Phosphorus absorption kinetics of some phosphate solubilizing bacterial strains isolated from river Ganga

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ABSTRACT

In this study, a total of twenty four phosphate solubilizing bacterial (PSB) colonies were isolated on the Pikovskaya’s agar medium from different effluent exposed sediment of river Ganga. The bacteria showing clear halo zones around their colonies were considered as phosphate solubilizers. Out of 24 phosphate solubilizing bacterial isolates, 4 potential isolates showing highest halo zone on Pikovskaya’s agar medium were selected for the phosphorus absorption kinetics study. Isolates RPSB6, SPSB13, JPSB16 and TPSB23 belong to genera *Pseudomonas*, *Enterobacter*, *Bacillus* and *Flavobacterium* respectively as identified by their physiological, morphological and biochemical characteristics. Among these 4 PSB isolates, *Bacillus* sp JPSB16 showed the maximum P absorption (Vmax) value of 72.4 (10⁻⁸ mg d⁻¹ number⁻¹) followed by *Flavobacterium* sp TPSB23 (65.1), *Enterobacter* sp SPSB13 (62.2) and *Pseudomonas* sp RPSB6 (58.7), as compared to 26.3 (10⁻⁸ mg d⁻¹ number⁻¹) for the average sediment microorganisms.

Keywords: Phosphate solubilizing bacteria, Phosphorus Absorption, River Ganga, West Bengal

Abbreviation: PSB- Phosphate Solubilizing Bacteria, P-Phosphorus

To Cite this Article:

1. INTRODUCTION

Phosphorus is an essential macromolecule and plays a vital role in the primary production in aquatic ecosystem. It is also an essential nutrient for aquatic life (Karl, 2000). Among various factors the bottom deposits play a significant role to maintain the phosphate concentration in water (Promod and Dhevendaran, 1987). Previous studies have indicated that the major part of available phosphate is locked up as insoluble inorganic and organic phosphorous compounds in the sediments. Sediments act as buffer on the phosphate concentration in the overlying water column (Carritt and Goodgal, 1954; Gesnner, 1960; Pomeroy et al., 1965; Promod and Dhevendaran, 1987). Seshadri et al., (2002) reported that phosphate ions are strongly adsorbed by sediments with a high content of silt and clay.

Microorganisms play an important role for transformation of phosphorous in water and sediments. Phosphate solubilizing bacteria and fungi solubilize inorganic phosphate compounds to their soluble form (Bardiya and Gaur, 1972; Wani et al., 1979). Bacteria of the genera such as *Bacillus*, *Pseudomonas*, *Rhizobium*, *Mesorhizobium*, *Enterobacter*, *Acinetobacter*, *Azotobacter*, *Flavobacterium*, *Klebsiella*, *Ewingia* and *Micrococcus* are regarded as efficient phosphate solubilizers (Villegas and Fortin, 2002).

Phosphate solubilization is a complex phenomenon, which is governed by physiological, nutritional and cultural conditions of the bacteria (Reyes et al., 1999). Phosphate solubilizing bacteria play an important role in regulating the inorganic phosphate concentration by secreting extracellular enzymes such as alkaline phosphatases. These enzymes are present in all organisms but only bacteria, fungi, actinomycetes and some algae are able to secrete them outside their cells (Jones, 2002). PSB have been found to produce some organic acids in order to solubilize inorganic phosphate compounds such as monocarboxylic, monocarboxylic hydroxy, monocarboxylic keto gluconic, decarboxylic, dicarboxylic hydroxy and tricarboxylic hydroxy acids (Goldstein, 1995; Kim et al., 1997).

In the aquatic ecosystem, PSB have been found in the water column (Promod and Dhevendaran, 1987, Paul and Sinha, 2013) and in sediments (Ayyakkannu and Chandramohan, 1971, Devendran et al., 1974). Phosphate solubilization is a complex biochemical phenomenon and it is important to understand the bacterial populations capable of phosphate solubilization, realizing the multiple roles the phosphate solubilizing bacteria play (Shri Ramanan and Kannan, 2011). This study aims to screen PSBs from various polluted site of river Ganga, which are capable of utilizing and transforming adsorbed P more effectively than the average microbial community, and to characterize and identify these phosphate solubilizing bacterial strains.

2. MATERIALS AND METHODS

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2.1. Sampling site and collection of sediment samples

Sediment samples were collected from near the effluent discharge point of rayon factory, Tribeni (S1); thermal power plant, Tribeni (S2); jute mill, Bansberia (S3) and sewage, Bansberia(S4), West Bengal of the river Ganga (Figure 1) with the help of Ekman dredge. The samples were aseptically transmitted to sterilized glass bottle and transported to the laboratory immediately for further analysis.

2.2. Isolation of phosphate solubilizing bacteria (PSB)

Water and sediment samples were aseptically transferred to the laboratory and serially diluted water samples were plated on Petri dishes containing Pikovskaya’s (PKV) agar medium consisting of ingredients in g/l: Glucose 10g; tri-calcium phosphate (TCP) 5g; ammonium sulphate 0.5g; sodium chloride 0.2g; potassium chloride 0.2g; magnesium sulphate 0.10g; yeast extract 0.5g; manganese sulphate trace; ferrous sulphate trace; agar agar 20g; manganese sulphate trace; ferrous sulphate trace; agar agar 20g; the pH was adjusted to 7.0 ± 0.2 before sterilization (Pikovskaya, 1948) by pour plate technique and incubated at 28 ± 2°C for 48-96h. The bacterial colonies showing clear zone around them were considered as phosphate solubilising bacteria (PSB) (De Freitas et al., 1997). Pure culture of the isolates were made by repeated subculturing for 2-3 times on fresh PKV plate and were maintained on PKV slants at refrigeration temperature. A total of 24 phosphate solubilizing bacterial colonies were isolated.

2.2.1. Characterization and identification of bacterial strains

Identification of phosphate solubilizing bacterial strains was performed by physiological, morphological characteristics and biochemical analysis comparing with standard methodology (Collins and Lyne, 1980), Physiological, morphological and biochemical tests of all the PSB isolates were carried out for their identification as per the procedures outlined in Bergey’s Manual of Systemic Bacteriology (Krieg and Holt, 1984).

### Table 1

<table>
<thead>
<tr>
<th>Test</th>
<th>Pseudomonas sp</th>
<th>Enterobacter sp SPSB13</th>
<th>Bacillus sp JPSB16</th>
<th>Flavobacterium sp TPSB23</th>
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<tr>
<td>Cell shape</td>
<td>Rod</td>
<td>Rod</td>
<td>Rod</td>
<td>Rod</td>
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<tr>
<td>Gram reaction</td>
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<td>-</td>
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<tr>
<td>Motility</td>
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<td>+</td>
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<td>+</td>
</tr>
<tr>
<td>Growth at 5%NaCl</td>
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<td>Catalase</td>
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<td>Oxidase</td>
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<td>IMViC test</td>
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<tr>
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<tr>
<td>Methyl red</td>
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<tr>
<td>Hugh-Leifson (O/F) reaction</td>
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<td>O/F</td>
<td>F</td>
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<tr>
<td>Utilization of carbon source</td>
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<tr>
<td>Glucose</td>
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<td>Lactose</td>
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<tr>
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</tbody>
</table>

+ indicates presence or positive reaction; - indicates absence or negative reaction; O = Oxidation; F= Fermentation

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SUMMARY OF RESEARCH
1. Four phosphate solubilizing bacterial isolates with potential phosphate solubilizing ability were screened for P-absorption kinetics study in river sediment.
2. Among these 4 phosphate solubilizing bacterial isolates Bacillus sp JPSB16 showed maximum P-absorption value.

FUTURE ISSUES
The isolated phosphate solubilizing bacterial strains may be exploited for release of phosphorus from complex river sediment, which may fertilize the aquatic bodies.

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Figure 2
Kinetics of P uptake by the selected PSB strains as compared with river sediment microorganisms.

From the phosphorus absorption kinetics study, it was observed that there was no significant difference in the absorption rate of P between the PSBs and the average river sediment microorganisms at very low P concentration range (< 0.2 mg L⁻¹). But, at higher P concentrations (0.2-2.0 mg L⁻¹), the PSBs had a greater uptake rate of P than the average river sediment microorganisms (Figure 2). Bacillus sp JPSB16 had a maximum P absorption (Vmax) value of 72.4 (10⁴ mg d⁻¹ number⁻¹) followed by Flavobacterium sp TPSB23 (65.1), Enterobacter sp SPSB13 (52.2) and Pseudomonas sp RPSB6 (58.7), as compared to 28.3 (10⁴ mg d⁻¹ number⁻¹) for the average sediment microorganisms (Figure 2). These results indicate that the phosphate solubilizing bacteria have a greater capacity for P uptake than the average river sediment microorganisms. Phosphate solubilizing bacterial isolates produces various types of organic acids which enhanced the dissolution of minerals by chelating with aluminium and iron, thus releasing the absorbed phosphorus. Moreover a large number of organic anions strongly compete with phosphate for adsorbing sites on surface of aluminium and iron oxides (Nagaraj et al., 1970; He et al., 1990; Violante et al., 1991) and release adsorbed P through ligand exchange reaction. Besides the release of organic acids the PSB had a greater capacity for P-transport from external sources into the cell and hence can more efficiently absorb and incorporate P into the cells. As a result, more P can be desorbed from the tightly bound sites by PSBs than the average sediment microorganisms.

4. CONCLUSION
The present study revealed that out of 24 bacterial strains 4 are found to be exhibiting higher P-absorption kinetics. Bacillus sp JPSB16 showed maximum P-absorption value. The study indicated that the phosphate solubilizing bacteria in river sediment exhibited higher capacity for phosphorus uptake over the average river sediment microorganisms which might be use to release phosphorus from sediment to overlying water so that phosphorus cycling in river water can be maintained.

15000 rpm for 15 min. Phosphorus concentration in the supernatant solution was determined, and the decrease in P concentration after microbial culture was considered to result from P absorption by the inoculated microorganisms. The experiment was repeated three times and average values of the three measurements were used for calculating P absorption kinetic parameters. The maximum absorption (Vmax) and the affinity constant (Km) were calculated based on the Michaelis–Menten equation [v = Vmax C/(Km+C), where v is the absorption rate and C is the initial P concentration].

3. RESULTS AND DISCUSSION
A total of 24 bacterial isolates were selected for the study. All isolates were screened for phosphate solubilizing activity on Pikovskaya’s agar. Four different isolates exhibiting maximum zone of phosphate solubilization on Pikovskaya’s agar after 48-96h incubation around the bacterial colonies were selected for phosphorus absorption kinetics study. Physiological, morphological and biochemical characterization of selected PSB strains performed were presented in Table 1. Based on these characters, the isolates were assigned to different genera like Pseudomonas, Enterobacter, Bacillus and Flavobacterium. These bacteria genera were well known identified as phosphate solubilizer by many researchers (Rodriguez and Fraga, 1999; Kumar et al., 2001).

The isolated phosphate solubilizing bacterial strains may be exploited for release of phosphorus from complex river sediment, which may fertilize the aquatic bodies.

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