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

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ABSTRACT

The germination of paddy seeds is vital for seedling establishment and subsequent development. Impaired rice seed germination often results from reduced levels of bioactive gibberellins (GAs) caused by environmental stress. This inhibition can be alleviated through methods such as hormone priming, nutrient priming, and osmopriming, which stimulate α-amylase activity and trigger other developmental processes. Recently, novel biopriming approaches have emerged, involving seed treatment with diverse plant growth-promoting rhizospheric microbes. Reports indicate that actinobacteria are among the least studied biopriming agents. In this study, approximately 43 actinomycete isolates were obtained from rhizospheric soils associated with the rice variety Indrayani. Data showed significant improvements in germination following seed biopriming with four multifunctional isolates, with *Streptomyces ardesiacus* strain I7 achieving the highest vigor index. *Streptomyces antibioticus* strain I4 demonstrated notable antimicrobial activity against *Xanthomonas oryzae pv. oryzae* (Xoo). Colonization of actinomycetes on rice roots was confirmed using scanning electron microscopy. These isolates hold potential for development into a commercial microbial consortium.

1. INTRODUCTION

Rice, Oryza sativa, is the primary staple food and the second most significant cereal crop in global trade. In India, rice production is predominantly during the monsoon season (June to October), rather than in the post-monsoon months (November to March). The projected increase in global population indicates a need for a 60% boost in rice production. Addressing the 'yield gap', the difference between current yields and the optimal potential, is essential to meeting this demand. Agriculture faces two primary challenges: rising food demand and ensuring high-quality nutrition. Besides yield, issues such as loss of aroma, flavor, and nutritional value significantly impact rice quality. To enhance sustainability and productivity, farmers should consider restoring the levels of indigenous Plant Growth-Promoting Rhizobacteria (PGPR) in the soil, offering a natural alternative to chemical fertilizers, which can degrade soil health (Adnan et al.2018, Sharif et al. 2014).

Seed bio-priming is a sophisticated biological treatment method in which seeds are soaked in water and subsequently inoculated with beneficial microorganisms (Fiodor et al. 2023). This technique significantly improves seed germination and seedling establishment under challenging conditions. It also enhances resistance to phytopathogens, stress tolerance, and aroma production, ultimately leading to higher crop yields and superior grain quality (Chakraborti et al. 2022, Chinachanta et al. 2021, Dhondge et al. 2021). Understanding the plant growth-promoting (PGP) properties of natural soil inhabitants and applying them to boost soil productivity is essential for increasing rice production efficiency and achieving

optimal quality. Thus, biopriming involves applying diverse plant growth-promoting rhizospheric microbes as a seed pre-treatment to promote plant immunity, increase yield, and improve agronomic traits such as grain size, aroma, and taste. Reports indicate that Actinobacteria are among the least studied biopriming agents.

Actinomycetes form a distinct group of bacteria classified within the phylum Actinobacteria. Rhizospheric Actinobacteria are widespread in the natural environment (Yadav et al. 2017). They are increasingly being recognized for their diverse agricultural applications. Research highlights their potential as microbiological inoculants by exploring mechanisms that enhance plant growth, such as biological nitrogen fixation, phosphate solubilization, siderophore production (Elshafie et al. 2023), phytohormone synthesis (El-Tarabily & Sivasithamparam 2006), and the production of beneficial bioactive compounds, including antimicrobials with significant potential for controlling phytopathogens (Kabdwal et al. 2023) and insect pests (Diab et al. 2024).

In India, Maharashtra is a major rice-producing state. The crop is cultivated both along the coast and in the hilly regions of the Western Ghats. Currently, rice production faces several challenges, including loss of aroma, moderate yields, and the threat of diseases. Therefore, the objective of our study was to isolate and identify actinomycete strains from the rice rhizosphere and evaluate their plant growth-promoting abilities to enhance paddy quality. Additionally, the research aimed to develop an ecologically sustainable and holistic seed biopriming method using a consortium of potent actinomycete strains for field application.

2. MATERIALS AND METHODS

2.1. Isolation of Indigenous Rhizospheric Actinomycetes

Actinomycetes were isolated from samples collected from various paddy fields of rice variety Indrayani in Bhor and Mulshi tehsils of Pune district. Sampling occurred at three distinct farming stages: bulk soil before sowing, rhizospheric soil from transplanted seedlings, and at harvest. One gram of each soil sample was suspended in 9 mL of sterile physiological saline and vortexed. The samples were then serially diluted up to 10^{-5}, and aliquots from the 10^{-4} and 10^{-5} dilutions (0.1 mL) were spread on Starch casein agar (SCA) supplemented with 50 $\mu g.mL^{-1}$ cycloheximide, produced by Hi Media Laboratories, Mumbai, India. Plates were incubated at 28 ± 2°C for seven days. Colonies exhibiting powdery and dry textures were isolated and preserved on ISP 4 (International Streptomyces Project 4) medium slants.

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 incubated at 28°C for 7 to 12 days with agitation at 120 rpm. After incubation, 0.5 mL of Nessler's reagent was added to each culture tube. A yellow to brown coloration indicated a positive result for ammonia production (Cappuccino & Sherman 1992). Ammonia-producing strains were then screened for plant growth-promoting (PGP) features.

2.2.2. Production of Indole Acetic Acid

Indole-3-acetic acid (IAA), an auxin produced by selected isolates, was analyzed using Salkowski's method. All isolates were cultured in International Streptomyces Project 2 (ISP 2) medium supplemented with 0.2% tryptophan and incubated at 28°C for 7 days. The cell-free supernatant was then mixed with 2 mL of Salkowski's reagent, which consisted of 50 mL of 35% perchloric acid and 1 mL of 0.5 M FeCl3 solution. The mixture was incubated in the dark for 30 min at room temperature. The development of a pink or red color indicated IAA production. Absorbance was measured at 535 nm. (Anwar et al.2016).

2.2.3. Gibberellic Acid (GA) Production

Actinomycete isolates were cultured in ISP-2 medium. GA was extracted from cell-free extracts with an equal volume of ethyl acetate (1:1). The organic layer was evaporated, and the dried extract was dissolved in 2 mL of ethanol before being combined with 1 mL of 2,4-Dinitrophenylhydrazine and 5% KOH. Absorbance was measured 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 the isolates was evaluated using Pikovskaya's agar medium. Each isolate was streaked onto a Pikovskaya agar plate and incubated at room temperature for seven days. The formation of clear halo zones around colonies indicated phosphate solubilization.

2.2.6. Amylase Production

Isolates were spot-inoculated onto starch agar plates and incubated at 28°C for 7 days. Following incubation, the plates were flooded with iodine solution and left undisturbed for

5-10 min. Subsequently, the iodine solution was drained, and the clear zones surrounding the colonies were recorded (Ekka & Namdeo 2018).

Phosphate solubilization, Siderophore production index, and amylase production index were calculated as follows:

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

2.2.7. Acetyl Pyrroline Production

Acetyl Pyrroline (2AP) is a key volatile component responsible for the 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 through ornithine decarboxylation, which raises the medium's pH and causes the indicator to change from yellow to purple. Detection of putrescine production was achieved using Moeller's decarboxylase broth supplemented with 1% ornithine, resulting in a color change from yellow to purple (Nassar et al. 2003).

2.2.9. Evaluation of Biocontrol Properties

The isolates were evaluated for their antimicrobial activity against the major rice pathogen Xanthomonas oryzae subspecies oryzae (Xoo), which causes leaf blight in rice, using the giant colony technique. The pathogen was obtained from the pathology department of the Agriculture Research Station at Mahatma Phule Krishi Vidyapeeth, Lonavala, Maharashtra. Isolates were streaked onto ISP 4 medium as a single, thick band at the center of the plate and incubated for five days at 28°C. After incubation, the pathogen was streaked in triplicate, perpendicular to the giant colony. The plates were then incubated for 48 h at room temperature. Inhibition of the pathogen's growth was taken as an indication of biocontrol activity. Secondary screening of antimicrobial producers was performed using the standard agar well diffusion assay described by Balouiri et al. (2015). Cultures were cultivated in International Streptomyces Project 2 (ISP 2) medium for ten days. Cell-free broth was obtained through sonication and filtration. Standard streptomycin (200 $\mu g.mL^{-1}$) served as a positive control, while sterile ISP 2 medium was used as a negative control.

2.3. Screening of Isolates for Seed Germination Ability

2.3.1. Seed Germination Bioassay

The growth-promoting activity of the isolates was assessed using the modified 'ragdoll' method (Sreevidya et al. 2016). One gram of paddy seeds, obtained from a local market, was sterilized with 70% alcohol and pre-soaked in water. The seeds were then treated with a spore suspension of individual actinomycetes at a concentration of 10^{-7} $CFU.mL^{-1}$ for 24 h. They were placed on one half of a wet paper towel, folded, rolled, and placed in a large petri dish. Control seeds were treated with sterile distilled water only. The dishes were sealed with parafilm and incubated at 28 ± 2°C with a light source for 10 days. After incubation, the root and shoot lengths of both treated and control seedlings were measured. Germination percentage and vigor indices were calculated using the respective formulas:

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

$$Vigor\ Index = Germination\ (\%) \times Average\ seedling\ length\ (cm) \quad \ldots(3)$$

2.3.2. Extraction and Estimation of Active Metabolites from Germinated Seeds

One gram of germinated seeds was crushed using 10 mL of sodium phosphate buffer at pH 7.0 (0.05 M) in a mortar and pestle. The metabolites were extracted overnight under cold conditions. The mixture was centrifuged at 5000 rpm for 15 min, and the supernatant was collected to obtain the crude seed extract. Quantitative assessment of α-amylase enzyme in both treated and control germinated seeds was performed using the standard DNSA method. The quantification of GA content in seed extracts was conducted using the DNPH method described by Graham and Thomas (1961).

2.3.3. Root Colonization Study

Bioprimed roots of treated paddy seeds, obtained during the germination study, were further examined for colonization by the respective actinomycetes using a scanning electron microscope (JEOL-JSM-6360A) at the Department of Physics, SPPU, Pune, India. Roots were cut into 3-4 mm pieces and fixed in 2.5% glutaraldehyde solution, then washed with phosphate buffer at pH 7 for 24 h at 4°C. The fixed roots were dehydrated with 75% ethanol and air-dried. Finally, the dehydrated roots were coated with platinum and observed under scanning electron microscopy.

2.3.4. Compatibility Testing of Potent Actinomycetes

Each potent actinomycete culture was streaked at the center using the same technique as the giant colony method. Other isolates to be tested were streaked perpendicular to it. The plates were incubated for five days. The growth of isolates at the crossing points of the paired streaks was considered an indication of compatibility (Fiodor et al. 2023).

2.3.5. Morphological Identification of Actinomycetes Using the Coverslip Culture Technique

Sterile cover slips were inserted at a 45° angle into each

quadrant of ISP 4 medium agar plates. The inoculum was streaked onto the agar at the base of the cover slip. Plates were then incubated at room temperature (28°–30°C) for 4-5 days, until the organism grew up to the cover slip. Gently, the cover slip was removed using sterile forceps, and a drop of crystal violet was applied to a slide. The cover slip was placed on the stain, and the sample was examined 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 through 16S rRNA analysis. Sequences obtained from HiMedia Laboratories were identified at the species level using the NCBI GenBank database and compared with the BLAST program. The nucleotide sequences were submitted to GenBank, and NCBI accession numbers were obtained (Cook & Meyers 2003). These sequences were aligned using Clustal W software, and a phylogenetic tree was constructed using the neighbor-joining method. Bootstrap analysis with 1000 replications was performed using MEGA 11 software to assess the reliability of the inferred phylogenetic relationships, illustrating the evolutionary history among the selected potent actinomycetes. In addition to NCBI, actinomycete genera were confirmed using the EZBiocloud database.

2.3.7. Statistical Analysis

The germination data were statistically analyzed using R software. Shoot and root lengths were subjected to one-way analysis of variance (ANOVA). Post hoc comparisons were carried out with Tukey's honest significant difference test to identify which actinomycete treatments were significantly

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

Isolate No.	API	PSI	SPI	IAA Production [µg .mL^{-1}]	GA Production [mg.mL^{-1}]	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±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±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±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±0.5	0.0
L1D*	2.5	1.1	1.9	4.80	49.53	1.0	1.0	3±0.2	0.0
L1E	2	0.0	0.0	0.0	0.0	1.0	1.0	0.0	0.0
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

Note:API:Amylase production index, PSI: Phosphate solubilization index; SPI: siderophores production index, IAA : Indole-3-acetic acid production,GA: Gibberellic acid production. Key: 1 = positive, 2 = moderately positive, 3 = strongly positive, 0 = negative.
Anti Xoo activity – Antimicrobial activity against Xanthomonas 0ryzae 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.

effective. The graphical representations included boxplots, bar plots with standard deviation bars, and mean difference plots with 95% confidence intervals. Boxplots were used to examine the distribution of shoot and root length measurements across different seed treatments.

3. RESULTS AND DISCUSSION

3.1. Isolation and Assessment of Various Plant Growth Promotion Properties

Out of 49 actinomycetes isolates, 50% demonstrated ammonia production, while 56% were found to produce IAA. Another study showed that 34% of actinomycete isolates from the soybean rhizosphere produced IAA and also promoted plant growth (Wahyudi et al. 2019). The present study revealed the diversity of rhizospheric actinomycetes, with a richness of biostimulatory features that support plant development. Table 1 presents the distribution of plant growth-promoting (PGP) properties among the actinomycetes isolates.

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

3.2. Germination Bioassay of Bioprimed Seeds

The potential of rhizospheric actinomycetes, i.e., in vivo growth-promoting abilities, was evaluated using a germination test with paddy seeds. One-way ANOVA confirmed an overall treatment effect on root length ($p < 0.01$ and 0.05). Table 2 presents the results of the one-way ANOVA assessing whether there are statistically significant differences in root and shoot length across treatment groups.

The F-values of root length and shoot length were 26.96 and 9.448, respectively. P-values of root length and shoot length are 1.15×10^{-15} and $2.17\times10^{-7,}$ which are less than

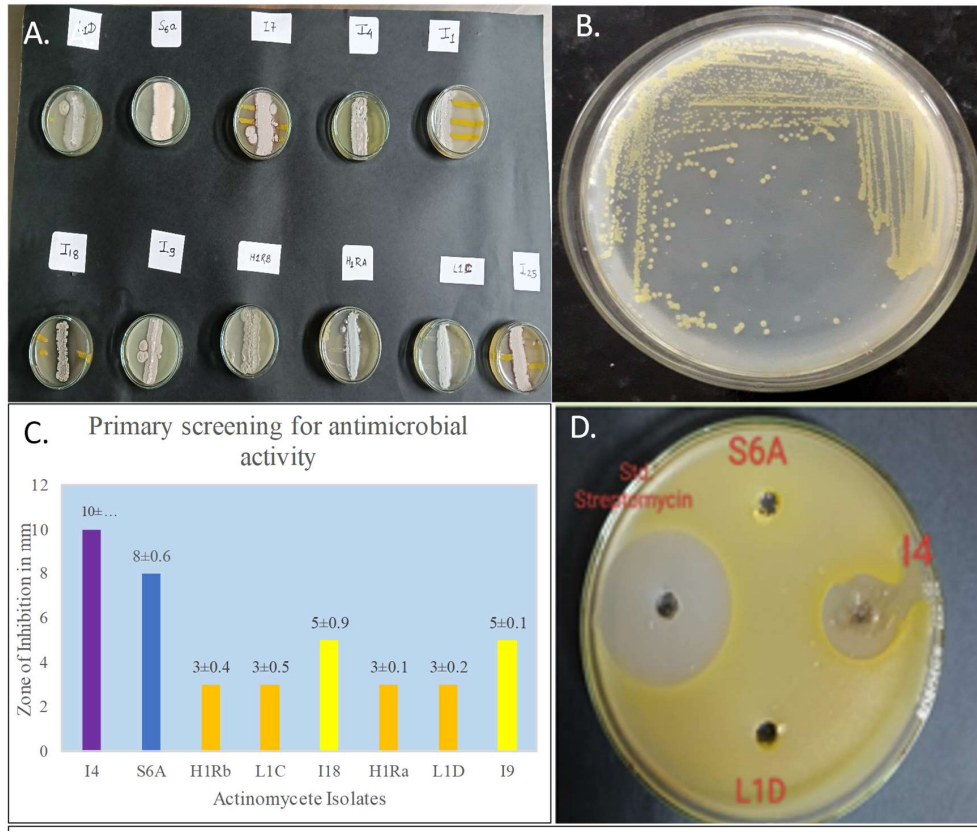


Fig. 1: Biocontrol property of Actinomycetes. A. Primary screening B. Plant pathogen *Xanthomonas oryzae* C. Antimicrobial activity of isolates D. Secondary screening of cell-free broths in comparison with standard streptomycin.

Table 2: One-way ANOVA analysis of root length.

	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		

 = 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 (Tables 3 and 4).

Tukey's HSD test identified S6A, I7, and L1D as significantly superior to the Control, which consisted of unprimed seeds (Table 3). Treatments I7, L1D, and S6A exhibited noticeably higher medians and smaller interquartile ranges compared to the Control. For isolates S6A, I7, and L1D, the confidence intervals do not include zero, indicating significant differences from the Control. In contrast, I1 and I4 have intervals that encompass zero, suggesting no significant difference from the Control. The data underscores S6A as the most effective treatment, demonstrating the greatest and most consistent improvement in root length.

Biopriming treatment also demonstrated a significant effect on shoot length ($p < 0.01$ and $p < 0.05$). The Tukey HSD post hoc test identified treatments I7, L1D, S6A, and H1Rb as significantly more effective than the control in promoting shoot elongation. As shown in Fig. 2, treatments I7 and S6A exhibited the highest shoot growth, indicating

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

Group 1	Group 2	Mean Diff	p-adj	Lower CIass	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 CIass	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

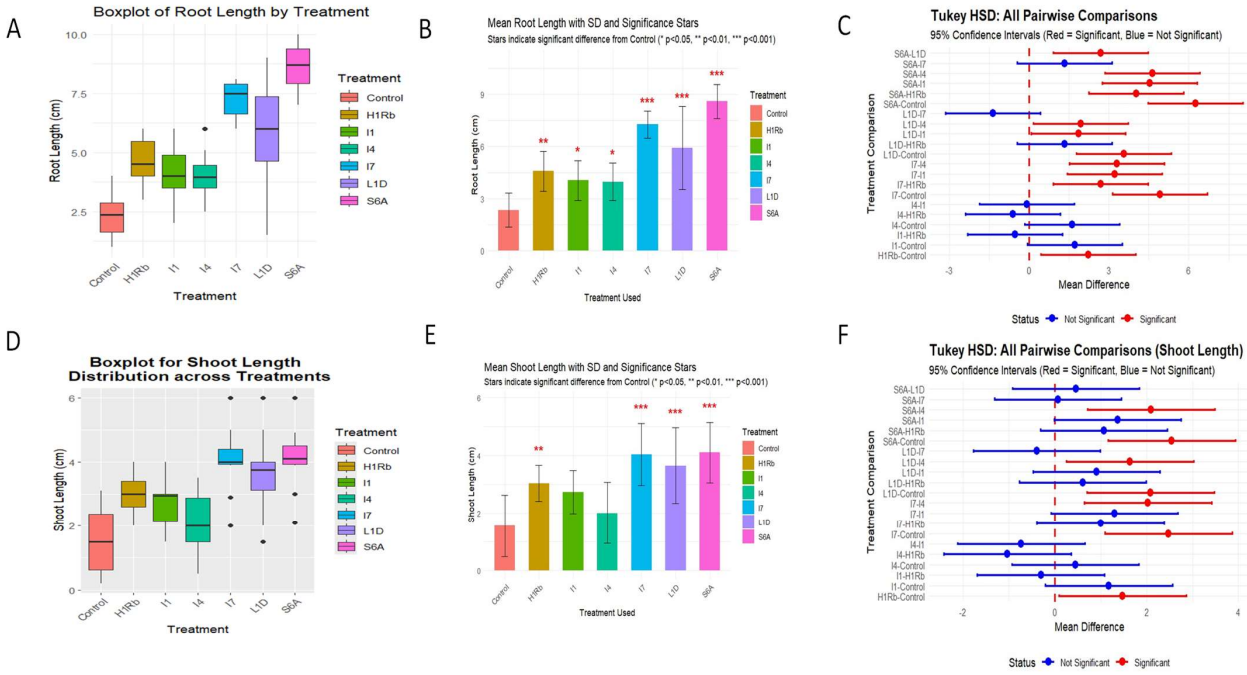


Fig. 2: Germination study of seeds biopriming with selective actinomycetes. (A), (B) & (C) Analysis of the effect of treatment on root length of seedlings. (D), (E) & (F) Shoot length analysis of seed treatment. Vertical bars represent ± standard error of the mean. H1Rb, I1, I4, I7, L1D and S6A represent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

their strong potential for application in enhancing plant development. An analogous experiment involving wheat seed inoculation with actinomycetes found that Streptomyces nobilis WA-3 led to notable increases in shoot length (65%) and root length (81%) during pot trials (Anwar et al. 2016). These results align with findings by Sreevidya et al. (2016), who reported a 17% increase in root length and a 3% increase in shoot length in chickpea after treatment with actinomycete isolates. Therefore, various biopriming treatments significantly enhanced root and shoot growth of seedlings at both 5% and 1% levels of significance.

Seeds primed with selected actinomycetes spores also showed improved vigor index and increased germination percentage in comparison with the control, as depicted in Fig. 3. Biopriming 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. The greatest improvement in germination percentage was shown by I7, i.e., by 44.67% in comparison with the control (Table 5). A similar study also showed that biopriming with five different bacterial isolates improved carrot seed germination and seedling growth. Bacterial genera *S. plymuthica* EDC15, *B. ambifaria* AF8II10, and *S. plymuthica* EEPC5 have shown 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 the endosperm is gradually hydrolyzed by α-amylase to supply energy for germination. 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 the role of GA in the enhancement of germination as well as in the induction of α-amylase. Seed treatment with potent *Streptomyces* strains was found to enhance the concentration of α-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 with higher GA_3 levels achieved in rice seeds, resulting in the subsequent induction of the expression of GA-responsive α-amylase genes (Wang et al. 2025). The activation of seed germination and early support for seedling growth under stressful conditions has been highlighted by seed biopriming (Prasad et al. 2016).

3.3. Suitability of Potent Actinobacteria for Agronomic Application

Findings of compatibility testing (Table 6) suggest that isolates I7, L1D, S6A, and I4 are relatively highly compatible with each other and can grow best in a consortium. However, I1 and H1Rb had non-compatibility with 16% & 83%

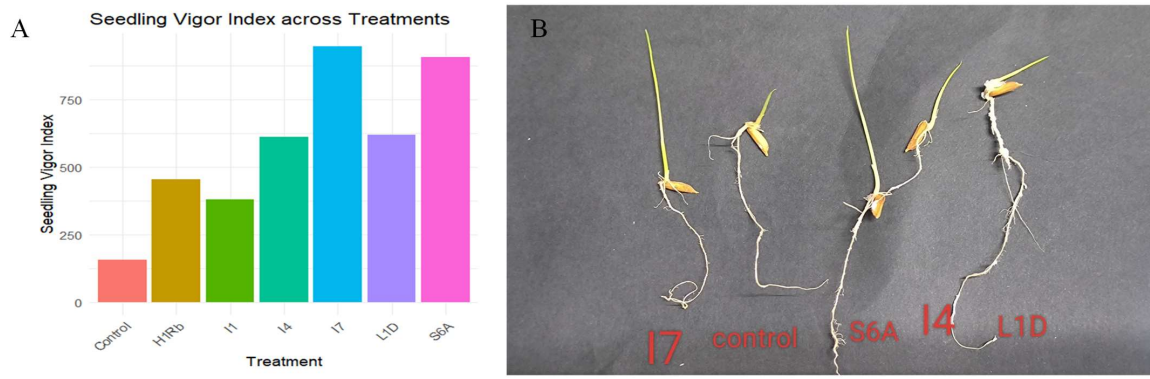


Fig. 3: A. Effect of seed treatment on seedling Vigor Index. B. Rice Seedlings showing plant growth promotion in germination study.

Table 5: Effect of treatment on germination of paddy seeds.

Seed treatment used	Concentration of GA in treated seeds [µg.g^{-1}]	Amylase activity U (in terms of maltose)	Germination Percentage [%]
Control	0.15±0.11	11.4	39.66±0.57
S6a	0.79 ±0.25	20.8	70.66±0.55
I1	0.56± 0.69	16.1	56.33±0.58
I7	0.90±1.1	30.6	84.33±0.57
I4	0.83± 0.68	27.9	81±1
L1D	0.69± 0.19	19.7	69.33±1.52
H1Rb	0.54± 0.29	18.5	64±1

Note: 1unit of Amylase enzyme = 10000 µM.min^{-1}.mL^{-1}

Table 6: Compatibility testing 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

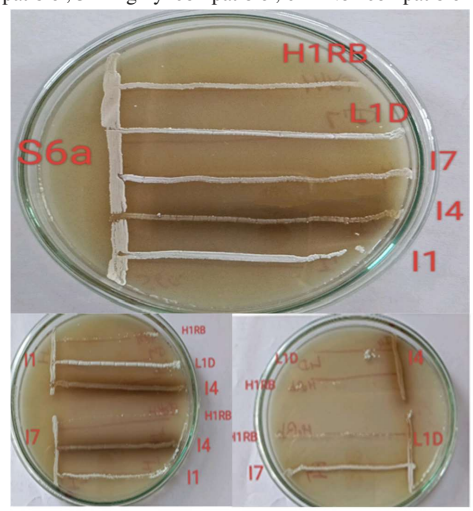
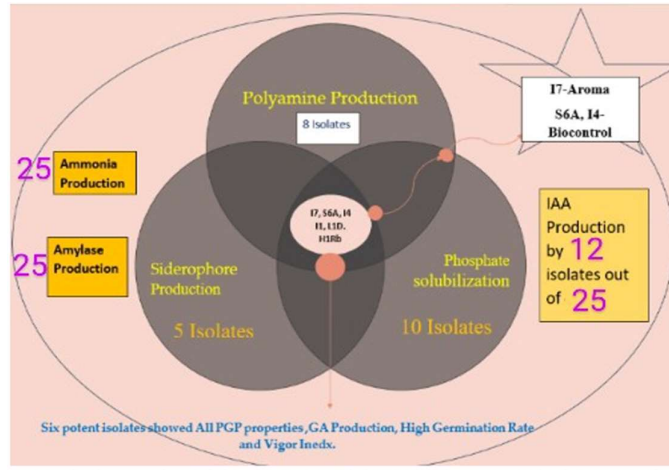


Fig. 4: Compatibility testing of Actinomycetes isolates H1Rb, I1, I4, I7, L1D and S6A.

actinomycetes, respectively, as in Fig. 4. S6A was highly compatible with all five other potent cultures.

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.

The SEM images of bioprimed roots showed that these *Streptomyces* strains can colonize the paddy roots without damaging them. 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 a significant reduction in the growth of *Cephalosporium maydis*, causing late wilt disease of maize in pot trials (El-Mehalawy et al. 2004). A similar study in



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

Fig. 5: Summary of research work.

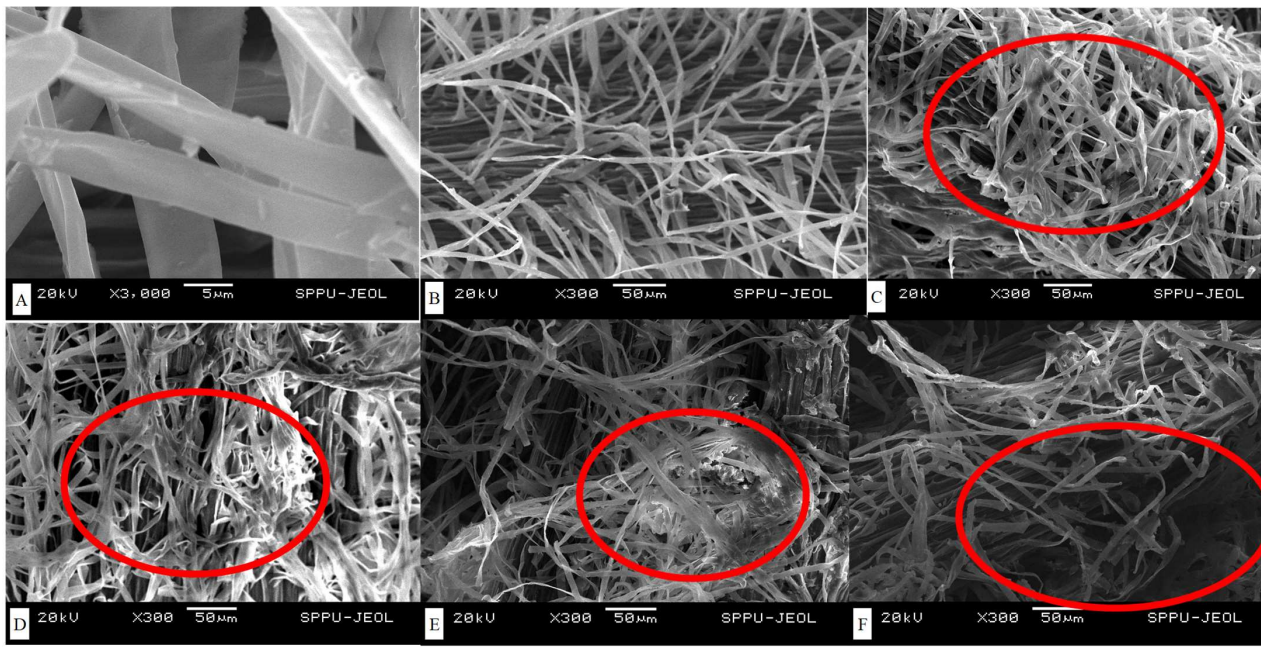


Fig. 6: A & B - Scanning electron micrographs of control roots at magnifications 3000X and 300X, respectively; C, D, E & F - SEM images of roots of seeds treated with actinomycetes L1D, S6A, I7 and I4, respectively. The highlighted area shows root colonization by the actinomycete species used.

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	*Streptomyces antibioticus* strain I4	PV652702	1409	100.00	*Streptomyces antibioticus* NBRC 12838(T)
2.	I7	*Streptomyces ardesiacus strain* I7	PV652733.1	1405 bp	99.72	*Streptomyces ardesiacus* NRRL B-1773
3.	L1D	*Streptomyces albogriseolus strain* L1D	PV652750.1	1434 bp	99.16	*Streptomyces albogriseolus* NRRL B-1305
4.	S6A	*Streptomyces tanashiensis strain* S6A	PV652742.1	1415bp	99.79	*Streptomyces hydrogenans* JCM 4771

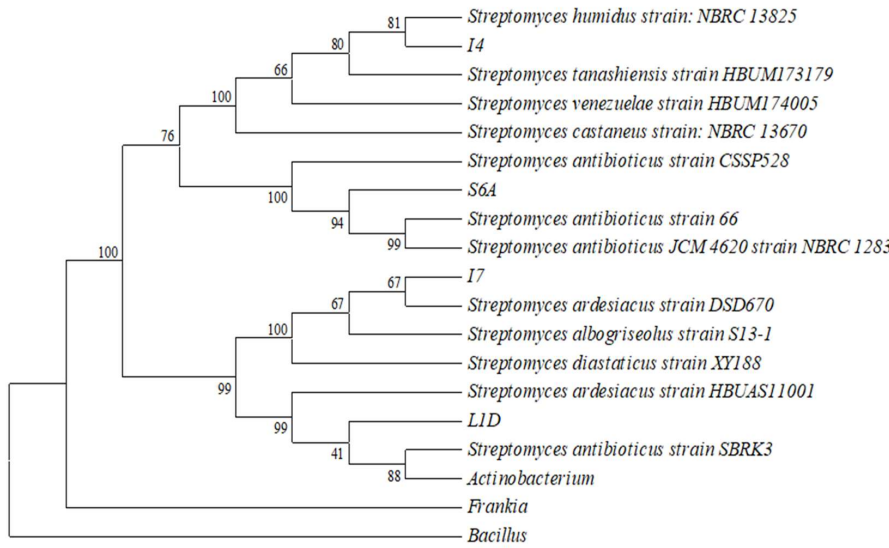


Fig. 7: Phylogenetic relationship between I4, S6A, I7, and L1D with representative microbial species based on full-length 16S rDNA sequences constructed using the neighbor-joining method.

chickpea showed that all 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).

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 the 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 GenBank, and NCBI accession numbers were obtained. The phylogenetic tree illustrates their genetic similarity and evolutionary relationship with other known actinomycetes. (Fig. 7).

4. CONCLUSIONS

Diversity was observed among Actinomycetes isolated from the rice (*Oryza sativa*) rhizosphere. A significant number of rhizospheric actinobacteria exhibited major plant growth-promoting properties. The present study showed that the production of growth hormones by microbes may enhance the germination rate. High GA content and increased α-amylase activity in extracts of germinated bio-primed seeds, compared with the control, highlight the potential role of actinomycetes, particularly Streptomyces, in increasing GA levels in the endosperm. *Streptomyces antibioticus* strain I4 has prominent biocontrol ability against bacterial leaf blight of rice. Production of 2AP by rhizospheric actinomycetes provides new insight into the enhancement of rice aroma. This study revealed the suitability of these

four multipotent actinomycetes, *Streptomyces antibioticus* strain I4, *Streptomyces ardesiacus* strain I7, *Streptomyces albogriseolus* strain L1D, and *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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6. REFERENCES

Adnan, M., Patel, M., Reddy, M.N., Khan, S., Alshammari, E., Abdelkareem, A.M. and Hadi, S., 2016. Isolation and characterization of effective and efficient plant growth-promoting rhizobacteria from rice rhizosphere of diverse paddy fields of Indian soil. *ARPN Journal of Agricultural and Biological Science*, 11(9), pp.373-379.

Anwar, S., Ali, B. and Sajid, I., 2016. Screening of rhizospheric actinomycetes for various in-vitro and in-vivo plant growth-promoting (PGP) traits and for agroactive compounds. *Frontiers in Microbiology*, 7, p.1334. [DOI]

Adnan, M.A., Shah, S., Khan, A., Fazli Wahid, M.I., Inayat-ur-Rahman, M.A.K. and Ali, R., 2018. Optimization of sowing time and seed rates can enhance wheat yield in semi-arid environment. *Philippine Agricultural Scientist*, 101(4). [DOI]

Asante, M.D., Asante, B.O., Acheampong, G.K., Offei, S.K., Gracen, V., Adu-Dapaah, H. and Danquah, E.Y., 2013. Farmer and consumer preferences for rice in the Ashanti region of Ghana: Implications for rice breeding in West Africa. *Journal of Plant Breeding and Crop Science*, 5(12), pp.229-238. [DOI]

Balouiri, M., Sadiki, M. and Ibnsouda, S.K., 2016. Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, 6(2), pp.71-79. [DOI]

Cappuccino, J.C. and Sherman, N., 1992. *Microbiology: A Laboratory Manual*. New York: Benjamin Cummings, pp.125-179.

Chakraborti, S., Bera, K., Sadhukhan, S. and Dutta, P., 2022. Bio-priming of seeds: Plant stress management and its underlying cellular, biochemical and molecular mechanisms. *Plant Stress*, 3, p.100052. [DOI]

Kabdwal, B.C., Sharma, R., Kumar, A., Kumar, S., Singh, K.P. and Srivastava, R.M., 2023. Efficacy of different combinations of microbial biocontrol agents against sheath blight of rice caused by *Rhizoctonia solani*. *Egyptian Journal of Biological Pest Control*, 33(1), p.29. [DOI]

Chinachanta, K., Shutsrirung, A., Herrmann, L., Lesueur, D. and Pathom-Aree, W., 2021. Enhancement of the aroma compound 2-acetyl-1-pyrroline in thai jasmine rice (*Oryza sativa*) by rhizobacteria under salt stress. *Biology*, 10(10), p.1065. [DOI]

Diab, M.K., Mead, H.M., Ahmad Khedr, M.M., Abu-Elsaoud, A.M. and El-Shatoury, S.A., 2024. Actinomycetes are a natural resource for sustainable pest control and safeguarding agriculture. *Archives of Microbiology*, 206(6), p.268.

Dhondge, H.V., Pable, A.A., Barvkar, V.T., Dastager, S.G. and Nadaf, A.B., 2021. Rhizobacterial consortium mediated aroma and yield enhancement in basmati and non-basmati rice (*Oryza sativa* L.). *Journal of Biotechnology*, 328, pp.47-58. [DOI]

Ekka, A. and Namdeo, N., 2018. Screening, isolation and characterization of amylase producing bacteria and optimization for production of amylase. *IOSR Journal of Biotechnology and Biochemistry*, 4, pp.50-56.

El-Mehalawy, A.A., Hassanein, N.M., Khater, H.M., Karam El-Din, E.A. and Youssef, Y.A., 2004. Influence of maize root colonization by the rhizosphere actinomycetes and yeast fungi on plant growth and on the biological control of late wilt disease. *Egyptian Journal of Microbiology*, 39(1), pp.45-62.

El-Tarabily, K.A. and Sivasithamparam, K., 2006. Non-streptomycete actinomycetes as biocontrol agents of soil-borne fungal plant pathogens and as plant growth promoters. *Soil Biology and Biochemistry*, 38(7), pp.1505-1520. [DOI]

Fiodor, A., Ajijah, N., Dziewit, L. and Pranaw, K., 2023. Biopriming of seed with plant growth-promoting bacteria for improved germination and seedling growth. *Frontiers in Microbiology*, 14, p.1142966. [DOI]

Graham, H.D. and Thomas, L.B., 1961. Rapid, simple colorimetric method for the determination of micro quantities of gibberellic acid. *Journal of Pharmaceutical Sciences*, 50(1), pp.44-48. [DOI]

Elshafie, H.S., Osman, A., El-Saber, M.M., Camele, I. and Abbas, E., 2023. Antifungal activity of green and chemically synthesized ZnO nanoparticles against *Alternaria citri*, the causal agent citrus black rot. *The Plant Pathology Journal*, 39(3), p.265. [DOI]

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. *International Journal of Systematic and Evolutionary Microbiology*, 53(6), pp.1907-1915. [DOI]

Mitchell, J.L. and Britt, E.M., 1981. A coverslip culture technique for preparing permanent fungus mounts. *Mycopathologia*, 76(1), pp.23-25. [DOI]

Nassar, A.H., El-Tarabily, K.A. and Sivasithamparam, K., 2003. Growth promotion of bean (*Phaseolus vulgaris* L.) by a polyamine-producing isolate of *Streptomyces griseoluteus*. *Plant Growth Regulation*, 40(2), pp.97-106. [DOI]

Rajendra Prasad, S., Kamble, U.R., Sripathy, K.V., Udaya Bhaskar, K. and Singh, D.P., 2016. Seed bio-priming for biotic and abiotic stress management. In: D.P. Singh, H.B. Singh and R. Prabha, eds., *Microbial Inoculants in Sustainable Agricultural Productivity: Vol. 1: Research Perspectives*. New Delhi: Springer India, pp.211-228. [DOI]

Sharif, M.K., Butt, M.S., Anjum, F.M. and Khan, S.H., 2014. Rice bran: a novel functional ingredient. *Critical Reviews in Food Science and Nutrition*, 54(6), pp.807-816. [DOI]

Sreevidya, M., Gopalakrishnan, S., Kudapa, H. and Varshney, R.K., 2016. Exploring plant growth-promotion actinomycetes from vermicompost and rhizosphere soil for yield enhancement in chickpea. *Brazilian Journal of Microbiology*, 47(1), pp.85-95. [DOI]

Van Overbeek, L.S. and Van Elsas, J.D., 1995. Root exudate-induced promoter activity in *Pseudomonas fluorescens* mutants in the wheat rhizosphere. *Applied and Environmental Microbiology*, 61(3), pp.890-898. [DOI]

Wahyudi, A.T., Priyanto, J.A., Fijrina, H.N., Mariastuti, H.D. and Nawangsih, A.A., 2019. *Streptomyces* spp. from rhizosphere soil of maize with potential as plant growth promoter. *Biodiversitas*, 20(9), pp.2547-2553.

Wang, W., Jiang, Q., Tan, B., Yang, H., Cao, F., Chen, J., Huang, M. and Zheng, H., 2025. The physio-biochemical changes induced by gibberellic acid seed priming in rice seeds against submergence stress: Consequences for starch degradation, energy metabolism and hormonal metabolism. *Rice*, 18, p.25. [DOI]

Yadav, A.N., Verma, P., Singh, B., Chauhan, V.S., Suman, A. and Saxena, A.K., 2017. Plant growth promoting bacteria: biodiversity and multifunctional attributes for sustainable agriculture. *Advances in Biotechnology & Microbiology*, 5(5), pp.1-16.