



Diversity of Indigenous Phosphate Solubilizing Microorganisms from Orange Rhizosphere in Kalmeshwar, Tahsil and Their Indole-3-Acetic Acid Production

Dhanshree M Ridhorkar^{1*} and Y. S. Banginwar²

¹Department of Molecular Biology and Genetic Engineering, RTMNU, Nagpur, India

²Shankarrao Bhoyar Patil Mahavidyalaya, Pulgaon, India

*Corresponding Author: Dhanshree M Ridhorkar, Department of Molecular Biology and Genetic Engineering, RTMNU, Nagpur, India.

Received: March 27, 2026

Published: April 22, 2026

© All rights are reserved by Dhanshree M Ridhorkar and Y. S. Banginwar.

DOI: 10.31080/ASMI.2026.09.1597

Abstract

Soil microorganisms play an essential role in converting fixed or insoluble forms of phosphorus into forms that are available for plant uptake. This group includes bacteria, fungi, actinomycetes, and arbuscular mycorrhizal fungi. Among these, phosphate-solubilizing microorganisms (PSM), particularly bacteria, are highly effective in improving phosphorus availability through the mineralization of organic matter and solubilization of inorganic phosphates present in the soil.

In addition to phosphorus solubilization, PSM possess several plant growth-promoting properties. They are capable of producing various bioactive compounds such as plant growth hormones (including indole-3-acetic acid (IAA), cytokinins, and gibberellins), vitamins, antibiotics, and siderophores. These substances not only stimulate plant growth but also help in suppressing soil-borne pathogens, improving soil health, and maintaining long-term soil fertility and productivity.

Indole 3-acetic acid, a crucial auxin, is vital for plant growth and development as well as in plant-microbe interactions. It acts as a signaling molecule that influences root architecture, enhances cell division, and promotes elongation of plant tissues. In the rhizosphere, many beneficial bacteria synthesize IAA using root exudates as substrates. This bacterial IAA production contributes to improved root development, increased nutrient uptake and enhanced tolerance to environmental stresses such as drought, salinity, and heavy metal toxicity.

The biosynthesis of IAA in bacteria is regulated by complex genetic pathways that respond to environmental conditions. These regulatory mechanisms influence plant hormonal balance and strengthen beneficial plant-microbe associations. While some pathogenic microorganisms manipulate IAA levels to favor infection, beneficial rhizobacteria utilize IAA production to promote plant growth, improve stress tolerance, and enhance nutrient use efficiency, thereby reducing the need for chemical fertilizers.

In the present study, soil samples were collected from the rhizosphere of orange trees in the Kalmeshwar tehsil. The bacterial isolates obtained were evaluated for their ability to solubilize phosphate. Further investigations were carried out to assess important plant growth-promoting traits, particularly the production of indole-3-acetic acid.

Keywords: PSM; Indole-3-Acetic Acid; Chemical Fertilizers

Introduction

Plant growth-promoting rhizobacteria (PGPR) are a diverse group of beneficial bacteria that inhabit the rhizosphere the region surrounding plant root. These microorganisms either live closely attached to root surfaces or exist freely in the soil and influence plant growth through direct and indirect mechanisms.

Several bacterial genera *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthobacter*, *Burkholderia*, *Bacillus*, and *Serratia Pseudomonas*, have been documented to promote plant growth [1,2]. PGPR are extensively documented to promote plant growth and are crucial in sustainable agriculture by improving nutrient availability and reducing the dependence on chemical fertilizers.

These helpful bacteria promote plant development through various processes such as nitrogen fixation, solubilization of essential minerals like phosphorus and iron, and production of plant growth regulators such as phytohormones. Among these, phosphorus is one of the most important macronutrients required for plant growth and development, constituting nearly 0.2% of plant dry weight. However, despite its abundance in soil, phosphorus is mostly present in insoluble forms, making it unavailable to plants. Typically, soil contains about 0.05% phosphorus, but only a very small fraction (around 0.1%) is accessible for plant uptake (Illmer and Schimier, 1995).

To overcome this limitation, large quantities of chemical phosphorus fertilizers are commonly applied in agriculture (Khan, *et al.* 2007). However, these soluble fertilizers are quickly converted into insoluble forms such as tricalcium phosphate [$\text{Ca}_3(\text{PO}_4)_2$], iron phosphate (FePO_4), and aluminum phosphate (AlPO_4). These forms cannot be easily absorbed by plants, leading to excessive fertilizer application and environmental concerns. Interestingly, the accumulated insoluble phosphorus in soils is sufficient to support crop production for many decades if it can be effectively utilized.

Phosphate-solubilizing PGPR offer a sustainable solution to this problem. These microorganisms convert insoluble phosphorus into plant-available forms through mechanisms such as the secretion of organic acids, chelation, and ion exchange. While many studies have reported the solubilization of calcium-bound phosphates,

fewer investigations have focused on the solubilization of iron and aluminum phosphates.

Previous studies have demonstrated the potential of phosphate-solubilizing bacteria in improving plant growth and phosphorus uptake. For instance, rhizobacteria isolated from field-grown plants have shown the ability to solubilize phosphate and enhance growth in crops like canola under controlled conditions. Similarly, research on sugarcane and soybean has indicated that these bacteria can improve soil phosphorus availability, crop yield, and nodulation.

In addition to nutrient solubilization, PGPR also promote plant growth through the production of plant hormones. Among these, indole 3-acetic acid is naturally occurring auxin that plays the crucial role in growth and advancement. IAA influences various physiological processes such as cell division, root elongation, and overall plant architecture. Microorganisms in the rhizosphere often produce IAA as a secondary metabolite, utilizing root exudates as substrates.

Many PGPR species, including *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Pseudomonas*, and *Rhizobium*, are known to produce IAA or similar compounds (Dubeikovsky, *et al.* 1993; Taghavi, *et al.* 2009). These hormones facilitate communication between plants and microorganisms, leading to enhanced root development, increased seed germination, improved nutrient uptake, and better overall plant growth (Moore, 1989; Lüthen, *et al.* 1999; Davies, 1995).

In the present study, soil samples were collected from the rhizosphere of orange plants in the Kalmeshwar region. The isolates obtained were evaluated for their ability to solubilize phosphate. Furthermore, their plant growth-promoting traits, particularly the production of indole-3-acetic acid, were analyzed to assess their potential in improving crop productivity.

Materials and Method

Isolation of bacteria

Sample collection and bacterial isolation

A soil sample collected from the rhizosphere of orange crop in Kalmeshwar, Tahsil possessed an intact root structure. The sample was meticulously gathered in plastic bags beneath aseptic environment.

Determination of phosphate solubilization

We evaluated all the rhizobacterial isolates we received for various traits that support plants. expand. We maintained each culture on modified *Pikovskaya agar* [3] containing insoluble tricalcium phosphate (TCP) and maintained it at $30 \pm 0.1^\circ\text{C}$ for 5 days to observe phosphate dissolution.

Detection of IAA production by the selected isolates

IAA Production was evaluated utilizing the qualitative method created by [4]. Bacterial cultures were introduced into nutrient broth containing tryptophan (1 mg/ml) and incubated at $35 \pm 2^\circ\text{C}$ for a duration of 7 days. Cultures were spun at 3000 rpm for 30 minutes. 2 mL of supernatant was combined with 2 drops of orthophosphoric acid and 4 mL of Salkowski's reagent (50 mL, 35% perchloric acid; 1 mL of 0.5 FeCl₃). A pink coloration signified the production of Indole Acetic Acid (IAA) [5].

Result

Isolation of bacteria

Eighty soil samples were effectively extracted from the rhizosphere soil of orange fields in Kalmeshwar Tahsil.

Determination of Phosphate solubilization

From 80 samples, the phosphate solubilizing ability of 60 isolated bacterial strains was determined through their phosphate solubilizing efficiency, observed by the formation of a clear zone around the colonies after 5 days of incubation. Among the six isolates, PSMKA1, PSMKA3, PSMK21, and PSMK41 demonstrated low phosphate solubilization, while PSMK35, PSMK37, PSMKA55, PSMKA58, and PSMKA60 exhibited significant phosphate solubilization zones. Maximum zone recorded by the isolate PSMKA52 is 27 mm.

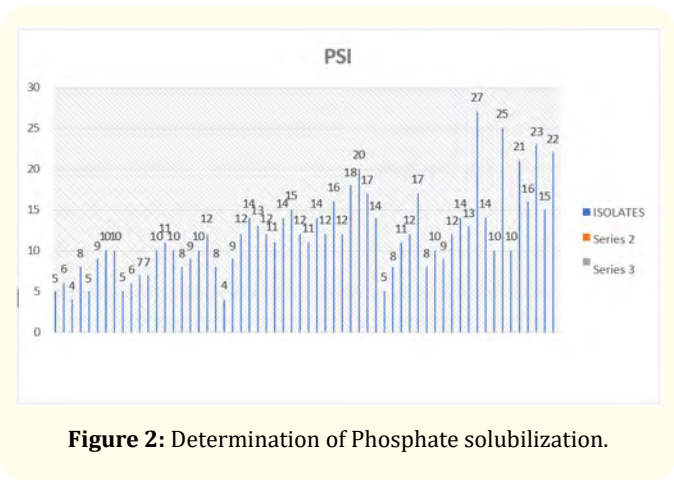


Figure 2: Determination of Phosphate solubilization.

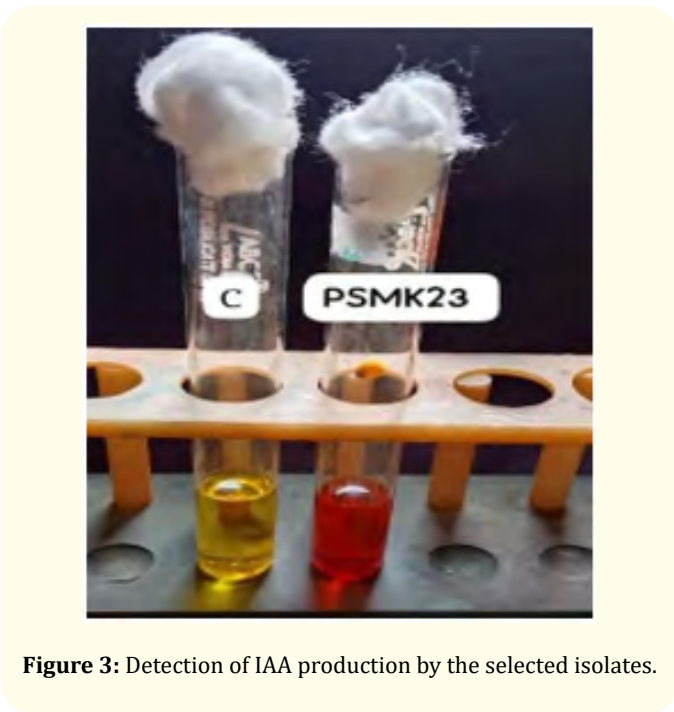


Figure 3: Detection of IAA production by the selected isolates.

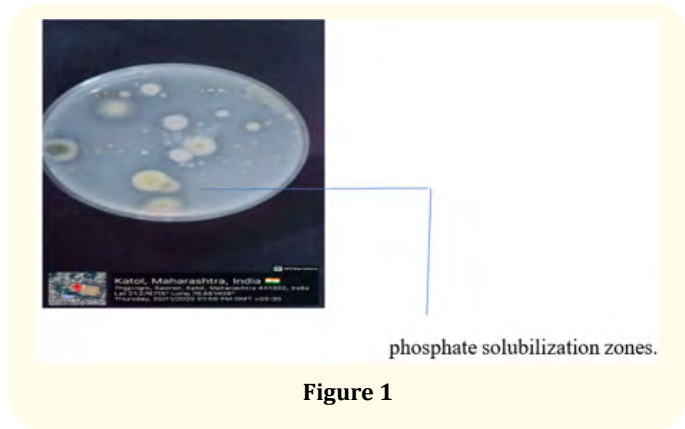


Figure 1

Sr. No.	Isolates	IAA production
	PSMKA 1	+
1	PSMKA2	-
2	PSMKA3	+
3	PSMKA4	+
4	PSMKA5	+
5	PSMKA6	+
6	PSMKA7	+
7	PSMKA8	-

8	PSMKA9	+
9	PSMKA10	+
10	PSMKA11	+
11	PSMKA12	+
12	PSMKA13	-
13	PSMKA 1	+
14	PSMKA14	+
15	PSMKA15	+
16	PSMKA16	+
17	PSMKA17	+
18	PSMKA18	+
19	PSMKA19	+
20	PSMKA20	++
21	PSMKA21	+
22	PSMKA22	-
23	PSMKA23	++
24	PSMKA24	+
25	PSMKA25	+
26	PSMKA26	+
27	PSMKA27	+
28	PSMKA28	+
29	PSMKA29	+
30	PSMKA30	-
31	PSMKA31	++
32	PSMKA32	-
33	PSMKA33	++
34	PSMKA34	-
35	PSMKA35	++
36	PSMKA36	-
37	PSMKA37	+
38	PSMKA38	++
39	PSMKA39	+
40	PSMKA40	++
41	PSMKA41	-
42	PSMKA42	+
43	PSMKA43	+
44	PSMKA44	+
45	PSMKA45	+
46	PSMKA46	--
47	PSMKA47	+
48	PSMKA48	-

49	PSMKA49	++
50	PSMKA50	+
51	PSMKA51	-
52	PSMKA52	+
53	PSMKA53	-
54	PSMKA54	+
55	PSMKA55	+
56	PSMKA56	+
57	PSMKA57	-
58	PSMKA58	+
59	PSMKA59	++
60	PSMKA60	-

Table 1: Detection of IAA production by the selected isolates.

This table demonstrates that the majority of the isolates demonstrate a positive outcome for indole production.

Result and Conclusion

Out of 80 samples, the capability of 60 isolated bacterial strains to solubilize phosphate was assessed by their phosphate solubilizing efficiency, noted by the appearance of a clear zone around the colonies following 5 days of incubation. Of the six isolates, PSMKA1, PSMKA3, PSMK21, and PSMK41 showed minimal phosphate solubilization, whereas PSMK35, PSMK37, PSMKA55, PSMKA58, and PSMKA60 displayed considerable phosphate solubilization zones. The highest zone observed by the isolate PSMKA52 is 27 mm. and Most of the isolates show a favorable result for indole synthesis.

The ability of certain bacteria to produce indole-3-acetic acid (IAA) offers significant potential for applications in biofertilization and biostimulation. This provides an environmentally friendly alternative to chemical fertilizers while enhancing plant tolerance to abiotic stresses such as drought, salinity, and heavy metal toxicity. In addition, microbially produced IAA plays an important role in plant signaling processes and may also influence plant-microbe interactions, including its possible involvement as a virulence factor in some cases.

The impact of microbial IAA on plant defense mechanisms and overall plant health highlights the need for further detailed research. A comprehensive understanding of the complex

interactions between IAA-producing bacteria and their host plants is essential for effectively utilizing these microorganisms in sustainable agricultural practices.

Bibliography

1. Kloepper J., *et al.* "Plant root- bacterial interactions in biological control of soilborne diseases and potential extension to systemic and foliar diseases". *Australas Plant Pathology* 28.1 (1999): 21-26.
2. Glick BR. "The enhancement of plant growth by free living bacteria". *Canadian Journal of Microbiology* 41 (1995): 109-114.
3. Pikovaskya RI. "Mobilization of phosphorus in soil in connection with the vital activity of some microbial species". *Mikrobiologiya* 17 (1948): 362-370.
4. Bric JM., *et al.* "Rapid in situ assay for indole acetic acid production by bacteria immobilized on a nitrocellulose membrane". *Applied and Environmental Microbiology* 57 (1991): 535-538.
5. Loper JE and MN Schroth. "Influence of bacterial source of indole-3-acetic acid of root elongation of sugar beet". *Phytology Pathology* 76 (1986): 386-389.
6. Ahmad F., *et al.* "Indole acetic acid production by the indigenous isolates of Azotobacter and fluorescent Pseudomonas in the presence and absence of tryptophan". *Turkish Journal of Biology* 29 (2005): 29-34.
7. Azziz G., *et al.* "Abundance, diversity and prospecting of culturable phosphate solubilizing bacteria on soils under crop-pasture rotations in a no-tillage regime in Uruguay". *Applied Soil Ecology* 61 (2012): 320-326.
8. Bhattacharya PN and Jha DK. "Plant growth promoting rhizobacteria (PGPR): emergence in agriculture". *World Journal of Microbiology and Biotechnology* 28.1327 (2012): 1350.
9. BS Saharan and V Nehra. "Plant Growth Promoting Rhizobacteria: A Critical Review". *Life Sciences and Medicine Research* 1 (2011).
10. Gordon SN and Weber RP. "Colorimetric estimation of indole acetic acid". *Plant Physiology* 26 (1951): 192-195.
11. MB Hossain., *et al.* "Evaluation of rock phosphate solubilization capacity by isolated strains and their effect on mung bean". *The Journal of Animal and Plant Sciences* 23.1 (2013): 251-255.
12. MA Sattar and A C Gaur. *Bangladesh Journal of Agriculture* 11.2 (1986).
13. Arshad M and Frankenberger WT. "Microbial production of plant growth regulators". In: *Soil Microbial Ecol.* Marcel Dekker Inc., New York. (1992).
14. Park KH., *et al.* "Conditions for soluble phosphate production by environment-friendly biofertilizer resources, Pseudomonas fluorescens". *Journal of Environmental Sciences* 17 (2008): 1033-1037.
15. Mehnaz S., *et al.* "Isolation, Characterization and Effect of fluorescent Pseudomonas on Micro propagated Sugarcane". *Canadian Journal of Microbiology* 55 (2009): 1007-1011.
16. Rodriguez H and Fraga R. "Phosphate; solubilizing bacteria and their role in plant growth promotion". *Biotechnology Advance* 17 (1999): 319-339.
17. Sadaf Shahab., *et al.* "Indole acetic acid production and enhanced plant growth promotion by indigenous PSBs". *African Journal of Agricultural Research* 4.11 (2009): 1312-1316.
18. S Reetha., *et al.* "Isolation of indole acetic acid (IAA) producing rhizobacteria of Pseudomonas fluorescens and Bacillus subtilis and enhance growth of onion (Allim cepa. L)". *International Journal of Current Microbiology and Applied Sciences* 3.2 (2014): 568-574.