(\text{cm}) \quad \dots(3)$$

### 2.3.2 Extraction and estimation of active metabolites from germinated seeds

One gram of the germinated seeds was crushed using 10ml of the sodium phosphate buffer of pH 7.0 (0.05 M ) in mortar and pestel. Overnight extraction of metabolites in cold condition was done. The content was centrifuged at 5000 rpm for 15 minutes and crude seed extract was obtained by collecting the supernatant (Asante et al. 2013).

Quantitative assessment of  $\alpha$ -amylase enzyme in both treated as well as control germinated seeds was performed using standard protocol of DNSA method (Ekka & Namdeo,2018). Quantitation of GA Content in seed extracts was done by DNPH method described by Graham & Thomas, 1961.

### 2.3.3 Root colonization study

Bioprimered roots of treated paddy seeds obtained in germination study were further examined for colonization of respective actinomycetes using scanning electron microscope (JEOL-JSM-6360A) at Department of Physics,SPPU,Pune,India. Roots were cut into pieces of 3-4 mm length and were fixed in 2.5% glutaraldehyde solution followed by washing with phosphate buffer of pH 7 for 24 h at 4°C. These fixed roots were dehydrated using 75% ethanol and air dried. Dehydrated roots were observed upon Platinum (Pt) coating under scanning electron microscopy.

### 2.3.4 Compatibility testing of potent actinomycetes

Each of potent actinomycetes cultures was streaked at center same as in giant colony technique. Other isolates to be tested were streaked at right angle to it .Plates were incubated for 5 days. The presence of growth of isolates under study at crossing points of the paired isolates was considered as compatibility (Fiodor et al. 2023).

### 2.3.5 Morphological identification of actinomycetes using coverslip culture technique

Sterile cover slips were stabbed in each quadrant of ISP 4 medium agar plates at 45° angle. The inoculum was streaked on agar at the base of the cover slip. Plates were incubated at room temperature 28°- 30°C for 4-5 days until the organism grow upto the cover slip. Cover slip was removed with a sterile forceps gently and a drop of crystal violet was placed on a slide place coverslip .Coverslip was put on stain and was observed under a microscope( Mitchell & Britt, 2004).

### **2.3.6 Molecular identification and phylogenetic analysis of the potent actinomycetes**

The selected actinomycetes were sent to Hi-Geno MB ,Himedia Laboratories,Pvt.Ltd.for identification by 16S rRNA analysis. The sequences obtained from Himedia Laboratories were identified at the species level using the NCBI GenBank database and were compared using the BLAST program.The nucleotide sequences were submitted to Gen-Bank and NCBI accession numbers were obtained (Cook & Meyers, 2003).These sequences were aligned using the Clustal W software and phylogenetic tree was prepared by neighbor-joining method.Bootstrap analysis with 1000 replications was performed using the MEGA 11 software to estimate the reliability of the inferred phylogenetic relationships within a tree, demonstrating evolutionary history between selected potent actinomycetes. Besides NCBI,the actinomycetes genera were also confirmed with EZBiocloud database.

### **2.3.7 Statistical analysis**

Statistical analysis of germination study was done by using R software. Shoot and root lengths were subjected to analysis of variance (one-way Anova). Further post-hoc comparisons were performed using Tukey's honestly significant difference test to know which actinomycetes treatment is significantly effective. Graphical analysis involves boxplots, bar plots with standard deviation, and mean difference plots with 95% confidence intervals. Boxplot were used to study distribution of shoot and root length values across seed treatment.

## **3. RESULTS AND DISCUSSION**

### **3.1 Isolation and assessment of various plant growth promotion properties**

Out of 49 actinomycetes 50% isolates showed ammonia production. 56% isolates were found to produce IAA. Another study showed 34% of Actinomycetes isolates from the soybean rhizosphere produced IAA and also showed plant growth promotion (Wahyudi et al.2019). Present study revealed the diversity of rhizospheric actinomycetes with abundance of biostimulatory features for plant development. Table 1 depicts distribution of PGP properties in actinomycetes isolates.

**TABLE 1: Major plant growth-promoting traits of actinobacterial isolates.**

Isolate No.	API	PSI	SPI	IAA Production ( $\mu\text{g} / \text{ml}$ )	GA Production (mg/ml)	Ammonia Production	Polyamine production	AntiXoo activity (Zone of inhibition)	Aroma Production
H1Rb	3.8	1.4	0.0	5.62	39.75	1.0	0.0	3 $\pm$ 0.4	0.0
H1Rc	2	1.2	0.0	0.0	0.0	1.0	0.0	0.0	0.0
I1	3.6	1.5	1.5	4.1	46.89	1.0	1	0.0	0.0
I2	2.1	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0
I4*	2.9	2.0	3.9	7.24	75.76	1.0	3	10 $\pm$ 0.5	0.0
I5	2	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0
I7*	2.8	1.2	4.1	3.79	82.6	2.0	3	0.0	3.0
I8	1.8	0.0	1.2	0.0	0.0	1.0	0.0	0.0	0.0
I18	2.1	0.0	0.0	0.0	0.0	1.0	1.0	5 $\pm$ 0.9	0.0
I26	2.2	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0
E3.3	2.4	0.0	1.3	3.79	0.0	1.0	0.0	0.0	0.0
E4.1	2	1.6	0.0	0.0	0.0	1.0	0.0	0.0	0.0
L1A	1.9	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0
L1B	1.9	0.0	1.4	5.01	0.0	1.0	0.0	0.0	0.0
L1C	2	0.0	0.0	0.0	0.0	1.0	0.0	3 $\pm$ 0.5	0.0
L1D*	2.5	1.1	1.9	4.80	49.53	1.0	1.0	3 $\pm$ 0.2	0.0
L1E	2	0.0	0.0	0.0	0.0	1.0	1.0	0.0	0.0

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S1a	2.1	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0
S2a	1.5	0.0	0.0	2.36	0.0	1.0	0.0	0.0	0.0
S4a	1.9	0.0	0.0	5.01	0.0	1.0	0.0	0.0	0.0
S4g	1.8	0.0	0.0	2.37	0.0	1.0	0.0	0.0	0.0
S6a*	3.8	1.8	1.9	5.62	64.75	1.0	0.0	8±0.6	0.0
S7a	1.1	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0

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Note:API: Amylase production index, PSI: Phosphate solubilization index; SPI: siderophores production index, IAA :Indol Acetic Acid production;,GA:Gibbrellic acid production Key: 1 = positive, 2 = moderately positive, 3 = strongly positive, 0 = negative.

Anti Xoo activity – Antimicrobial activity against *Xanthomonas Oryzae* showing inhibition zone in cm.

All values given in the column are the average of three replicates.Asteric denotes multifunctional isolates.

\* denotes potent microbes with maximum Plant growth promoting traits.

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Only six (24%) actinomycetes were found to produce major PGP characteristics such as ammonia,putrescine,siderophore, phosphatase ,amylase, IAA and GA except production of aroma and antimicrobial activity against *Xanthomonas oryzae*. A distinct feature of aroma production was shown only by isolate I7. However strong biocidal property against *Xanthomonas oryzae* have been shown by I4 (Fig.1). Isolate no. S6A,I4,I7 showed all PGP properties.Higher amounts of IAA were produced by I4(7.2479 µg / ml),H1RB (5.6219 µg / ml) and L1B (5.0121µg / ml).Gibberellin plays major role in germination. (Kaneko M. et.al 2002) Table 1 shows significant GA secretion in the media by all six selected isolates.

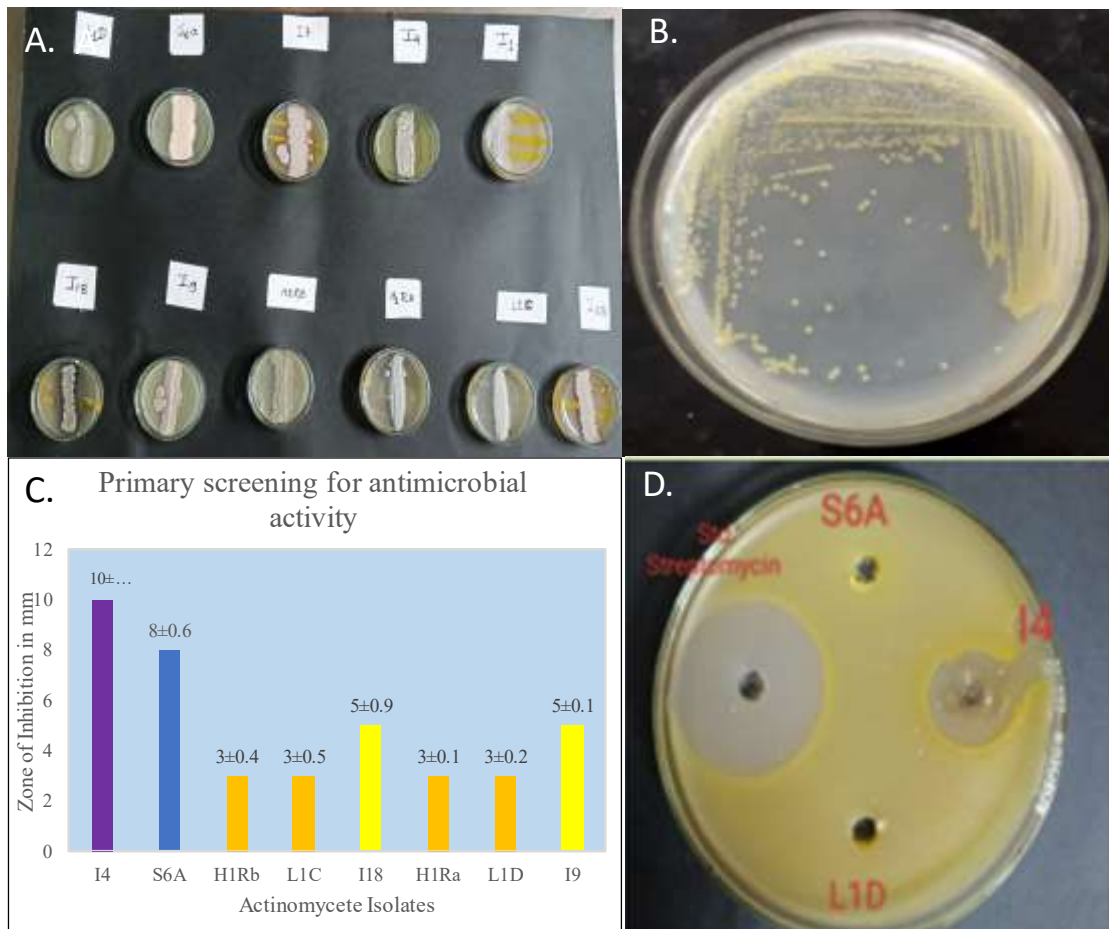


Fig:1-Biocontrol property of Actinomycetes. A. Primary screening B.Plant pathogen *Xanthomonas oryzae* C. Antimicrobial activity of isolates D. Secondary screening of cell free broths in comparison with standard streptomycin

### 3.2 Germination bioassay of bioprimered seeds

The potential of rhizospheric actinomycetes i.e. in vivo growth-promoting abilities were evaluated by germination test using paddy seeds. One way ANOVA confirms overall effect of treatment on root length ( $p < 0.01$  &  $0.05$ ).

The table 2 shows the results of the one-way ANOVA to assess whether there are statistically significant differences in root and shoot length across treatment groups.

	Source	df	Sum of Square	Mean Square	F	P value (>F)
Root length in cm	Treatment	6	276.5189	46.08648	26.9619	1.15e-15 ***
	Residuals	63	107.687	1.709317		

Shoot length in cm	Treatment	6	58.5589	9.76	9.448	2.17e-07
	Residuals	63	65.079	1.033		

The F-value of root length and shoot length was 26.96 and 9.448 respectively. P-values of root length and shoot length are  $1.15 \times 10^{-15}$  and  $2.17 \times 10^{-7}$  which are less than  $\alpha = 0.05$  & 0.001, indicating a highly significant effect of treatment on root length. This means we reject the null hypothesis, confirming that not all group means are equal. The significant difference justifies proceeding with Tukey's HSD for pairwise comparisons.

**Table.3 Tukey's Honest Significant Difference (HSD) test (Root length comparison)**

Group 1	Group 2	Mean Diff	p-adj	Lower Class	Upper Class	Significant
Control	H1Rb	2.23	0.0055	0.45	4.01	✓ Yes
Control	I7	4.92	0	3.14	6.7	✓ Yes
Control	L1D	3.56	0	1.78	5.34	✓ Yes
Control	S6A	6.25	0	4.47	8.03	✓ Yes
H1Rb	I7	2.69	0.0004	0.91	4.47	✓ Yes
H1Rb	S6A	4.02	0	2.24	5.8	✓ Yes
I1	I7	3.21	0	1.43	4.99	✓ Yes
I1	S6A	4.54	0	2.76	6.32	✓ Yes
I4	I7	3.3	0	1.52	5.08	✓ Yes
I4	S6A	4.63	0	2.85	6.41	✓ Yes
L1D	S6A	2.69	0.0004	0.91	4.47	✓ Yes

**Table.4 Tukey's Honest Significant Difference (HSD) test (Shoot length comparison)**

Group 1	Group 2	Mean Diff	p-adj	Lower Class	Upper Class	Significant
Control	H1Rb	1.48	0.0286	0.0957	2.8643	✓ Yes
Control	I7	2.48	0	1.0957	3.8643	✓ Yes
Control	L1D	2.09	0.0004	0.7057	3.4743	✓ Yes
Control	S6A	2.55	0	1.1657	3.9343	✓ Yes
I4	I7	2.03	0.0006	0.6457	3.4143	✓ Yes

I4	L1D	1.64	0.0104	0.2557	3.0243	✓ Yes
I4	S6A	2.1	0.0004	0.7157	3.4843	✓ Yes
Control	H1Rb	1.48	0.0286	0.0957	2.8643	✓ Yes
Control	I7	2.48	0	1.0957	3.8643	✓ Yes
Control	L1D	2.09	0.0004	0.7057	3.4743	✓ Yes
Control	S6A	2.55	0	1.1657	3.9343	✓ Yes

Tukey's HSD test identified S6A, I7, and L1D as significantly superior to Control ie unprimed seeds (Table .3). Treatments I7, L1D, and S6A have visibly higher medians and smaller interquartile ranges compared to the Control. For Isolates S6A, I7, and L1D, the confidence intervals do not cross zero, indicating significant differences from Control. I1 and I4 have intervals that include zero, implying no significant difference from the Control. The data highlights S6A as the most effective treatment with the largest and reproducible improvement in root length.

Biopriming treatment had also showed significant effect on shoot length also ( $p < 0.01$  &  $0.05$ ). Tukey HSD post hoc test of shoot length identified treatments I7, L1D, S6A, and H1Rb as significantly more effective than the Control in enhancing shoot length. Fig. 2 represents that treatments I7 and S6A stand out with the highest shoot growth, suggesting strong potential for application in enhancing plant growth. The similar experiment involved inoculating wheat seeds with the actinomycetes showed that *Streptomyces nobilis* WA-3 resulted in significant increases in shoot length (65%) and root length (81%) during pot trial (Anwar et al. 2016). These findings are consistent with the study done by Sreevidya et al. 2016 which showed that actinomycete isolates exhibited 17% increase in root lengths and 3% increase in shoot lengths in chickpea .

Hence, root and shoot growth of seedlings were significantly found to increase at both 5% & 1% level of significance with respect to various biopriming treatments done.

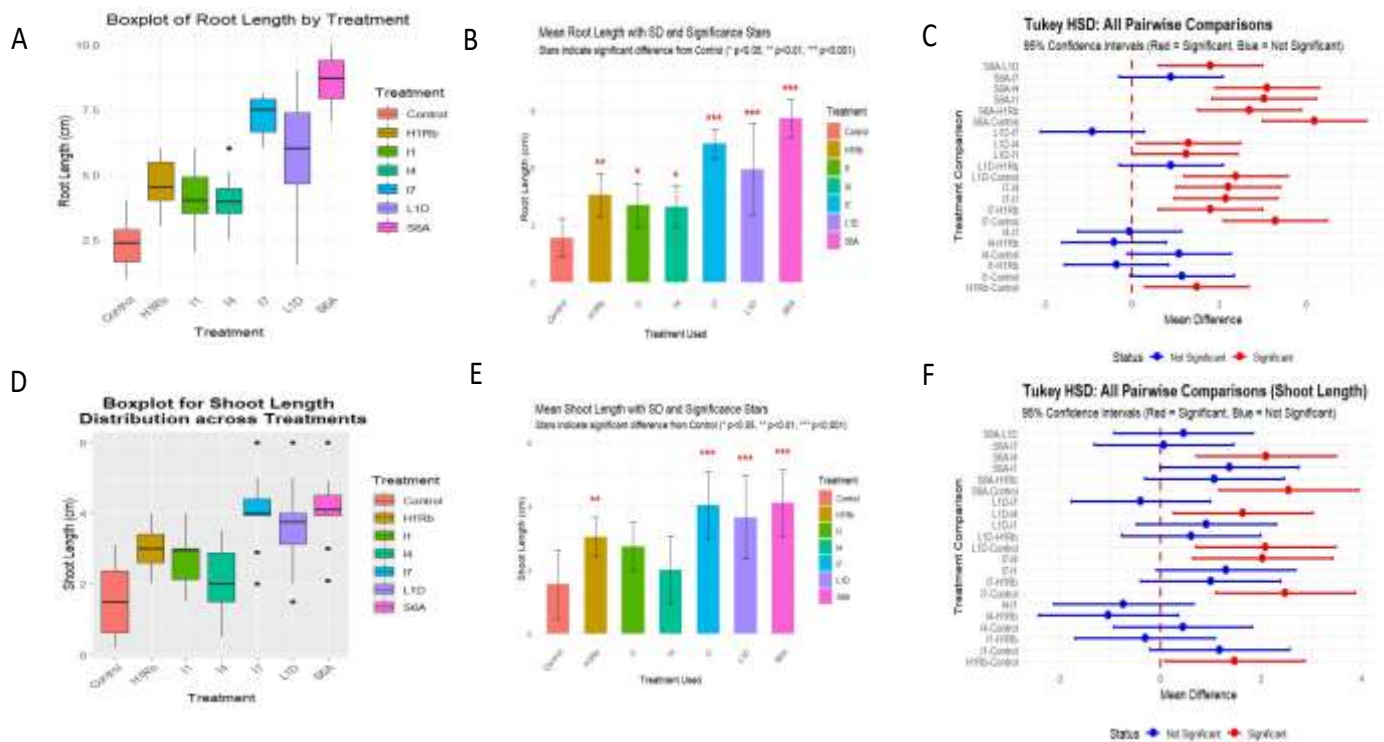


Fig. 2: Germination study of seeds bioprimered with selective actinomycetes. (A),(B)&(C) Analysis of effect of treatment on root length of seedlings. (D),(E)&(F) Shoot length analysis of seed treatment. Vertical bars represent  $\pm$  standard error of mean. H1Rb, I1, I4, I7, L1D and S6A represent selective actinomycetes. There were 10 replicates for the control and each treatment.

Seeds primed with selected actinomycetes spores also showed improved vigor index increased germination percentage in comparison with control as depicted in Fig.3. Bioprimering with actinomycetes may lead to enhanced germination, subsequent protection against major diseases and finally may enhance crop yield and may improve other agronomic traits too. Highest improvement in germination percentage was shown by I7 i.e., by 44.67% in comparison with control (TABLE. 5). Similar study also showed that bioprimering with five different bacterial isolates improved carrot seed germination and seedling growth. Bacterial genera *S. plymuthica* EDC15, *B. ambifaria* AF8II10, and *S. plymuthica* EEPC5 has showed an impact on seed germination by increasing it by 8.22, 10.22, and 10.32%, respectively (Fiodor et al.2023). The synthesis of active GA in the epithelium is important for-amylase expression in the endosperm. Starch from endosperm is gradually hydrolyzed by  $\alpha$ -amylase to supply energy for germination. (Kaneko M. et.al 2002) One possible explanation for the increased germination rate could be improved GA content of treated paddy seeds due to possible GA secretion by actinomycete strains. Increased amylase content of seeds

supports role of GA in enhancement of germination as well as in induction of  $\alpha$ -amylase. Seed treatment with potent *Streptomyces* strains found to enhance concentration of  $\alpha$ -amylase in germinating seeds. GA Priming significantly promoted seed germination and seedling growth under submergence compared to no priming and hydro-priming. This improvement was associated to higher GA<sub>3</sub> levels achieved in rice seeds, resulting subsequent induction of the expression of GA-responsive  $\alpha$ -amylase genes (Wang et al.2025). The activation of seed germination and early support for seedling growth under stressful conditions have been highlighted by seed biopriming (Prasad et al.2016).

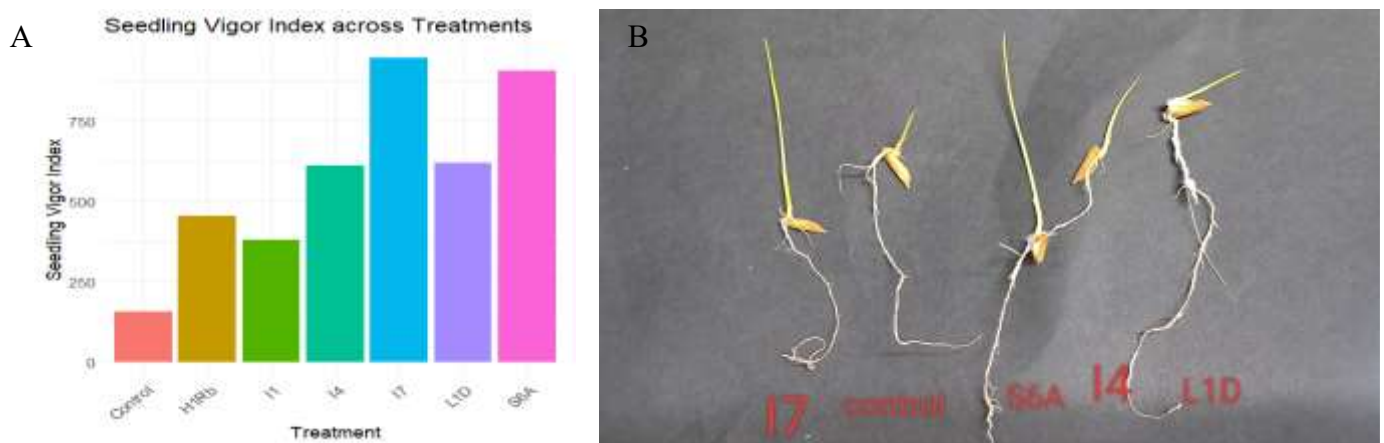


Fig. 3: A. Effect of seed treatment on seedling Vigor Index B.Rice Seedlings showing plant growth promotion in germination study

**TABLE. 5 :Effect of treatment on germination of paddy seeds**

Seed treatment used	Concentration of GA in treated seeds ( $\mu\text{g/g}$ )	Amylase activity U (in terms of maltose)	Germination percentage(%)
Control	0.15 $\pm$ 0.11	11.4	39.66 $\pm$ 0.57
S6a	0.79 $\pm$ 0.25	20.8	70.66 $\pm$ 0.55
I1	0.56 $\pm$ 0.69	16.1	56.33 $\pm$ 0.58
I7	0.90 $\pm$ 1.1	30.6	84.33 $\pm$ 0.57
I4	0.83 $\pm$ 0.68	27.9	81 $\pm$ 1
L1D	0.69 $\pm$ 0.19	19.7	69.33 $\pm$ 1.52

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H1Rb	0.54± 0.29	18.5	64±1
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Note: 1unit of Amylase enzyme= 10000  $\mu$ M/min/ml

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### 3.3 Suitability of potent actinobacteria for agronomic application

Findings of compatibility testing (TABLE 6) suggests that isolates I7, L1D, S6A, and I4 are relatively highly compatible with each other and can grow best in consortium. However I1 and H1Rb had non compatibility with 16% & 83% actinomycetes respectively as in Fig .4 S6A was highly compatible with all other five potent cultures.

**Table 6 : Cultural compatibility test of selected PGP Actinomycetes**

Sr No.	Isolate No.	S6A	I1	I7	I4	L1D	H1Rb
1	S6A	3	3	3	3	3	3
2	I1	3	3	3	3	3	1
3	I7	3	3	3	3	3	1
4	I4	3	3	3	3	2	0
5	L1D	3	3	3	2	3	2
6	H1Rb	3	1	1	0	2	3

Key: 1 = less compatible , 2 = moderately compatible , 3 = highly compatible , 0 = Non compatible

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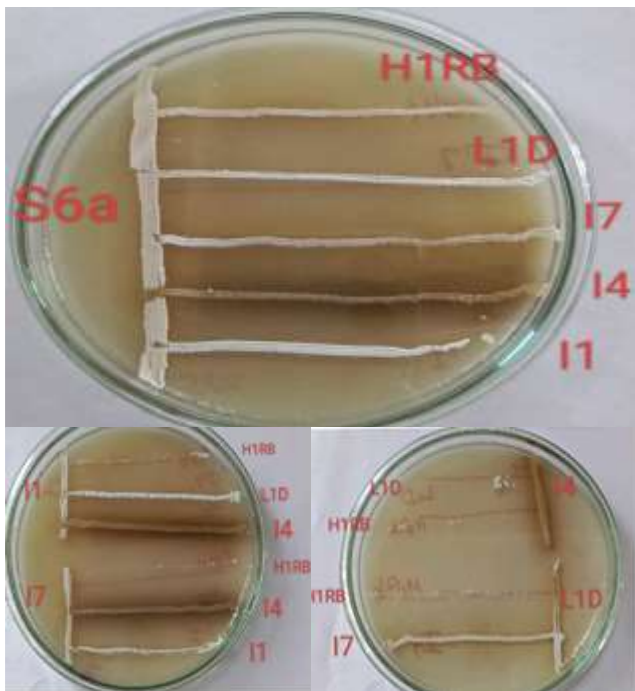
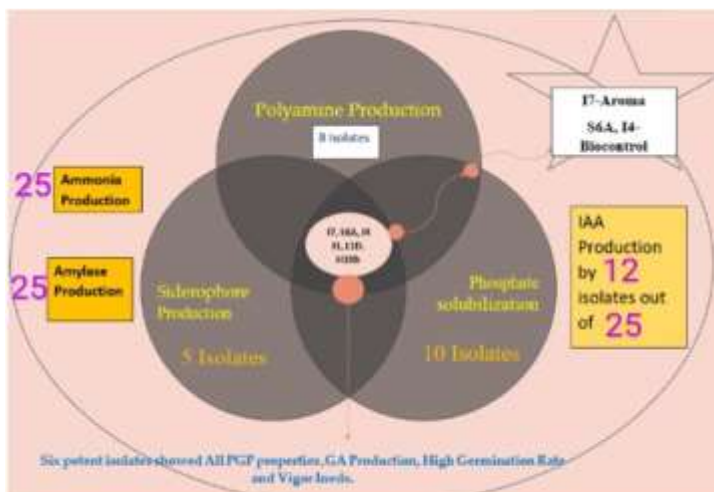


Fig 4 : Compatibility testing of Actinomycetes isolates H1Rb,I1,I4,I7,L1D and S6A

Summary of research work depicted in Fig 5 and findings of compatibility testing revealed that four potent isolates are highly suitable in consortium preparation for further pot trials .



Strategical screening of the most potential PGP actinomycete isolates out of 49 isolates from different paddy fields

Fig. 5: Summary of Research Work

The SEM images of bioprimered roots showed that these *Streptomyces* strains can colonize the paddy roots with out damaging it.

Fig.6 shows that all three potent strains of *Streptomyces* were found to establish extensive colonization and biofilm formation in

comparison with control roots. However *Streptomyces antibioticus* strain I4 showed relatively less extent of biofilm formation. *Streptomyces graminofaciens*, *S. rochei*, *S. annulatus*, *S. gibsonii*, *Candida glabrata*, *C. maltosa*, *C. slooffii* and *Rhodotorula* have been reported for root colonization in maize leading to significant reduction in the growth of *Cephalosporium maydis*, causing late wilt disease of maize in pot trials ( El-Mehalawy et al. 2004). Similar study in chickpea showed that all the four *Streptomyces* strains VAI-7 ,VAI-40,SAI-13 and SAI-29 colonized the roots of chickpea(Sreevidya et al. 2016). Root exudates contain aromatic and phenolic chemicals, sugars, amino acids, organic acids, and carbohydrates that function as chemoattractants to aid in root colonization (Van & Van, 1995).

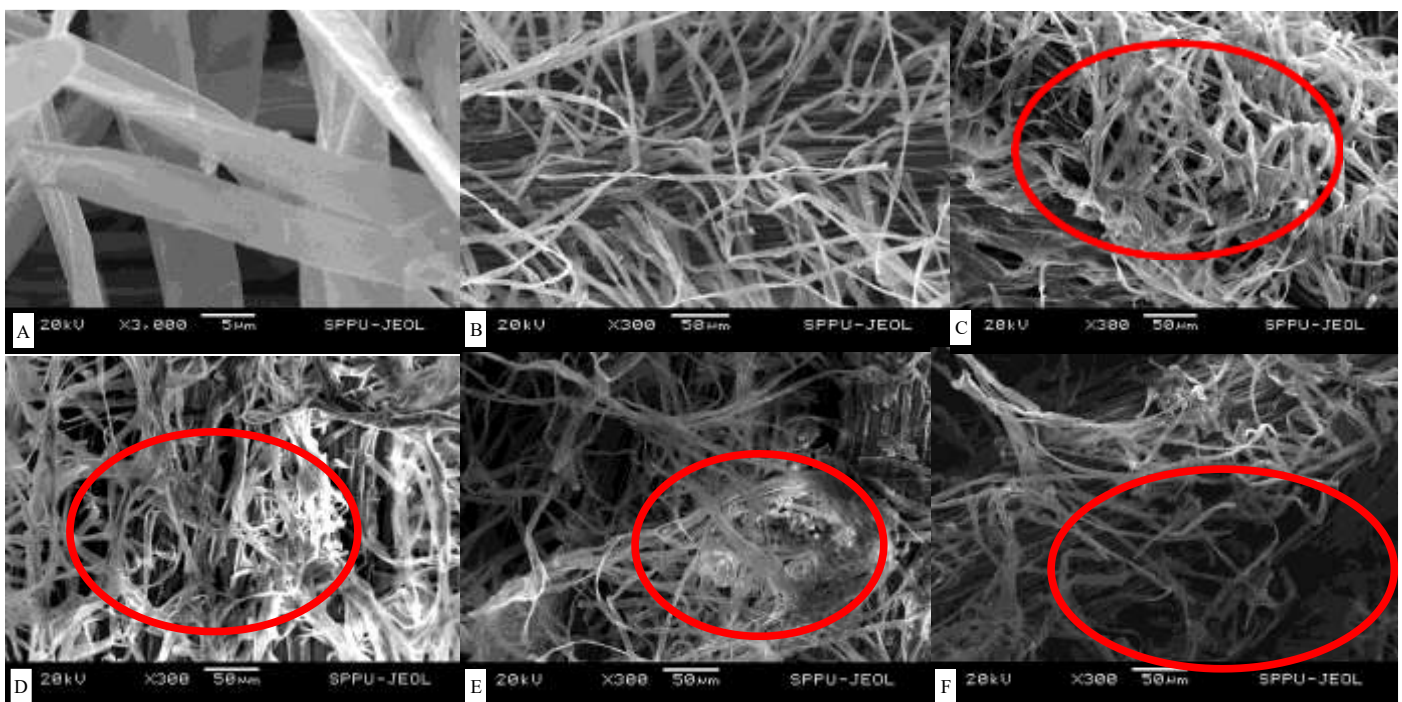


Fig. 6 : A & B- Scanning electron micrographs of control roots at magnification 3000X and 300X respectively; C,D,E &F- SEM images of roots of seeds treated with actinomycetes L1D, S6A, I7 & I4 respectively. Highlighted area shows root colonization by actinomycete species used.

**3.4 Morphological and molecular identification of potent actinomycetes-** The most promising plant growth-promoting bacteria were studied for their morphological and colony characteristics . Morphology in coverslip cultures confirmed their thin mycelial structure with long spiral spore chains . All four future members of bioconsortium were found to be from the genus

*Streptomyces*. Details of blast analysis are listed in TABLE. 7. The nucleotide sequences of these isolates were submitted to Gen Bank and NCBI accession numbers were obtained. Phylogenetic tree illustrates their genetic similarity and evolutionary relationship with other known actinomycetes.(Fig. 7)

**Table 7:Homology of Actinomycetes-**

Sr No.	Isolate Name	Identity Result (NCBI Database)	Accession Number	16 S r RNA length	Percent Similarity	Type strain (EZBiocloud)
1.	I4	<i>Streptomyces antibioticus</i> strain I4	PV652702	1409	100.00	<i>Streptomyces antibioticus</i> NBRC 12838(T)
2.	I7	<i>Streptomyces ardesiacus</i> strain I7	PV652733.1	1405 bp	99.72	<i>Streptomyces ardesiacus</i> NRRL B-1773
3.	L1D	<i>Streptomyces albogriseolus</i> strain L1D	PV652750.1	1434 bp	99.16	<i>Streptomyces albogriseolus</i> NRRL B-1305
4.	S6A	<i>Streptomyces tanashiensis</i> strain S6A	PV652742.1	1415bp	99.79	<i>Streptomyces hydrogenans</i> JCM 4771

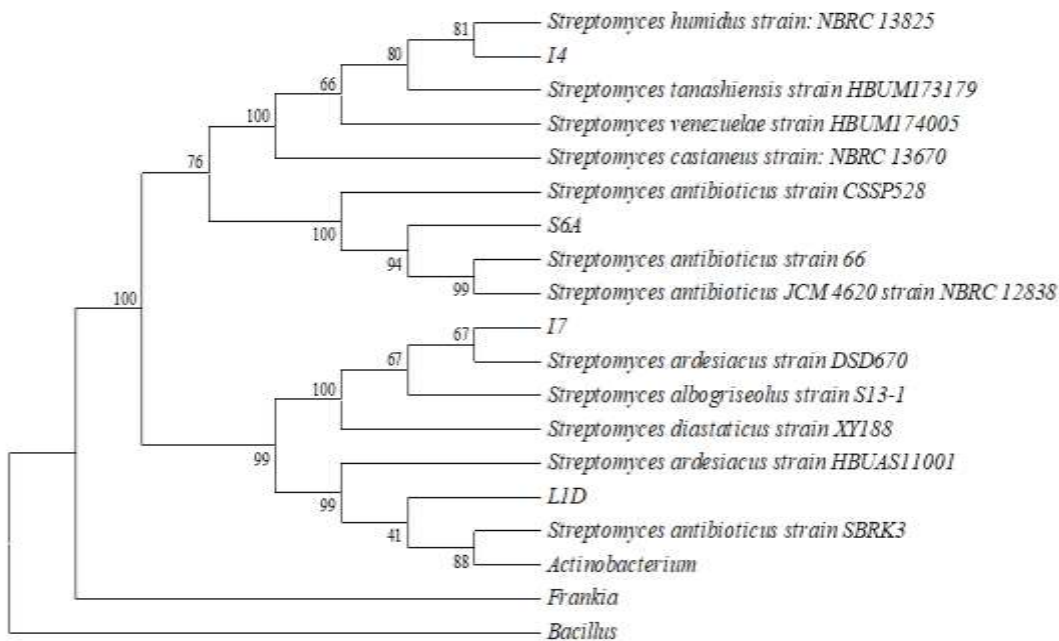


Fig.7: Phylogenetic relationship between I4, S6A, I7, and L1D with representative microbial species based on full length 16S rDNA sequences constructed using the neighbor-joining method.

#### 4 .CONCLUSIONS

Diversity was found amongst Actinomycetes isolated from the rhizosphere of rice (*Oryza sativa*). Significant number of rhizospheric actinobacteria showed major plant growth-promoting properties. Present study showed production of growth hormones by microbes may enhance germination rate. High GA content and increased  $\alpha$ -amylase activity in the extracts of germinated bio-primed seeds in comparison with control, highlights the probability of role of actinomycete in increasing level of GA in endosperm. *Streptomyces antibioticus* strain I4 has prominent biocontrol ability against bacterial leaf blight of rice. Production of 2AP by rhizospheric actinomycetes provides a new insight into the enhancement of rice aroma. This study revealed suitability of these four multipotent actinomycetes *Streptomyces antibioticus* strain I4, *Streptomyces ardesiacus* strain I7, *Streptomyces albogriseolus* strain L1D, *Streptomyces tanashiensis* strain S6A as a biopriming tool for seeds. These four *Streptomyces* strains need to be further developed as biopriming agents for sustainable paddy farming.

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**Conflicts of interest-**There is no conflict of interest.

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