RESEARCH PAPER

Isolation, Biochemical Profiling and Molecular Characterization of Phosphate Solubilizing Microorganisms with Antimicrobial Activity Against Plant Pathogens from Orange Rhizosphere of Bhiwapur Tahsil, Nagpur District, Maharashtra, India

Dhanshree M. Ridhorkar *[1] , Y.S. Banginwar [2]

[1]Department of Molecular Biology and Genetic Engineering, RTMNU, Nagpur
[2] Shankarrao Bhoyar Patil Mahavidyalaya, Pulgaon
email: omshreesainathom@gmail.com,

Abstract
The present investigation focused on the exploration of native phosphate solubilizing microorganisms associated with orange rhizospheric soil collected from Bhiwapur Tahsil of Nagpur District, Maharashtra. The study aimed to isolate efficient phosphate solubilizing microorganisms and evaluate their biochemical characteristics, molecular identity and antimicrobial potential against selected plant pathogens. Soil samples collected from citrus orchards were processed using serial dilution techniques and cultured on selective Pikovskaya's medium. Distinct colonies producing phosphate solubilization zones were purified and subjected to cultural, morphological and biochemical examination. The isolates were further screened for phosphate solubilization efficiency and plant growth promoting attributes including indole acetic acid production, siderophore secretion HCN Production and ammonia production.
Antagonistic activity of the selected isolates was evaluated against important phytopathogens such as Fusarium oxysporum and Xanthomonas citri under laboratory conditions. Molecular identification was carried out through 16S rRNA gene amplification and sequence analysis. The study revealed that several isolates belonging to *Bacillus subtilis* genera possessed efficient phosphate solubilization ability along with notable antimicrobial activity. Among the isolates, PSMB37 demonstrated comparatively higher phosphate solubilization and pathogen inhibition efficiency.
The findings indicate that orange rhizospheric microorganisms from Bhiwapur Tahsil may serve as promising candidates for the development of eco-friendly biofertilizer and biocontrol formulations for sustainable citrus cultivation.
Keywords: Phosphate solubilizing microorganisms, orange rhizosphere, antimicrobial activity, Bacillus, Pseudomonas, 16S rRNA, biocontrol, plant growth promoting rhizobacteria.
How to cite this article: Ridhorkar DM, Banginwar YS. Isolation, Biochemical Profiling and Molecular Characterization of Phosphate Solubilizing Microorganisms with Antimicrobial Activity Against Plant Pathogens from Orange Rhizosphere of Bhiwapur Tahsil, Nagpur District, Maharashtra, India. Int J Drug Deliv Technol. 2026;16(55s): 628-639. DOI: 10.25258/ijddt.16.55s.64

1. Introduction

Sustainable agricultural practices increasingly depend upon beneficial soil microorganisms capable of improving nutrient availability and suppressing phytopathogens. Among the essential plant nutrients, phosphorus is considered one of the major limiting factors for crop productivity because a substantial proportion of soil phosphorus remains unavailable in insoluble complexes [1]. Continuous dependence on chemical fertilizers to compensate for phosphorus deficiency often leads to deterioration of soil fertility and environmental imbalance. Consequently, microbial approaches for nutrient mobilization are gaining considerable attention in modern agriculture. Phosphate solubilizing microorganisms constitute an important group of rhizospheric microbes capable of transforming insoluble phosphate compounds into plant-available forms through the production of organic acids and other metabolites [2,3]. In addition to nutrient mobilization, many phosphate solubilizers are recognized for their multiple plant growth promoting properties including phytohormone production, siderophore secretion and biological suppression of plant pathogens [4]. These characteristics make them valuable candidates for integrated nutrient and disease management programs.

*Author for Correspondence: omshreesainathom@gmail.com,

The citrus rhizosphere represents a dynamic microbial environment enriched with diverse bacterial populations that influence plant growth and soil health. Orange cultivation occupies an important position in the agricultural economy of the Vidarbha region of Maharashtra, especially in Nagpur District. Bhiwapur Tahsil is known for citrus cultivation due to its favorable agroclimatic conditions. However, citrus orchards frequently encounter yield losses due to fungal and bacterial diseases caused by pathogens such as Fusarium oxysporum and Xanthomonas citri. Isolation of indigenous rhizospheric microorganisms adapted to local soil conditions may provide efficient strains for agricultural application. Native phosphate solubilizing microorganisms with antimicrobial potential can contribute simultaneously to nutrient availability and disease suppression. Therefore, the present work was undertaken to isolate phosphate solubilizing microorganisms from orange rhizosphere soils of Bhiwapur Tahsil and characterize them using biochemical and molecular approaches while evaluating their antagonistic activity against selected plant pathogens.

2. Objectives

1. To isolate phosphate solubilizing microorganisms from orange rhizospheric soil of Bhiwapur Tahsil.
2. To characterize the isolates based on morphological and biochemical properties.
3. To evaluate phosphate solubilization efficiency of the isolates.
4. To determine plant growth promoting traits of selected isolates.
5. To assess antagonistic activity against important plant pathogens.
6. To perform molecular characterization using 16S rRNA gene sequencing.

3. Materials and Methods

3.1 Study Area

The present study was conducted using orange orchards located in different villages of Bhiwapur Tahsil, Nagpur District, Maharashtra, India. The region is characterized by tropical climatic conditions suitable for citrus cultivation.

3.2 Collection of Soil Samples

Rhizospheric soil samples were collected from healthy orange plants at a depth of 5–15 cm using sterile polythene bags. Samples were transported to the laboratory and stored at 4°C until further processing.

3.3 Isolation of Phosphate Solubilizing Microorganisms

Isolation of phosphate solubilizing microorganisms was carried out using serial dilution and spread plate techniques on Pikovskaya's agar medium [1,10]. One gram of soil sample was suspended in 9 mL sterile distilled water and serially diluted up to 10−6 dilution. Aliquots from appropriate dilutions were spread onto sterile Pikovskaya's agar plates and incubated at 28 ± 2°C for 48–72 h. Colonies showing clear halo zones around the growth were considered positive for phosphate solubilization and purified by repeated streaking.

3.4 Morphological Characterization

Morphological characterization of bacterial isolates was carried out according to standard microbiological methods [16]. The isolates were examined for colony morphology including shape, size, color, elevation, margin and texture. Gram staining and microscopic observations were performed to determine cellular morphology.

3.5 Biochemical Characterization

Biochemical characterization was performed following standard protocols [17].

Biochemical characterization of isolates was carried out using standard microbiological procedures. The following tests were performed:

- Gram staining
- Catalase test
- Indole test
- Methyl red test
- Voges-Proskauer test
- Citrate utilization test
- Motility test
- Sugar fermentation tests

3.6 Qualitative Phosphate Solubilization Assay

Qualitative phosphate solubilization assay was performed using Pikovskaya's agar medium [1]. Qualitative phosphate solubilization was determined on Pikovskaya's agar medium by measuring halo zone diameter around bacterial colonies after incubation.

Phosphate solubilization efficiency (PSE) was calculated using the formula:

PSE (%) = (Halo zone diameter / Colony diameter) × 100

3.7 Plant Growth Promoting Traits

Plant growth promoting traits were analyzed using standard microbiological methods [2,7].

3.7.1 Indole Acetic Acid Production

IAA production assay was performed using standard colorimetric procedure [19]. IAA production was determined using Salkowski reagent after incubation in tryptophan supplemented broth.

3.7.2 Ammonia Production

Ammonia production was determined using standard microbiological procedure [17]. Ammonia production was detected by inoculating isolates in peptone water followed by addition of Nessler's reagent.

3.7.3 Siderophore Production

Siderophore production was analyzed using Chrome Azurol S agar method [20]. Siderophore production was evaluated using Chrome Azurol S agar medium.

3.7.4 Hydrogen cyanide (HCN) production

Hydrogen cyanide (HCN) production by bacterial isolates was determined using the qualitative method. The isolates were streaked on nutrient agar medium supplemented with glycine (4.4 g/L). A sterile Whatman No. 1 filter paper soaked in picric acid and sodium carbonate solution was placed inside the lid of each Petri plate. The plates were sealed and incubated at 28 ± 2°C for 48–72 h. Development of orange to reddish-brown coloration on the filter paper indicated HCN production by the isolates.[24]

3.8Antagonistic Activity Against Plant Pathogens

Antagonistic activity assays were carried out using standard biocontrol evaluation procedures [12].

3.8.1 Antifungal Activity

Antifungal activity against Fusarium oxysporum was evaluated using dual culture technique [21]. Antagonistic activity against Fusarium oxysporum was assessed using dual culture technique on potato dextrose

agar plates. Percentage inhibition of fungal growth was calculated.

3.8.2 Antibacterial Activity

Antibacterial activity against Xanthomonas citri was determined by agar well diffusion method [22]. Antibacterial activity against Xanthomonas citri was determined using agar well diffusion method. Zone of inhibition was measured after incubation.

3.9Molecular Characterization

Molecular characterization using 16S rRNA gene sequencing was performed using standard PCR amplification method [23].

Genomic DNA from selected efficient isolates was extracted using standard protocols. Amplification of 16S rRNA gene was performed using universal primers 27F and 1492R.

PCR amplification conditions included:

- Initial denaturation: 95°C for 5 min
- 35 cycles of:
  - Denaturation at 95°C for 30 sec
  - Annealing at 55°C for 30 sec
  - Extension at 72°C for 1 min
- Final extension at 72°C for 10 min

Amplified PCR products were analyzed by agarose gel electrophoresis and sequenced. Sequence similarity was analyzed using NCBI BLAST.

3.10 Statistical Analysis

All experiments were carried out in triplicates and results were expressed as mean ± standard deviation

4. Results

4.1 Isolation of Phosphate Solubilizing Microorganisms

A total of 80 soil samples were collected from the orange rhizosphere of Bhiwapur Tahsil. From these samples, 36 phosphate-solubilizing microbial isolates were successfully obtained. The isolates exhibited clear halo zones on Pikovskaya's agar medium, indicating their ability to solubilize insoluble phosphate and demonstrating phosphate-solubilizing activity..

4.2 Morphological and Biochemical Characterization

The isolates showed considerable variation in colony morphology, pigmentation and biochemical characteristics. Most efficient isolates were Gram-positive rod-shaped bacteria belonging to Bacillus spp.

Table 1. Morphological and Biochemical Characteristics of Selected Isolates

Sr.No.		Gram reaction	shape	Indole	MR	VP	Citrate	Motility	Endospore Staining	Lactose fermentation	Sucrose fermentation	Manitol fermentation	Glucose fermentation	catalase
1	PSMRU1	Gram +ve	Rod	-	+	-	+	+	+	-	+	+	+	+
2	PSMRU2	Gram -ve	Cocci	-	-	+	+	-	-	+	+	-	+	-
3	PSMRU3	Gram +ve	Rod	-	+	-	-	+	+	-	-	+	+	+
4	PSMRU5	Gram -ve	Rod	+	-	+	+	+	-	+	+	+	+	+
5	PSMRU7	Gram +ve	Cocci	-	+	-	-	-	-	+	-	-	+	+

6	PSMRU9	Gram -ve	Rod	+	+	-	+	+	-	+	+	-	+	+
7	PSMRU10	Gram +ve	Rod	-	-	+	+	+	+	-	+	+	+	+
8	PSMRU12	Gram -ve	Cocci	-	+	-	-	-	-	+	-	+	+	-
9	PSMRU13	Gram +ve	Rod	-	+	-	+	+	+	-	+	-	+	+
10	PSMRU18	Gram -ve	Rod	+	-	+	+	+	-	+	+	+	+	+
11	PSMRU19	Gram +ve	Cocci	-	+	-	-	-	-	-	+	+	+	+
12	PSMRU20	Gram -ve	Rod	+	+	-	+	+	-	+	-	-	+	+
13	PSMRU21	Gram +ve	Rod	-	-	+	+	+	+	-	+	+	+	+
14	PSMRU22	Gram -ve	Cocci	-	+	-	+	-	-	+	+	-	+	-
15	PSMRU23	Gram +ve	Rod	-	+	-	-	+	+	-	-	+	+	+
16	PSMRU25	Gram -ve	Rod	+	-	+	+	+	-	+	+	+	+	+

4.3 PHOSPHATE SOLUBLIZATION INDEX OF ISOLATES

17	PSMRU29	Gram +ve	Cocci	-	+	-	-	-	-	+	-	-	+	+
18	PSMRU33	Gram -ve	Rod	+	+	-	+	+	-	+	+	-	+	+
19	PSMRU34	Gram +ve	Rod	-	-	+	+	+	+	-	+	+	+	+
20	PSMRU35	Gram -ve	Cocci	-	+	-	-	-	-	+	-	+	+	-
21	PSMRU36	Gram +ve	Rod	-	+	-	+	+	+	-	+	-	+	+
22	PSMRU37	Gram -ve	Rod	+	-	+	+	+	-	+	+	+	+	+
23	PSMRU40	Gram +ve	Cocci	-	+	-	-	-	-	-	-	+	+	+
24	PSMRU41	Gram -ve	Rod	+	+	-	+	+	-	+	+	-	+	+
25	PSMRU42	Gram +ve	Rod	-	-	+	+	+	+	-	+	+	+	+
26	PSMRU43	Gram -ve	Cocci	-	+	-	+	-	-	+	-	-	+	-
27	PSMRU44	Gram +ve	Rod	-	+	-	-	+	+	-	+	+	+	+

28	PSMRU45	Gram -ve	Rod	+	-	+	+	+	-	+	+	+	+	+
29	PSMRU48	Gram +ve	Cocci	-	+	-	-	-	-	+	-	+	+	+
30	PSMRU51	Gram -ve	Rod	+	+	-	+	+	-	+	+	-	+	+
31	PSMRU52	Gram +ve	Rod	-	-	+	+	+	+	-	+	+	+	+
32	PSMRU53	Gram -ve	Cocci	-	+	-	-	-	-	+	-	-	+	-
33	PSMRU57	Gram +ve	Rod	-	+	-	+	+	+	-	+	-	+	+
34	PSMRU58	Gram -ve	Rod	+	-	+	+	+	-	+	+	+	+	+
35	PSMRU61	Gram +ve	Cocci	-	+	-	-	-	-	-	+	+	+	+
36	**PSMRU65**	**Gram -ve**	**Rod**	+	+	-	+	+	-	+	-	-	+	+

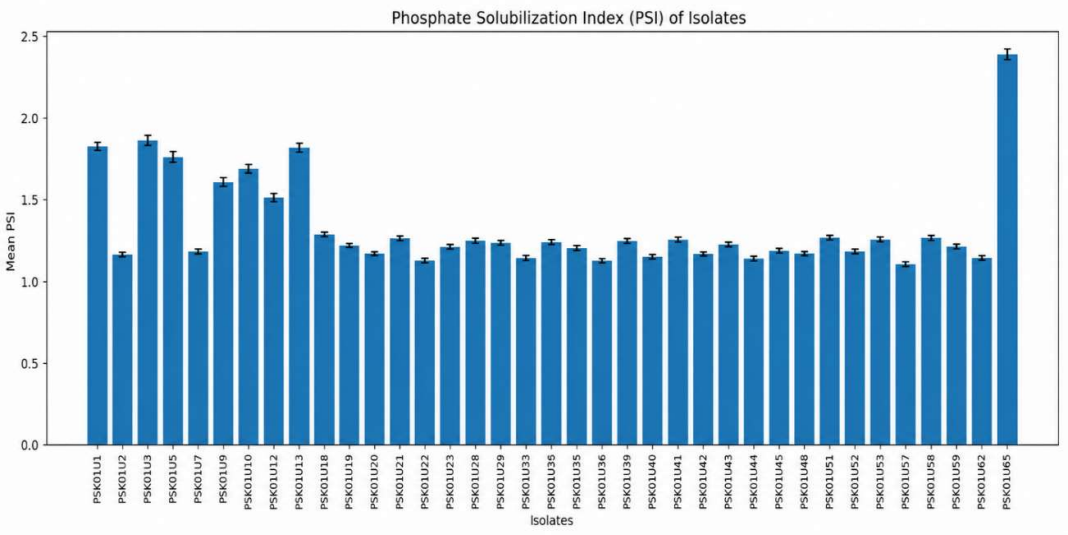


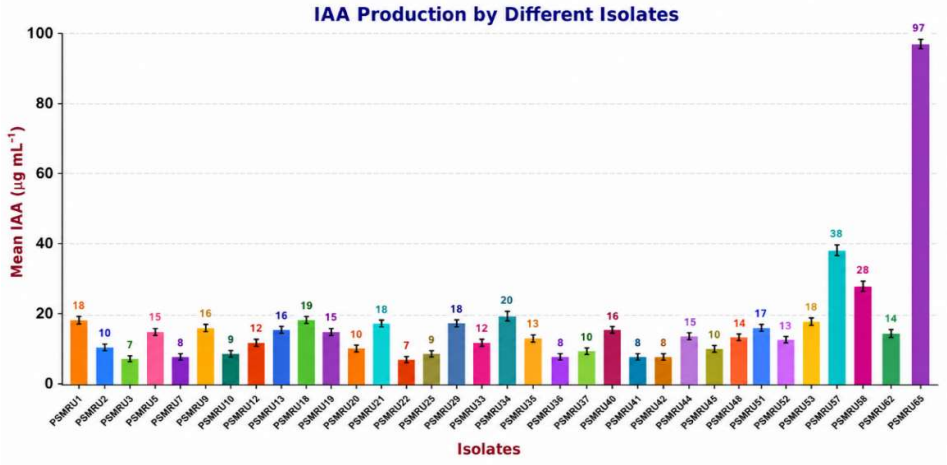
Fig No.1 PHOSPHATE SOLUBLIZATION INDEX OF ISOLATES

Among all isolates, **PSMRU65 s**howed the highest phosphate solubilization index (~2.38), indicating excellent phosphate-solubilizing potential.
Other isolates with comparatively high PSI values include:
PSMRU3 (~1.85)
PSMRU1 (~1.82)
PSMRU13 (~1.81)
PSMRU5 (~1.76)
PSMRU10 (~1.69)
Most remaining isolates exhibited moderate PSI values ranging from 1.10 to 1.30.

4.4 Plant Growth Promoting Traits

4.4.1 IAA Production of Different isolate

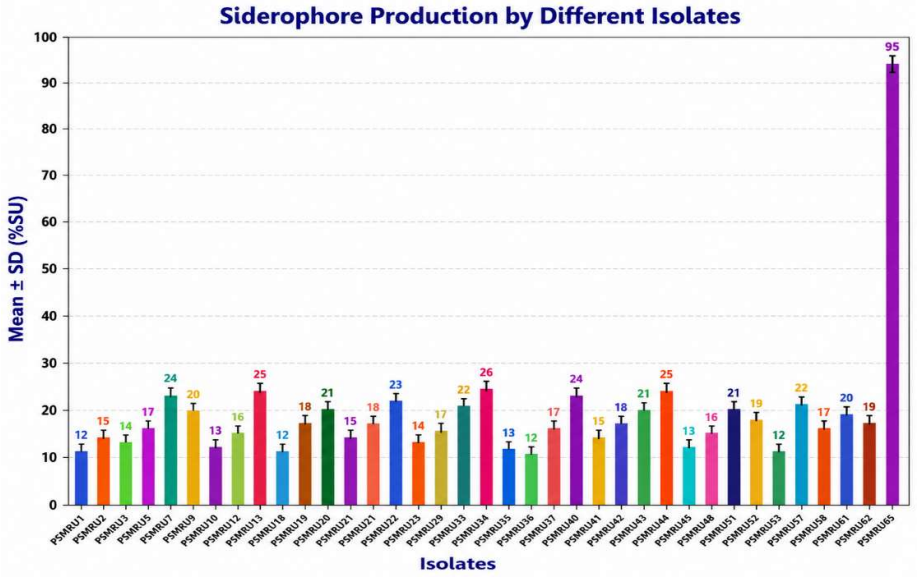
Several isolates exhibited important plant growth promoting traits including IAA production, ammonia production and siderophore production.



FigNo.2 IAA Production of Different isolate

The study indicates considerable variation in IAA production among the isolates. Among all tested isolates, **PSMRU65 was identified as the most efficient IAA producer**, followed by **PSMRU58** and **PSMRU65**

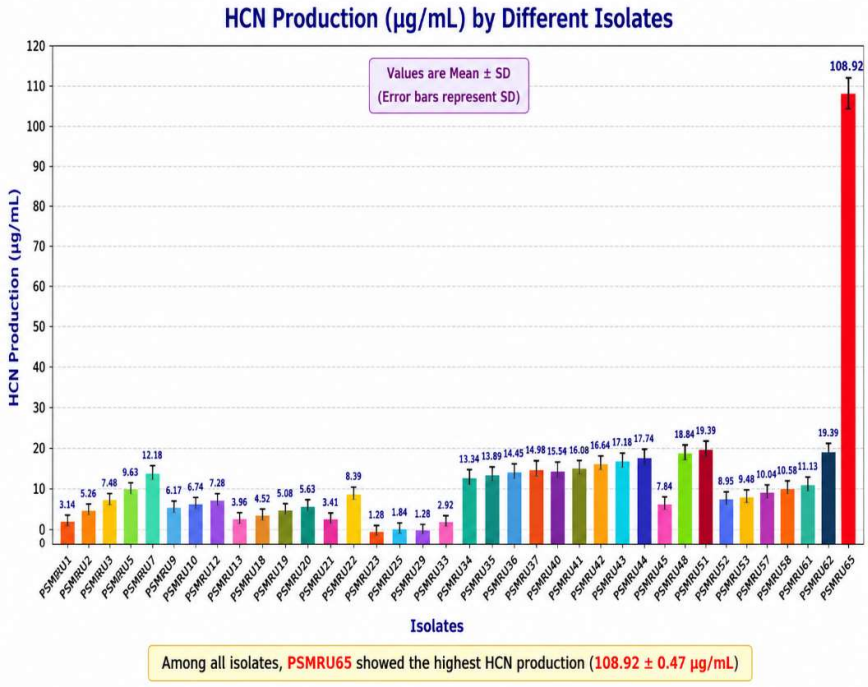
4.4.2 Siderophore Production of Different isolate



FigNo.3 Siderophore Production of Different isolate

Among all isolates, PSMRU65 showed exceptionally high siderophore production (approximately 95 %SU), indicating its strong iron-chelating ability and superior plant growth-promoting potential. And Lower siderophore production was observed in isolates such as PSMRU1, PSMRU20, PSMRU33, and PSMRU62.

4.4.3 HCN Production of Different isolate



FigNo.4 HCN Production of Different isolate

Among all isolates, PSMRU65 showed the highest HCN production: HCN=108.92±0.47 µg/mL Moderate to high HCN production was observed in isolates PSMRU34 to PSMRU62. Lowest HCN production was recorded in: PSMRU23 = 1.28 ± 0.27 µg/mLand PSMRU29 = 1.28 ± 0.27 µg/Ml

.

4.4.4 HCN Production of Different isolate

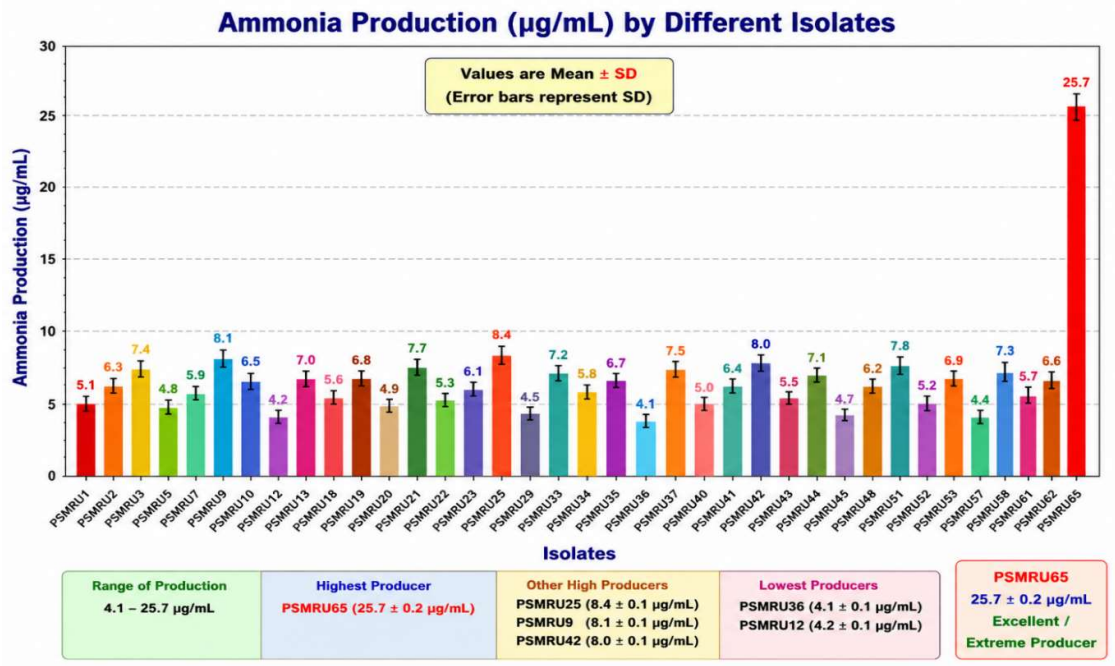


Fig No.5 Ammonia Production of Different isolate

Most isolates exhibited low ammonia production ranging between 4–8 µg/mL. Among all isolates, PSMRU65 showed the highest ammonia production 25.7±0.2 µg/mLand was categorized as an Excellent / Extreme Producer.

4.5 Antagonistic Activity Against Plant Pathogens

Antifungal Activity of Isolate PSMRU65 Against Test Pathogen

Sr.No.	Isolate	Control Growth (mm)	T_1 (mm)	T_2 (mm)	T_3 (mm)	Mean ± SD (mm)	% Inhibition	Activity
1	PSMRU65	90	42	40	41	41.0 ± 1.0	54.44	Excellent
2	PSMRU65	90	39	38	40	39.0 ± 1.0	56.67	Excellent
3	PSMRU65	90	44	45	43	44.0 ± 1.0	51.11	Excellent

Summary Statistics

Overall Mean Inhibition (%) : 54.07
Highest Inhibition (%) : 56.67
Lowest Inhibition (%) : 51.11
Activity Grade : Excellent

Activity Interpretation

The isolate PSMRU65 exhibited strong antifungal activity against the test pathogen with an average inhibition of 54.07%, which falls under the Excellent category.

$$\% \text{ Inhibition} = \frac{(\text{Control Growth} - \text{Treatment Growth})}{\text{Control Growth}} \times 100$$

Control Growth (mm) = 90 mm

Table No. 2 Antifungal Activity of Isolate PSMRU65 Against Test Pathogen *Fusarium oxysporum*

The table represents the antifungal activity of isolate PSMRU65 against the test fungal pathogen using the dual culture assay method. The antifungal potential was evaluated by measuring the radial growth . The isolate PSMRU65 exhibited excellent antifungal activity, effectively suppressing the growth of the test pathogen by more than 50%. This strong antagonistic effect may be attributed to the production of antimicrobial metabolites, siderophores, hydrogen cyanide (HCN), ammonia, and other plant growth-promoting substances. h inhibition of the pathogen in comparison with the control plate.

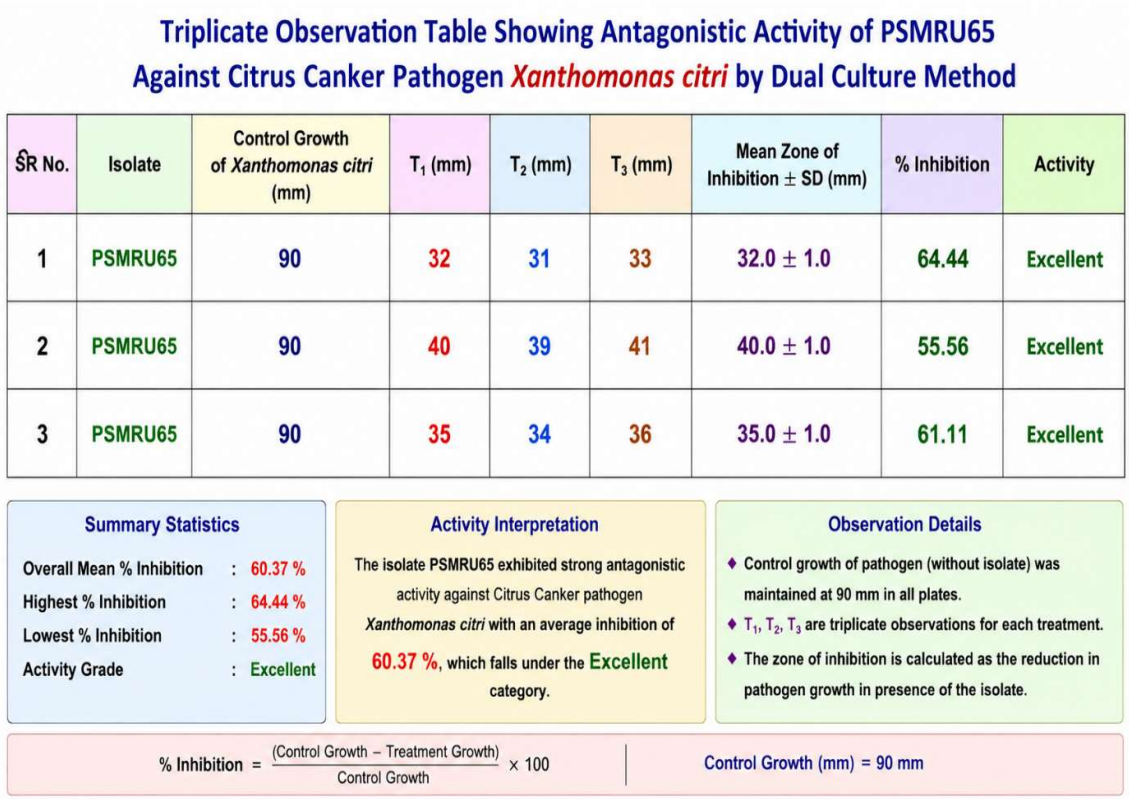


Table No. 3 Showing Antagonistic Activity of PSMRU65 Against Citrus Canker Pathogen Xanthomonas citri by Dual Culture Method

The table represents the antagonistic activity of isolate PSMRU65 against the citrus canker pathogen Xanthomonas citri using the dual culture method. The inhibitory effect of the isolate was determined by comparing the radial growth of the pathogen in treated plates with the untreated control.

The isolate PSMRU65 demonstrated excellent antagonistic activity against the citrus canker pathogen Xanthomonas citri. The inhibition percentage above 50% indicates strong antimicrobial potential. This activity may be associated with the production of antibacterial metabolites such as siderophores, hydrogen cyanide (HCN), ammonia, antibiotics, and extracellular enzymes.

4.6 Molecular Characterization

The obtained 16S rRNA gene sequence of isolate PSMRU65 was compared with sequences available in the NCBI GenBank database using the BLAST analysis tool. The sequence analysis revealed that the isolate showed maximum similarity with: PSMRU65≡*Bacillus subtilis* The sequence was submitted to the GenBank database and assigned the accession number: PZ414096

Table 5. Molecular Identification of Efficient Isolates

Isolate	Closest Organism	Accession Number
PSMRU65	*Bacillus subtilis*	PZ414096

5. Discussion

The present study demonstrated the occurrence of diverse phosphate solubilizing microorganisms in the orange rhizosphere soils of Bhiwapur Tahsil. Development of halo zones on Pikovskaya's agar medium indicated the ability of the isolates to mobilize insoluble phosphate compounds through extracellular metabolic activity. Variations observed among isolates in phosphate solubilization efficiency suggest the presence of physiologically diverse microbial populations in citrus rhizosphere soils.

Biochemical profiling revealed that the majority of efficient isolates belonged to ***Bacillus subtilis*** which are commonly associated with rhizospheric plant growth promotion and biological control activities [8,13]. These microorganisms are known to survive under varied environmental conditions and produce metabolites beneficial for plant health.

The selected isolates exhibited multiple plant growth promoting attributes including indole acetic acid production, HCN production ammonia release and siderophore secretion. Such characteristics are important for improving nutrient uptake, root development and rhizospheric competitiveness. The antagonistic activity observed against Fusarium oxysporum and Xanthomonas citri may be associated with the production of antimicrobial compounds, hydrolytic enzymes or competition for nutrients and ecological niches.

Molecular characterization through 16S rRNA sequencing confirmed the taxonomic identity of efficient isolates was *Bacillus subtilis* reported as beneficial rhizospheric bacteria with phosphate solubilization and biocontrol potential [14,15]. The recovery of such microorganisms from citrus rhizosphere soils indicates their possible adaptation to orchard ecosystems of the Vidarbha region.

Overall, the findings support the potential application of indigenous phosphate solubilizing microorganisms as environmentally safe alternatives to chemical fertilizers and pesticides in citrus cultivation systems.

6. Conclusion

The present study successfully isolated and characterized phosphate solubilizing microorganisms from orange rhizospheric soils of Bhiwapur Tahsil, Nagpur District. The isolates exhibited significant phosphate solubilization, plant growth promoting traits and antagonistic activity against important plant pathogens. Molecular characterization identified efficient isolates as *Bacillus subtilis*. These microorganisms may be utilized as eco-friendly biofertilizers and biocontrol agents for improving orange production and soil fertility.

Further field studies are recommended to evaluate the performance of these isolates under natural environmental conditions.

References

1. Pikovskaya RI. Mobilization of phosphorus in soil in connection with vital activity of some microbial species. Microbiologiya. 1948;17:362-70.
2. Glick BR. Plant growth-promoting bacteria: mechanisms and applications. Scientifica. 2012;2012:1-15.
3. Rodriguez H, Fraga R. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv. 1999;17(4-5):319-39.
4. Vessey JK. Plant growth promoting rhizobacteria as biofertilizers. Plant Soil. 2003;255:571-86.
5. Kloepper JW, Schroth MN. Plant growth-promoting rhizobacteria on radishes. In: Proceedings of the IV International Conference on Plant Pathogenic Bacteria. 1978. p. 879-82.
6. Lucy M, Reed E, Glick BR. Applications of free living plant growth-promoting rhizobacteria. Antonie Van Leeuwenhoek. 2004;86:1-25.
7. Ahmad F, Ahmad I, Khan MS. Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. Microbiol Res. 2008;163:173-81.
8. Compant S, Duffy B, Nowak J, Clément C, Barka EA. Use of plant growth-promoting bacteria for biocontrol of plant diseases. Appl Environ Microbiol. 2005;71:4951-9.
9. Sharma SB, Sayyed RZ, Trivedi MH, Gobi TA. Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. Springerplus. 2013;2:587.
10. Nautiyal CS. An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbiol Lett. 1999;170:265-70.
11. Patel DK, Archana G, Kumar GN. Variation in the nature of organic acid secretion and mineral phosphate solubilization by Citrobacter sp. DHRSS. Curr Microbiol. 2008;57:324-8.
12. Whipps JM. Microbial interactions and biocontrol in the rhizosphere. J Exp Bot. 2001;52:487-511.
13. Lugtenberg B, Kamilova F. Plant-growth-promoting rhizobacteria. Annu Rev Microbiol. 2009;63:541-56.
14. Saharan BS, Nehra V. Plant growth promoting rhizobacteria: a critical review. Life Sci Med Res. 2011;21:1-30.
15. Gupta G, Parihar SS, Ahirwar NK, Snehi SK, Singh V. Plant growth promoting rhizobacteria (PGPR): current and future prospects for development of sustainable agriculture. J Microb Biochem Technol. 2015;7:96-102.
16. Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST. Bergey's manual of determinative bacteriology. 9th ed. Baltimore: Williams and Wilkins; 1994.
17. Cappuccino JG, Sherman N. Microbiology: a laboratory manual. 10th ed. Pearson Education; 2014.

18. Jackson ML. Soil chemical analysis. New Delhi: Prentice Hall of India Pvt. Ltd.; 1973.
19. Gordon SA, Weber RP. Colorimetric estimation of indoleacetic acid. Plant Physiol. 1951;26:192-5.
20. Schwyn B, Neilands JB. Universal chemical assay for the detection and determination of siderophores. Anal Biochem. 1987;160:47-56.
21. Dennis C, Webster J. Antagonistic properties of species groups of Trichoderma. Trans Br Mycol Soc. 1971;57:25-39.
22. Perez C, Pauli M, Bazerque P. An antibiotic assay by agar-well diffusion method. Acta Biol Med Exp. 1990;15:113-5.
23. Weisburg WG, Barns SM, Pelletier DA, Lane DJ. 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol. 1991;173:697-703
24. 24 Lorck, H. (1948). Production of hydrocyanic acid by bacteria. Physiologia Plantaru 1(2), 42–146.