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Phosphate-solubilizing Endophytic Bacteria Isolation from Maize Plant

[1]Joshi RD, [1]Kulkarni SS[1,] Bhate MA and [2]Bhusare DU

[1]Department of Microbiology, Shri Shivaji College, Parbhani. (MS), India
[2]Department of Microbiology, Digambarrao Bindu ACS College, Bhokar (MS), India
Email: bhusaredu@gmail.com

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Abstract

In a solid culture medium supplemented with different inorganic sources of phosphorus, fifty isolates of endophytic bacteria isolated from Maize plant parts were evaluated for their ability to solubilize phosphate. Using a pikovskaya selective medium and the plate assay method, phosphate solubilizing bacterial endophytes were screened. Colony morphology, physiological parameters, and biochemical features were used to characterize endophytic bacterial isolates. Phosphorus from tricalcium phosphate was solubilized by all 50 isolates tested on solid medium. When $Ca_3 (PO_4)_2$ was utilized as a P source, five of these isolates demonstrated excellent phosphate solubilizing activity in solid media. With a maximum phosphate solubilization index, Bacillus sp. showed maximal phosphate solubilizing activity on the fourth day of incubation (DAI). Endophytic isolates are reported in this investigation.

Keywords: phosphorus, Maize plant, Colony morphology, physiological parameters.

1. Introduction

Phosphorus is a key macronutrient that influences plant growth and metabolism. It's also a finite natural resource that's increasingly being recognized as a new problem for global sustainable development. Because there isn't enough phosphorus to meet all of a plant's demands. Phosphorus deficiency is common in subtropical forest soils, limiting plant growth and productivity [1].

Phosphate fertilizers (P-fertilizers) are necessary for healthy plant growth because they provide phosphorus as a nutrient. The majority of fertilizer-derived phosphorus either precipitates with aluminum, iron, calcium, and organic matter in the soil's solid phase or is adsorbed on the surface of clay particles [2].

The low ion-exchange capacity of acidic tropical soils limits the utilization of natural sources of phosphate fertilizers. Chemical fertilizers are expensive and contribute to eutrophication. PSB (phosphate-solubilizing bacteria) could play a key role in delivering phosphate to plants in an environmentally favorable and long-term manner [3]. PSB, or phosphobacteria, is a type of bacteria.

PSBs are a type of bacterium that can dissolve insoluble phosphate, fix nitrogen, and secrete auxin, all of which help plants develop. Plant growth has been the focus of PSB research in recent years. It has been demonstrated that phosphate-solubilizing bacteria (PSB) inoculated seed or soil improves the solubilization of fixed soil phosphorus and applied phosphates, resulting in improved plant performance. PSB uses organic acids and extracellular enzymes that are released into the soil to solubilize phosphorus from organic and inorganic sources [2, 4]. PSB produces organic acids with low molecular weight, which solubilize mineral phosphates and lower soil pH.

PSB produces low-molecular-weight organic acids, which dissolve mineral phosphates and lower soil pH [3]. PSB has been extensively investigated as a biofertilizer and inoculant to boost crop yields. Different bacteria species have been identified as P-fertilizers, including *Azotobacter, Bacillus subtilis, Bacillus cereus, Bacillus megaterium, Pseudomonas putida, Pseudomonas aeruginosa, Enterobacter aerogenes,* and *Micrococcus luteus* [5]. As the endophytes plant contact is the product of an evolutionary process controlled by genes from both species, endophytic PSB is more competitive than non-endophytic or facultative microbes inside the host plant. The majority of soil bacteria are endophytes, meaning they can grow inside plant tissue and support plant growth by phosphorus solubilization.

2. Materials and Method

Various Maize plant samples, such as root, inner bark, leaves, flowers, and so on, were collected and processed promptly from each mature, healthy plant. Five points were sampled in three replicates for composite sampling.

The plant samples were initially subjected for surface sterilization as per the methodology given by Costa et al., [6] along with some modifications. The surface sterilized plant parts viz. leaves and flowers were further ground with 6ml 0.9% NaCl solution using sterile pestle and mortar and kept aseptically for 15-20 min. for the release of endophytic bacteria from host tissue. The tissue extract was diluted with 0.9% NaCl solution and plated on nutrient agar medium plates. The plant parts viz. inner bark, roots were cut into pieces with sterile knife to excise inner tissues. The excised inner tissues were further inoculated on nutrient soy agar medium plates. All the plates were incubated at 300 C for 3-5 days. After incubation, various colonies were selected showing different morphological and growth characters. The colonies were further maintained as pure culture on nutrient agar slants for further use [6].

Screening of Phosphate Solubilizing Endophytic bacteria

Each of the endophytic bacterial isolates were tested separately for their ability to solubilize phosphate on Pikovskaya agar (10 g glucose, 5 g $Ca_3(PO4)2$, 0.5 g $(NH_4)_2SO_4$, 0.2 g KCl, 0.1 g $MgSO_4$. $7H_2O$, 0.5 g yeast extract, 25 mg $MnSO_4$, 25 mg $FeSO_4$, and 20 g agar- agar in 1 L pH - 7) incubated for 3 d at 28°C .

Colonies forming a clear halo zone around each colony are screened as PSM after incubation at appropriate temperature. Pure cultures of such colonies are further processed for identification through biochemical and molecular characterization. P solubilizing ability of a particular PSM can be assessed in terms of the solubilization index (SI), the ratio of total diameter, i.e., clearance zone and the colony diameter. phosphate SI can be determined using the following formula [7].

$$SI = \frac{\text{colony diameter + halozone diameter}}{\text{Colony diameter}}$$

Characterization of Phosphate solubilizing endophytic bacterial isolates

Morphological characterization of Phosphate solubilizing strains were done by maintaining pure Cultures of bacteria and observing its colony characteristics on nutrient agar plate. Biochemical characterization was done by preferred conventional methods using different cultural media.

3. Results and Discussion

After five days of incubation on Pikovskaya agar medium, fifty endophytic bacteria isolated from maize roots (30), leaves (12), and sap (08) were examined for their phosphate solubilization efficiency based on growth or the presence of a distinct halo zone surrounding the colonies (Table 1). Thirty bacterial isolates thrived on solid medium and produced the halo that is commonly seen following phosphate solubilization. Twenty phosphate solubilizing bacterial endophytes were isolated from root samples, six from leaves, and four from sap samples, out of a total of thirty. Bacillus sp, Pseudomonas sp, Serratia sp, Enterobacter sp, and Klebsiella sp were the likely endophytic bacterial species with Phosphate solubilizing activity identified from Maize plants. The majority of the bacterial strains found belonged to the *Bacillus sp.*

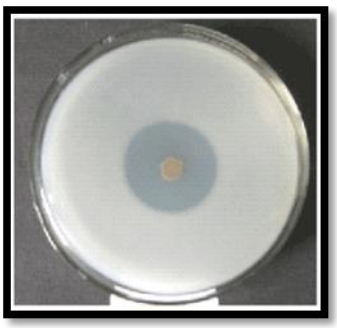
Table No. 1 Isolation of Endophytic bacteria from Maize plant

Sr.No.	Isolate	Plant Source	Phosphate solubilization Ability	Sr.No.	Isolate	Plant Source	Phosphate solubilization Ability
1.	MR1	MR	Negative	26.	MR26	MR	Negative
2.	MR2	MR	Positive	27.	MR27	MR	Negative
3.	MR3	MR	Positive	28.	MR28	MR	Negative
4.	MR4	MR	Positive	29.	MR29	MR	Negative
5.	MR5	MR	Positive	30.	MR30	MR	Negative
6.	MR6	MR	Positive	31.	ML31	ML	Negative
7.	MR7	MR	Negative	32.	ML32	ML	Negative
8.	MR8	MR	Positive	33.	ML33	ML	Positive
9	MR9	MR	Positive	34.	ML34	ML	Positive
.10	MR10	MR	Positive	35.	ML35	ML	Negative
11.	MR11	MR	Positive	36.	ML36	ML	Positive
12.	MR12	MR	Positive	37.	ML37	ML	Negative
13.	MR13	MR	Negative	38.	ML38	ML	Positive
14.	MR14	MR	Positive	39.	ML39	ML	Negative
15.	MR15	MR	Positive	40.	ML40	ML	Positive
16.	MR16	MR	Positive	41.	ML41	ML	Negative
17.	MR17	MR	Positive	42.	ML42	ML	Positive
18.	MR18	MR	Positive	43.	MS43	MS	Positive
19.	MR19	MR	Negative	44.	MS44	MS	Negative
20.	MR20	MR	Positive	45.	MS45	MS	Positive
21.	MR21	MR	Positive	46.	MS46	MS	Negative
22.	MR22	MR	Positive	47.	MS47	MS	Positive
23.	MR23	MR	Positive	48.	MS48	MS	Negative
24.	MR24	MR	Positive	49.	MS49	MS	Negative
25.	MR25	MR	Negative	50	MS50	MS	Positive

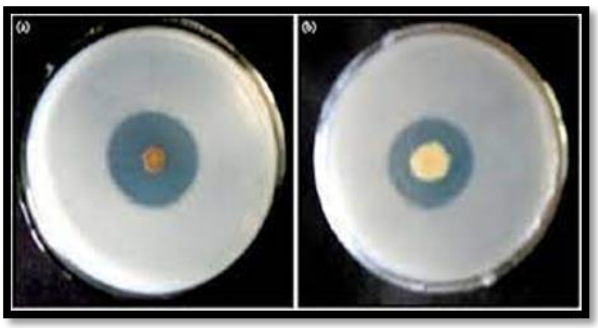
Five isolates, including *Bacillus* and *Pseudomonas* sp., displayed a maximal zone of solubilization on Pikovskaya agar medium, as evaluated by the estimated solubilization index for each phosphate solubilizing bacterial endophyte. (Table -2)

Table 2 -: Solubilization Index of Phosphate Solubilizing bacterial endophyte

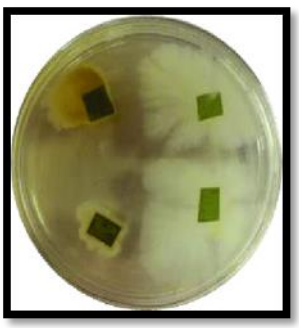
Sr. No.	Isolate	Colony Diameter(mm)	Halozone Diameter (mm)	Solubilization Index	Probable strain	Sr. No.	Isolate	Colony Diameter	Halozone Diameter	Solubilization Index	Probable strain
1.	MR2	10	22	3.2	*Bacillus*	16.	MR20	11	15	2.36	*Micrococcus*
2.	MR3	11	20	2.81	*klebsiella*	17.	MR21	13	18	2.38	*Serratia*
3.	MR4	8	15	2.87	*Bacillus*	18.	MR22	15	21	2.4	*klebsiella*
4.	MR5	14	19	2.35	*Bacillus*	19.	MR23	12	17	2.41	*Serratia*
5.	MR6	18	22	2.22	*Enterobacter*	20.	MR24	16	20	2.25	*Micrococcus*
6.	MR8	12	23	2.91	*Bacillus*	21.	ML33	18	23	2.27	*Enterobacter*
7.	MR9	13	20	2.53	*klebsiella*	22.	ML34	21	25	2.19	*Enterobacter*
8.	MR10	15	20	2.33	*klebsiella*	23.	ML36	10	25	3.5	*Pseudomonas*
9.	MR11	10	26	3.6	*Pseudomonas*	24.	ML38	19	23	2.21	*Micrococcus*
10.	MR12	17	21	2.23	*Enterobacter*	25.	ML40	11	16	2.45	*klebsiella*
11.	MR14	14	21	2.5	*Bacillus*	26.	ML42	11	12	2.09	*Bacillus*
12.	MR15	13	20	2.53	*Serratia*	27.	MS43	13	16	2.23	*Micrococcus*
13.	MR16	14	20	2.42	*Bacillus*	28.	MS45	17	22	2.29	*Enterobacter*
14.	MR17	14	19	2.35	*Serratia*	29.	MS47	12	20	2.66	*Bacillus*
15.	MR18	13	18	2.38	*Bacillus*	30.	MS50	18	23	2.27	*Enterobacter*



Photoplate 1 MR-11



Photoplate 2 MR-36, MS-47



Photoplate 3 Endophytes

The phosphate-solubilizing ability of endophytic PSB from maize was demonstrated in vitro by dissolving of Tri-calcium phosphate in the current investigation. The PSBs chosen could be employed as bioinoculants to boost maize production and phosphorus uptake.

Although the formation of a clear halo zone around colonies on Pikovskaya agar medium is a good preliminary criterion for selecting isolates with phosphate-solubilizing activity, it should not be considered the only test for determining phosphate solubilization, so all of the isolates were also tested in liquid medium for their ability to solubilize phosphorus [8-12]. As prospective phosphate solubilizers, microorganisms capable of forming a halo due to the solubilization of organic acids in the surrounding media were chosen (13, 14).

4. Conclusion

Phosphate solubilizing microorganisms are an effective technique to transform insoluble Phosphate molecules into plant-available Phosphate, resulting in increased plant growth, production, and quality. The most efficient P solubilizers for increasing Phosphate levels in soil include *Bacillus, Pseudomonas, Klebsiella, Serratia, and Enterobacter*. PSM accelerates plant growth by supplying easily absorbed P and increasing the production of plant growth hormones like IAA and GA. Furthermore, PSM promotes plant growth by producing siderophore, which improves nitrogen fixation efficiency. PSMs could be a viable alternative to inorganic phosphate fertilizers for meeting plant P requirements and increasing production in sustainable agriculture.

Conflicts of interest: The author stated that no conflicts of interest.

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