ORIGINAL RESEARCH PAPER

Microbiology

BIOCONTROL POTENTIAL OF *STREPTOMYCES* SPP. AGAINST MAJOR BACTERIAL PLANT PATHOGENS

KEY WORDS: Biocontrol agents, Phytopathogens, PGPB , Biofertilizer.Streptomyces

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ABSTRACT

The excessive use of chemical fertilizers in agriculture has led to environmental deprivation and reduced crop production. This study focuses on the isolation and characterization of soil-dwelling Streptomyces spp., Pseudomonas and Bacillus spp., as potential biocontrol agents against Xanthomonas spp. and Ralstonia spp. Rhizosphere soil samples were collected and subjected to serial dilution and culturing. The isolates were identified through morphological, biochemical, and enzymatic profiling. Phytopathogens were isolated from infected citrus and potato samples and confirmed via selective media and biochemical tests. The soil isolates were evaluated for plant growth-promoting (PGP) traits such as phosphate and zinc solubilization, indole-3-acetic acid (IAA), siderophore, ammonia, and hydrogen cyanide production. Antagonistic assays demonstrated inhibitory effects of the isolates on the pathogens, particularly Bacillus sp. against Xanthomonas spp. Seed germination studies using groundnut and green gram revealed improved germination rates and vigor index following bacterial treatment, with Pseudomonas sp. showing the highest efficacy. These findings underscore the potential of Streptomyces, Pseudomonas, and Bacillus spp. as biocontrol agents and biofertilizers.

INTRODUCTION

The continuous growth in the human population increases the demand for food (FAO, 2017). Human activities addressing food shortages have negatively impacted the natural environment (Tilman *et al.,* 2011). Plants are vulnerable to environmental changes, making them prone to diseases under stressful conditions (Boyer, 1982). To enhance crop yield, chemical fertilizers were widely adopted (Savci, 2012). However, excessive use of fertilizers affects soil properties and reduces crop productivity (Guo *et al.*, 2010).

Scientific research has identified plant growth-promoting bacteria (PGPB), which serve as biological fertilizers (Vessey, 2003). *Streptomyces* sp. produce antibiotics that inhibit harmful pathogens (Barka *et al.,* 2016). Beneficial bacteria support host plants by enhancing growth and providing protection against diseases (Glick, 2012). *Streptomyces* contribute to plant growth promotion by producing phytohormones such as indole-3-acetic acid (IAA) (Jog *et al.*, 2014). They also produce antagonistic compounds like siderophores and antibiotics (Butt *et al.*, 2023).

Plant diseases account for significant yield loss (Gohel *et al.*, 2006). *Streptomyces* species produce antimicrobial compounds protecting plants from pathogens (Vurukonda *et al.,* 2018). Despite their potential, commercialization remains limited due to challenges in formulation and field performance (LeBlanc, 2022).

MATERIALS AND METHODS

COLLECTION OF SOIL SAMPLE

Rhizosphere soil samples were collected from garden lands in and around Thindal, Erode District, Tamil Nadu, India. (Wang *et al.*, 2022)

SERIAL DILUTION & STAINING TECHNIQUE

Serial dilution was prepared by adding 1 g soil sample to 9 ml sterile water and diluted up to 10^{-10}. Dilutions were spread on starch casein agar and nutrient agar plates and incubated at 30°C for 7 days. Colonies were selected for further studies and gram staining was performed. (Chouyia *et al.*, 2022).

ISOLATION OF PHYTOPATHOGENS

Citrus canker infected leaves and fruits were collected and processed. Suspensions were streaked on YDA medium and incubated at 25–28°C for 2–5 days. Potato brown rot samples were streaked on TTC medium and incubated at 28±2°C. (Pang *et al.*, 2024).

IDENTIFICATION OF ISOLATES

Basic identification was done using Gram staining and biochemical tests including IMViC, carbohydrate fermentation, catalase, urease, starch hydrolysis, KOH solubility, fluorescence, levan production, and nitrate reduction tests. (Berza *et al.*, 2022).

ASSESSMENT OF GROWTH PROMOTING CHARACTERS

The isolates were screened for siderophore production, phosphate solubilization, IAA production, EPS production, zinc solubilization, ammonia production, nitrogen fixation, potassium solubilization, and HCN production. (Albertini *et al.,* 2022).

ANTAGONISTIC ACTIVITY

Antimicrobial activity of Pseudomonas, *Streptomyces*, and *Bacillus* isolates was tested against *Xanthomonas* sp. and *Ralstonia* sp. (Wang *et al.,* 2022).

ENZYME ACTIVITY

Amylase, protease, lipase, catalase, and cellulase activities were assessed using standard methods. (Kaur and Kaur, 2022).

PHYTOTOXICITY

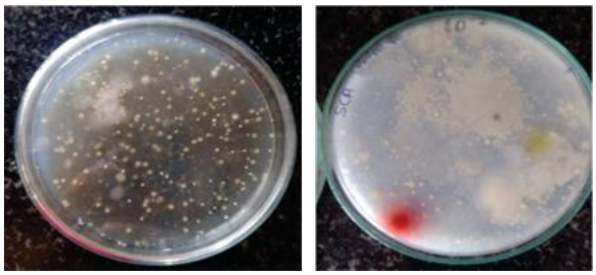
Groundnut and green gram seeds treated with bacterial isolates were incubated under laboratory conditions. Germination percentage and vigor index were determined. (Bourak *et al.,* 2024).

RESULT

ISOLATION OF SOIL BACTERIA

Streptomyces isolates showed varied pigmentation and gram-positive filamentous branching mycelia. Two additional isolates were identified as gram-positive and gram-negative rods.

Plate :1 Screening of *strepyomyces sp* on Starch casein agar



BIOCHEMICAL CHARACTERISTICS

The isolates reduced nitrate, utilized citrate, and hydrolyzed casein and starch. S1 was identified as Pseudomonas sp., S2 as Streptomyces sp., and S3 as Bacillus sp.

Table :1 Biochemical characterization of soil isolates

S.NO	Test parameters	S1	S2	S3
1	Indole	-	-	-
2	Methyl red	-	-	-
3	Voges proskauer	-	-	-
4	Citrate utilization test	-	-	-
5	Urease	-	-	-
6	Glucose fermentation	+	+	+
7	Mannitol fermentation	+	-	+
8	Sucrose fermentation	+	+	+
9	Starch hydrolysis	-	-	-
10	Casein hydrolysis	+	+	+
11	Nitrate reduction test	+	-	+
12	Probable identify to genus level	Pseudomonas sp	Streptomyces sp	Bacillus sp

ISOLATION OF PHYTOPATHOGENS

Xanthomonas spp. isolated from citrus samples produced pale yellow and dark yellow colonies on YDA medium. Ralstonia spp. isolated from potato samples produced pink-centered colonies on TTC medium.

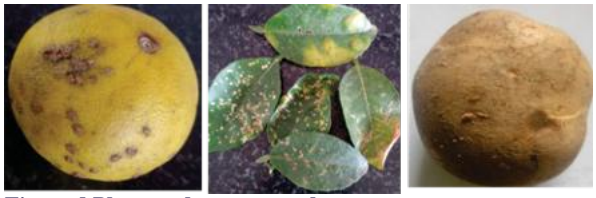


Figure 1 Phytopathogen sample

Table 2 Plant Samples

S.NO	Sample	No.of.isolates	Sample code
1	Lemon leaf	1	X1
2	Lemon fruit	1	X2
3	potato	1	X3

Table 3 Number of Isolates from Each Plant Species

S.NO	Isolates	Colony characteristics	Gram's reaction
1	X1	Pale yellow, dry colonies(YDA)	Gram negative rod
2	X2	Dark yellow, mucoid colonies (YDA)	Gram negative rod
3	X3	Pink spot in the middle of the colony and a white edge(TTC)	Gram negative rod

PLANT GROWTH PROMOTING CHARACTERS

All isolates showed positive results for IAA production, HCN production, ammonia production, zinc solubilization, phosphate solubilization, and siderophore production. Nitrogen fixation and potassium solubilization were absent.

Table 4:Plant growth Promoting Charecteristics for the isolate

S.no	Test parameters	Colour appearance	Pesudomonas	Streptomyces	Bacillus
1	Indole acetic acid	Pink color	+	+	+
2	Nitrogen fixation test	Growth formation	-	-	-
3	Hydrognen cyanide test	Red colour	+	+	+
4	Ammonia production	Dark brown colour	+	+	+
5	Zinc solubilization	Zone formation	+	+	+
6	Phosphate solubilization	Zone formation	+	+	+
7	Potassium solubilization	Zone formation	-	-	-
8	Eps production	Zone formation	+	-	+
9	siderophore	Reddish brown	+	+	+

ASSESSMENT OF PLANT GROWTH PROMOTING CHARACTERS OF SOIL ISOLATES:

Table 4 and Plate 4 revealed that isolate was positive for growth promoting characters. i.e., isolate produced clear zone on Pikovska's agar that confirmed phosphate solubilization character. On addition of Salkowski reagent into 72 hrs old king's broth culture its produce pink red colour indicated the production IAA and while adding Nessler's reagent into culture broth a development of Faint yellow to Dark brown present ammonium production. At last development of Orange to Red colour on Whats man Filter paper:1 that was placed on the lid of petriplate which was streaked by isolate as a positive result for HCN production (plate 4)

ENZYME ACTIVITY

Cellulase and catalase activity were positive in all isolates. Lipase activity was observed only in Bacillus sp.

ANTAGONISTIC ACTIVITY

Maximum inhibition against Xanthomonas sp.1 was shown by Bacillus sp. (15 mm), while Pseudomonas sp. showed 14 mm inhibition against Xanthomonas sp.2.shown in Table 6.

Table 6

S.NO	Soil isolate	Zone of inhibition		
		Xanthomonas sp 1	*Xanthomonas sp 2*	*Ralstonia*
1	*Pseudomonas sp*	10mm	14mm	R
2	*Streptomyces sp*	R	13mm	11mm
3	*Bacillus sp*	15mm	10mm	R

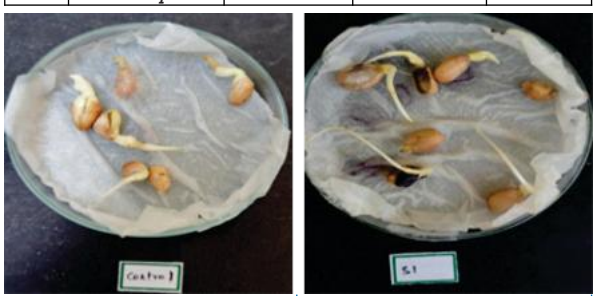


Plate 2 Effect of bacterial seed treatment on germination and vigor index (Ground nut)

SEED GERMINATION STUDY

All treatments improved germination and vigor index in groundnut and green gram. Pseudomonas sp. treatment showed the highest vigor index. Plate 2

Table :7 Effect of bacterial seed treatment on germination and vigor index (Ground nut)

S.NO	Growth measurement	Bacteria used for seed treatment		
		Pseudomonas sps	***Streptomyces sps***	***Bacillus sp***
1	Growth rate(%)	100	100	100
2	Relative shoot length(%)	80.35	117.85	91.05
3	Relative root length(%)	86.43	77.32	95.54
4	Vigor index	124.45	84.85	109.28

The vigor index increased up to 124.45% and 139.62% in Pseudomonas sp treated, 84.85% and 101.37%in Streptomyces sp treated,109.28%and 73.27% in Bacillus sp treated Ground nut and greengram under laboratory condition compared to control. Streptomyces sp, Pseudomonas sp ,Bacillius sp treatment improved the growth rate of ground nut and green gram seed 100%under lab condition .

Plate: 3 Effect of bacterial seed treatment on germination and vigor index (Green Gram)

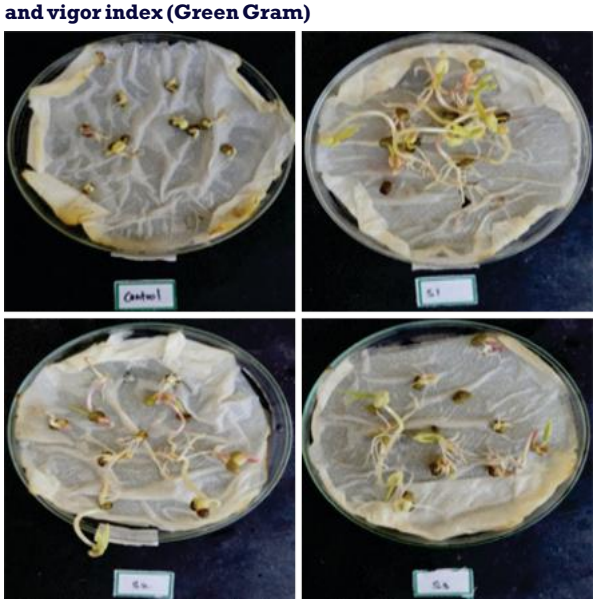


Table :8 Effect of bacterial seed treatment on germination and vigor index (Green gram)

S.NO	Growth measurement	Bacteria used for seed treatment		
		Pseudomonas sps	**Streptomyces sps**	**Bacillus sp**
1	Growth rate(%)	100	100	100
2	Relative shoot length(%)	71.62	98.64	136.48
3	Relative root length(%)	80	93.33	86.66
4	Vigor index	139.62	101.37	73.27

The vigor index increased up to 124.45% and 139.62% in *Pseudomonas* sp treated, 84.85% and 101.37%in *Streptomyces* sp treated,109.28%and 73.27% in *Bacillus* sp treated Ground nut and greengram under laboratory condition compared to control. *Streptomyces* sp, *Pseudomonas* sp ,Bacillius sp treatment improved the growth rate of ground nut and green gram seed 100%under lab condition .

DISCUSSION

The phenotypic, microscopic, and biochemical characterization of the isolates confirmed the presence of *Streptomyces* spp., *Pseudomonas* sp., and *Bacillus* sp. based on their distinct colony morphology, pigmentation, filamentous structures, spore formation, Gram reaction, and biochemical profiles. *Streptomyces* isolates showed characteristic aerial and substrate mycelia, while *Pseudomonas* sp. and *Bacillus* sp. exhibited typical Gram-negative and Gram-positive rod morphology respectively.

All isolates demonstrated metabolic versatility through positive casein and starch hydrolysis, nitrate reduction, and citrate utilization tests, whereas negative indole and Voges–Proskauer tests aided in genus-level identification according to Bergey's Manual of Systematic Bacteriology. The successful isolation of *Xanthomonas* spp. from citrus and *Ralstonia* spp. from potato rot confirmed the occurrence of important phytopathogens, identified through colony morphology on selective media, biochemical assays, sugar fermentation, and KOH string tests, although the Gram reaction inconsistency observed for *Xanthomonas* in the KOH test was noted. The isolates also exhibited several plant growth-promoting traits including phosphate and zinc solubilization, IAA production, ammonia and HCN production, which contribute to enhanced nutrient availability and suppression of soil-borne pathogens.

Although nitrogen fixation was absent, EPS production by *Pseudomonas* and *Bacillus* sp. indicated their biofilm-forming ability and improved rhizosphere colonization. Similar findings were reported by Mendes R (2024), who highlighted the role of exopolymeric genes in tomato rhizosphere colonization, and by Ma *et al.* (2022), who described the regulation of biofilm exopolysaccharide biosynthesis and degradation in *Pseudomonas* aeruginosa, emphasizing the importance of EPS production in plant–microbe interactions.

CONCLUSION

This study, focused on the in vitro activities of a potential PGP of *Streptomyces* sp, *Pseudomonas* sp, *Bacillus* sp from Rhizosphere soil a safe potential biocontrol agent against *Xanthomonas* sp and *Ralstonia* sp. These analyses facilitated the best selection of the PGP *Streptomyces* candidates. Plant growth promotion assays also demonstrated the growth-promoting effects of seeds treated with the *Streptomyces* spp. *Pseudomonas* sp, *Bacillus* sp, and treatments were supported by their enzymatic activities and PGP traits. Results have indicated the potential of *Streptomyces* sp, *Pseudomonas* sp, *Bacillus* sp as a biocontrol agent against *Xanthomonas* sp, *Ralstonia* sp and plant growth-promotors in Green gram and Ground nut offering the applicability of *Streptomyces* spp. in improving plant production.

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