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Research Article

STUDIES ON PHOSPHATE SOLUBILIZING POTENTIAL OF MARINE *PSEUDOMONAS* ISOLATES

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ABSTRACT

Phosphorus is one of the essential macronutrients in plant nutrition and is involved in many processes, including energy transfer reactions, development of reproductive structures, crop maturity, root growth and protein synthesis. The fertile cultivable soils rapidly becoming saline and alkaline due to non-judicious irrigation and application of chemical fertilizers in soils making the essential nutrients like phosphorous unavailable to plants. It triggered an urgent need to develop a phosphate solubilizing microbial (PSM) bio - fertilizer that can be used in saline and alkaline soils.

Marine environment is one of the most diverse habitats with respect to its characteristic biotic and abiotic content. Marine bacteria are known for their adaptability to extreme environments with saline and alkaline conditions.

The present study deals with the isolation, characterization and investigation of phosphate solubilizing ability of marine *Pseudomonas* isolates. Four *Pseudomonas* isolates were obtained by screening using Pikovskayas agar medium and identified on the basis of cultural, morphological and biochemical characterization. The phosphate solubilization activity determined by measuring the diameter of zone of solubilization and quantity of soluble phosphate released in the medium. *Pseudomonas* RSML04 is found to be more significant phosphate solubilizer, exhibiting 04 phosphate solubilization index and 68 ppm release of phosphate. *Pseudomonas* RSML04 showed a potential to be used as PSM under saline and alkaline conditions.

Keywords: Phosphate solubilizing bacteria (PSB), Marine *Pseudomonas*, phosphate solubilization index.

1. INTRODUCTION

Phosphorous is considered to be one of the crucial growth limiting macronutrients for plants as it is required for many processes, including energy transfer reactions, development of reproductive structures, crop maturity, root growth and protein synthesis. Generally, 400 and 1,200 mg kg^{-1} of phosphorus is present in soil out of which approximately1 mg kg^{-1} or less is in soluble form [1, 2]. The orthophosphates, H_2PO_4- and HPO_4, and certain organic phosphorus compounds are available forms of phosphorous in soil for absorption by plants [3, 4].

Upon released in to soil, the protoplasmic organic phosphorous converts in to inorganic phosphoric acid which reacts with iron, aluminium, magnesium and calcium present in soil becoming insoluble salts [5].

To fulfill plants' nutritional requirement, phosphorous is regularly added to soils in the form of synthetic chemical fertilizers [6]. However, 75-90% of added phosphorous is precipitated by metal-cation complexes and very little is available for absorption by plants [7]. These insoluble complexes of mineral phosphates in the soil limit its direct absorption by plant root [8].

The imprudent addition of chemical fertilizer in modern agricultural practices started during green revolution has led to soil and water pollution creating serious ecological challenges. Therefore, solubilization and mineralization of insoluble phosphorous in soil by phosphate-solubilizing bacteria (PSB) is an ecofriendly approach for replenishing the phosphorous deficiency in plants [9, 10]. Solubilization of inorganic phosphate by producing low molecular weight organic acids, such as citric, oxalic, malic and glucoronic acid and enzyme, phosphatases and phytases mediated, mineralization of organic phosphorus are the principle mechanisms of phosphate solubilization by microorganisms in soil [11, 12]. The organic acids trap phosphate-bound cations through their hydroxyl and carboxyl groups transforming them into bioavailable forms [13].

The marine environment comprises about 70% of Earth surface, and possesses vast ecological, chemical and biological diversity. The marine microorganisms inhabiting under saline and alkaline conditions are characterized by exclusive metabolic, functional and structural properties [14-16]. The phosphate-solubilizing ability and soil application for plant growth promotion by terrestrial bacterial isolates were extensively studied however applications of marine bacteria exhibiting high potential under extreme environmental conditions needs to be more explored. The phosphate-solubilizing strains of bacteria, actinomycetes and fungi showing good potential of phosphate dissolution were isolated from the marine environment [17, 18]. The physicochemical properties of marine water from Alibaug mangrove forest revealed high values of hardness, salinity with Na^+ and Cl^- are dominant cations and anions, respectively [19]. The present study aims to the isolation, characterization and testing phosphate solubilizing potential of *Pseudomonas* isolates from marine water sample of Alibaug surrounded by Arabian Sea coast.

2. MATERIAL AND METHODS

2.1. Isolation of marine bacteria

The marine water sample is collected from Alibaug surrounded by Arabian Sea coast, in a sterile wide mouth glass bottle of 250 ml capacity from about 12 cm depth and carried to the laboratory within 24 hrs. The water sample was subjected to isolation of marine bacteria on Zobell marine agar media (Himedia2216) at 28±2°C.

2.2. Screening for marine *Pseudomonas* isolates

The isolated bacterial strains were subjected for growth on citrimide agar (Himedia M024). The plates were incubated at 28±2°C for 48 hr for pigment formation.

2.3. Biochemical characterization of *Pseudomonas* isolates

Biochemical characters were determined as described in Bergey's Manual of Determinative Bacteriology.

2.4. Screening of *Pseudomonas* isolates for PSM activity

All isolates were subjected for screening phosphate solubilizing potential on Pikovskayas agar medium having composition per liter of Dextrose (10g), calcium phosphate (5g), Ammonium sulphate (0.5 g) potassium chloride (0.2g) magnesium sulphate (0.1 g) manganese sulphate (0.0001g) ferrous sulphate (0.0001g) Agar-Agar (15g), pH of medium is adjusted to 7.5± 2. The spot inoculation of isolates on Pikovskayas agar was followed by incubating plates at 28±2°C for 72 hr. Upon incubation, colonies having halo cleared zone around them are considered as P solubilization. All the colonies with solubilizing potential were selected and maintained on nutrient agar slants.

2.5. Qualitative test

A 18hrs. grown culture of the bacterial isolates were spot inoculated on Pikovskaya's agar (amended with 0.5g/l of Cl and pH adjusted to 7.5). The plates were incubated at 28± 2°C for 72 hrs. and were observed for zone of phosphate solubilization.

2.6. Determination of phosphate solubilization index (PSI)

The phosphate solubilization index was determined by following formula

Solubilizing efficiency index (S.E) = Z/C
Z = Solubilization zone (mm), C = Colony diameter (mm)

2.7. Quantitative estimation

The Barton's reagent method (at 430nm) as described in the literature [20] was used. The amount of phosphate solubilized in the medium by the bacterial isolates was calculated by comparing with the standard curve obtained with K_2HPO_4 in ppm.

3. RESULTS AND DISCUSSION

3.1. Isolation of marine bacterial isolates

The marine water sample collected from Alibaug surrounded by Arabian Sea coast showed pH 7.6 and salinity 39.4 ppt. Lotfinasabasl (2013) reported average pH 7.8 and average salinity 48.93 ppt in the same habitat in mangrove forest surface water. In total sixteen bacterial isolates of marine bacteria were obtained on Zobell marine agar medium.

3.2. Screening for marine *Pseudomonas* isolates

Four colonies with yellowish green pigmentation were observed on citrimide agar indicating presence of *Pseudomonas* species. The gram negative, non-spore forming short rods were observed.

3.3. Biochemical characterization of *Pseudomonas* isolates

The biochemical characterization according to Bergey's Manual of Determinative Bacteriology indicated that the isolates may be of genus *Pseudomonas* (Table 1) that may be further confirmed using molecular techniques.

3.4. Screening of *Pseudomonas* isolates for PSM activity

The halo zone around the colonies on the Pikovskaya's agar indicated the phosphate solubilizing ability of selected *Pseudomonas* isolates. The Strain RSML4 exhibited maximum zone of phosphate solubilization. (Photo: 1).

3.5. Determination of phosphate solubilization index (PSI) and quantitative estimation

The concentration of soluble phosphate released was determined by Barton's reagent method. All the four isolates showed release of phosphate in the medium. (Table 2) Phosphate solubilization potential of *P. aeruginosa* has also reported [21]. The strain RSML4 released maximum 68 ppm of soluble phosphate in the medium exhibiting highest efficiency. Astriani [22] reported highest 51.45 ppm of phosphate solubilization activity. Various phosphate-solubilizing bacterial strains were reported from the rhizosphere of species of mangroves in a semiarid coastal lagoon which include genera of *Bacillus*, *Paenibacillus, Vibrio, Xanthobacter, Enterobacter, Kluyvera, Pseudomonas, and Chryseomonas, Aspergillus*. [17].

Table 1: Biochemical characterization of marine bacterial isolates

S. No.	Character	RSML1	RSML 2	RSML 3	RSML 4
1.	**Size (in mm)**	2	3	2	2
2.	**Shape**	Rod	Rod	Rod	Rod
3.	**Color**	Yellowish	Greenish		Greenish
4.	**Margin**	Entire	Entire	Entire	Entire
5.	**Surface**	Smooth	Smooth	Smooth	Smooth
6.	**Elevation**	Flat	Flat	Flat	Flat
7.	**Opacity**	Opaque	Opaque	Opaque	Opaque
8.	**Consistency**	Sticky	Sticky	Sticky	Sticky
9.	**Gram's Nature**	Gram Negative	Gram negative	Gram Negative	Gram Negative
10.	**Motility**	Motile	Motile	Motile	Motile
11.	**Glucose**	A	A	A	A
12.	**Lactose**	-	-	-	-
13.	**Mannitol**	A	A	A	A
14.	**Maltose**	-	A	A	-
15.	**Catalase**	+	+	+	+
16.	**Oxidase**	+	+	+	+
17.	**Indole**	+	+	+	+
18.	**MR**	+	+	+	+
19.	**VP**	-	-	-	--
20.	**Citrate Utilization**	+	+	+	+
21.	**H_2S**	+	+	+	+
22.	**Nitrate reduction**	-	-	-	-
23.	**Identified As**	*Pseudomonas* sp.	*Pseudomonas* sp.	*Pseudomonas* sp.	*Pseudomonas* sp.
24.	**pH fall after 96 hr.**	*5.7*	6.7	*6.2*	*4.2*

+: Test Positive, - : Test Negative, A: acid Production

Table 2: Phosphate solubilization ability of marine *Pseudomonas* isolates

Bacterial Strain	Diameter of colony	Diameter of halo around colony	PSI	Phosphate released in medium in Burton's method (ppm)
Pseudomonas RSML 01	5mm	8mm	1.6	52
Pseudomonas RSML 02	5mm	10mm	2	46
Pseudomonas RSML 03	4mm	6mm	1.5	29
Pseudomonas RSML 04	5mm	20mm	4	68

PSI: Phosphate Solubilization Index

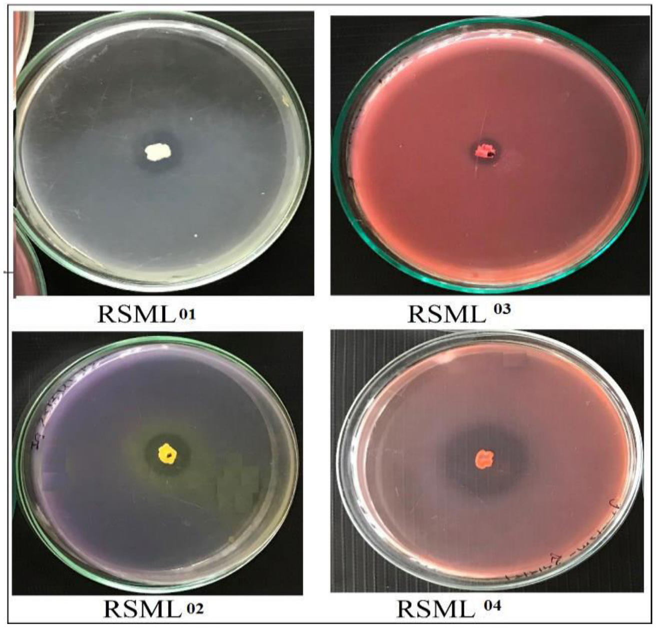

Fig. 1: Marine *Pseudomonas* isolates showing halo zones of phosphate solubilization

4. CONCLUSION

In the present investigation it is found that, the strain RSML4 exhibited considerable phosphate solubilizing activity as its intrinsic potential of plant growth promotion and therefore can be used as PSM bio-fertilizer. Present strain will be a promising conditioner for soil with incremented saline conditions, in order to emphasize sustainable ecofriendly agriculture practices. Future aspect of present studies will be commercial production of PSM bio-fertilizer and its wide field trials.

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