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***Research Article***

# PHOSPHATE SOLUBILIZATION POTENTIAL AND PHOSPHATASE ACTIVITY OF SOME BACTERIAL STRAINS ISOLATED FROM THERMAL POWER PLANT EFFLUENT EXPOSED WATER OF RIVER GANGA

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## ABSTRACT
Water samples from the Bandel thermal power plant effluent exposed water of river Ganga at Tribeni West Bengal, India were collected monthly to enumerate phosphate solubilizing bacteria (PSB) population between January 2011 and December 2011. The population density of PSB ranged from 2.07 $x10^3$ cells $ml^{-1}$ to 2.57$x10^3$ cells $ml^{-1}$. PSB was highest in the month of July and lowest in the month of February. Out of 24 PSB isolates, 5 isolates exhibiting highest phosphate solubilisation index (SI) ranged from 3.000-2.714. A significant variation (159.84-201.38 µg P $ml^{-1}$) in the capacity to solubilize phosphate by the isolates of PSB was noted. *Flavobacterium* sp TPSB23 showed highest phosphatase activity of 31.24 µmoles/g/h followed by *Bacillus* sp TPSR21 (27.12 µmoles/g/h). In all the PSB isolates, decrease in pH was observed in liquid Pikovskaya's medium ranging from 3.66 to 4.17 from initial pH of 7.0±0.2.

***Key Words:*** *Phosphate Solubilizing Bacteria, Phosphatase Activity, River Ganga, Solubilization Index*

## INTRODUCTION
Phosphorus is an essential nutrient to all form of life, being a structural and functional component of organisms. Phosphorus normally exists as phosphates in nature in a variety of organic and inorganic forms, primarily in either insoluble or very poorly soluble inorganic forms. The phosphate content in water depends upon various factors, of which the bottom deposits play a major role. Different studies (Rochford, 1950; Seshappa, 1953; Balasubramanyan, 1961; Pomeroy *et al.*, 1965) indicated clearly the nature of phosphate exchange between the sediment and the water, suggesting that the sediment act as a buffer on the phosphate concentration in the overlying water.

The phosphate solubilizing microorganisms convert insoluble P into soluble form (Rodriguez and Fraga, 1999) by acidification, chelation and exchange reactions (Gerke, 1992) in the periplasm, which act as an indicator for routine isolation and selection procedure of phosphate solubilizers (Illmer and Schinner, 1992). Different groups of phosphate solubilizing microorganisms, particularly bacteria and fungi, have been reported to solubilize inorganic phosphate compounds to soluble (Bardiya and Gaur, 1972; Wani *et al.*, 1979). Bacteria belonging to the genera *Bacillus, Pseudomonas, Rhizobium, Mesorhizobium, Enterobacter, Acinetobacter, Azotobacter, Flavobacterium, Klebsiella, Erwinia and Micrococcus* have been reported as efficient phosphate solubilizers (Villegas and Fortin, 2002).

Phosphate solubilizing bacteria (PSB) have the capability to produce extracellular enzymes such as phosphatase (George *et al.*, 2002), able to mineralize phosphate compounds. Phosphatase-catalysed reactions are involved in the hydrolysis of both esters and anhydrides of $H_3PO_4$ (Tabatabai, 1994). Phosphatases are generally classified as acid and alkaline phosphatases. Acid phosphatases are produced by both microorganisms and higher plants but alkaline phosphatases are mainly produced by microorganisms (Tabatabai and Bremner, 1969). Activity of phosphatase enzyme is affected by different factors, such as, the amount and type of substrate (Fitriatin *et al.*, 2008), pH, temperature, property of inhibitor and activator, concentration of enzyme and product, and also the kind of solvent used (Saparatka, 2002).

Some reports are available on the occurrence and distribution of phosphate solubilizing microbes in the marine environment (Ayyakkannu and Chandramohan, 1971; Venkateswaram and Natarajan, 1983; De Souza *et al.,* 2000; Seshadri *et al.,* 2002). But very few attempts have been made to isolate and characterize the potential PSB from the riverine ecosystems (Paul and Sinha, 2013). Hence, little information is available concerning phosphate solubilizing bacteria and about their phosphatase activity. The present study was undertaken to study in detail about the distribution and population density phosphate solubilizing bacteria (PSB) from thermal power plant effluent exposed water of river Ganga at Tribeni, West Bengal, India were enumerated and PSB isolates were also screened for their phosphatase activity under *in vitro* conditions.

## MATERIALS AND METHODS

### *Sampling*

Water samples were collected in every month from the Bandel thermal power plant effluent exposed water of river Ganga at Tribeni (23°00'56"N and 88°24'54"E), West Bengal, India (Figure 1) for a period of one year (January-December, 2011). Water samples collected in sterilized McCartney bottles were transported to the laboratory in an icebox immediately after collection for further studies.

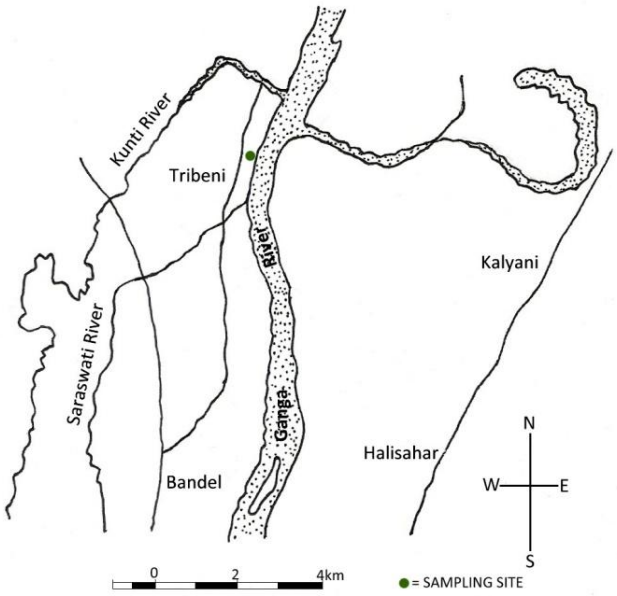


**Figure 1: Map of the river Ganga showing sampling site**

### *Analysis of Physicochemical Parameters*

Water samples were analyzed for the following physicochemical parameters; temperature, pH, dissolved oxygen (DO) and phosphate. The physicochemical parameters were analysed as per standard methods (APHA, 2005).

### *Enumeration of Phosphate Solubilizing Bacteria (PSB)*

Serial dilutions of the water sample were made and one ml of aliquots of $10^2$-$10^5$ dilutions were transferred to Petri plates containing Pikovskaya's agar media consisting of ingredients in g/l: Glucose 10g; tri-calcium phosphate 5g; ammonium sulphate 0.5g; sodium chloride 0.2g; potassium chloride 0.2g; magnesium sulphate 0.1g; yeast extract 0.5g; manganese sulphate trace; ferrous sulphate trace; agar 15 g; the pH was adjusted to 7.0 ± 0.2 before sterilization (Pikovskaya, 1948) for enumerating PSB. Plating was done in triplicate and incubated at $28^0C \pm 2^0C$ for PSB. After 120 h of incubation CFUs of PSB were recorded. A total of 24 phosphate solubilizing bacterial colonies were isolated. Out of 24 bacterial

isolates, 5 isolates having larger halo zones were selected for further study. Physiological, morphological and biochemical tests of the selected PSB strains were carried out for their identification as per the procedures outlined in Bergey's Manual of Systemic Bacteriology as in (Krieg and Holt, 1984).

***Determination of Phosphate Solubilization Potential***

Quantitative estimation of phosphate solubilization on Pikovskaya's agar media was measured in terms of solubilisation index (SI). Phosphate solubilization index was determined by the following formula (Edi-Premono, 1996).

$$\text{Solubilization Index (SI)} = \frac{\text{Colony diameter + Halo zone diameter}}{\text{Colony diameter}}$$

The qualitative estimation of phosphate solubilization potential of selected strains of PSB was measured *in vitro* by determining available phosphate in the Pikovskaya's broth amended or supplemented with tricalcium phosphate as substrate. The flasks were inoculated with phosphate solubilizing bacterial culture broth (OD=2 at $A_{600}$). The flasks were incubated at 28 ± 2°C for 5 days and centrifuged at 10000 rpm for 10 minutes. Available phosphate was determined in supernatant as per the procedure outlined by Strickland and Parsons (1972). After incubation for 5 days, pH of the medium was recorded with a digital pH meter (Jenway 3510).

***Determination of Phosphatase Activity***

To determine the phosphatase enzyme activity, experiments were set up using phosphate solubilizing bacterial broth cultures in Erlenmeyer flasks with and without adding phosphorous source (β-glycerophosphate used as a substrate). Phosphate solubilizing bacterial culture filtrates were centrifuged and subjected to estimate phosphatase activity following the protocol of Tabatabai and Bremner (1969).

## RESULTS AND DISCUSSION

The physico-chemical parameters of thermal power plant effluent exposed water of river Ganga at Tribeni are presented in Table 1. The temperature of river water oscillated between 22.0$^0$C and 33.5$^0$C with the average value of 28.83$^0$C throughout the period of study. The pH of the river water fluctuated between 7.26 and 7.44 with the mean value of 7.32. Similarly the dissolved oxygen (DO) value ranged between 3.68 mg/l and 4.40 mg/l with average value of 3.96 mg/l. The phosphate concentration ranged between 0.504 mg/l to 0.648 mg/l with the mean value of 0.579 mg/l. The physic-chemical parameters found in this region of the river Ganga favours the growth and activity of phosphate solubilizing bacteria.

**Table 1: Physicochemical parameters of river water monitored during January-December, 2011**

| | Parameters | | | |
|---|---|---|---|---|
| **Months** | **Temperature ($^0$C)** | **pH** | **D.O. (mg/l)** | **Phosphate (mg/l)** |
| **January** | 22.0 | 7.31 | 4.32 | 0.504 |
| **February** | 24.0 | 7.30 | 4.40 | 0.532 |
| **March** | 27.0 | 7.41 | 4.22 | 0.580 |
| **April** | 30.5 | 7.39 | 3.96 | 0.568 |
| **May** | 33.0 | 7.44 | 3.98 | 0.596 |
| **June** | 33.5 | 7.37 | 3.72 | 0.620 |
| **July** | 32.0 | 7.32 | 3.68 | 0.648 |
| **August** | 32.0 | 7.27 | 3.80 | 0.616 |
| **September** | 30.5 | 7.30 | 3.74 | 0.620 |
| **October** | 30.0 | 7.26 | 3.70 | 0.588 |
| **November** | 28.0 | 7.28 | 3.92 | 0.552 |
| **December** | 23.5 | 7.28 | 4.14 | 0.524 |

Population dynamics of PSB in this study site are presented in figure 2. It is generally observed that there was a significant difference of phosphate solubilizing bacterial population in river water. Throughout the year of survey PSB population remained almost between 2.07 x$10^3$ cells $ml^{-1}$ to 2.57x$10^3$ cells $ml^{-1}$ with the highest value during July and lowest during February. The mean population of PSB was 2.30x$10^4$ cells $ml^{-1}$. This variation in the population of PSB might be affected by various factors such as nutrients, pH, organic matter and some enzyme activities in water column. Chen *et al.*, (2006) also showed that the phosphate solubilization activity of isolated strains was related to the release of organic acids and subsequent reduction of pH.

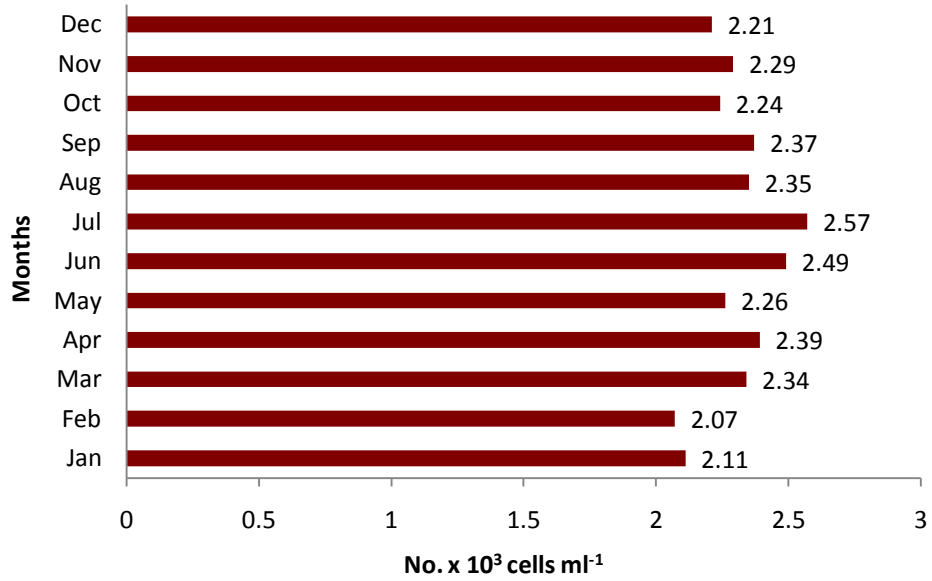


**Figure 2: Phosphate solubilizing bacterial (PSB) count in river water (January-December, 2011)**

All the isolated PSB strains were found to be potent phosphate solubilizers showing clear halo zone around their colonies in Pikovskaya's agar medium. Among these 5 potent PSB isolates, strains *Flavobacterium* sp TPSB23 showed the maximum phosphate solubilization index of 3.00 followed by *Bacillus* sp TPSR21 (2.857) (Figure 3).

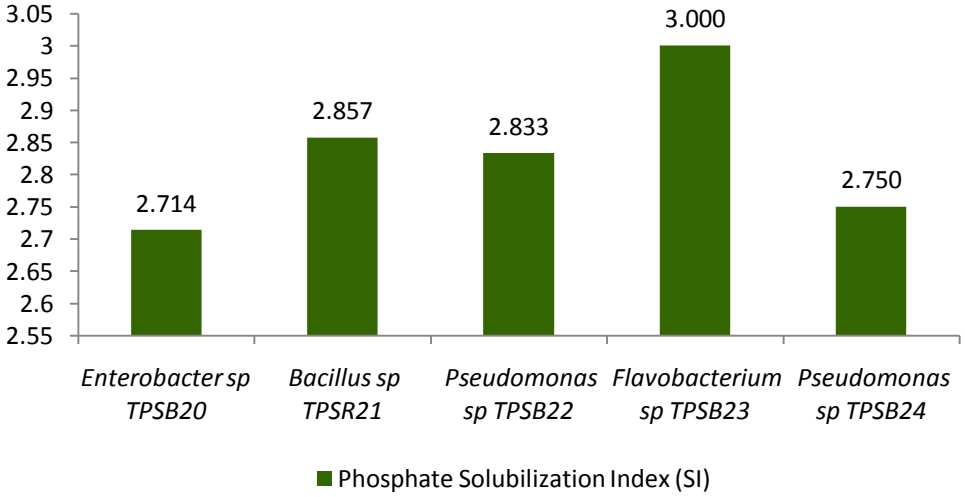


**Figure 3: Phosphate solubilising index (SI) of the PSB isolates**

The phosphate solubilizing efficiency of isolated PSB strains in Pikovskaya's broth indicated that all the strains solubilized inorganic phosphate efficiently in the medium (Table 2). Among the 5 PSB strains, *Flavobacterium* sp TPSB23 showed maximum (201.38 µg P $ml^{-1}$) phosphate solubilizing efficiency followed by *Bacillus* sp TPSR21 and *Pseudomonas* sp TPSB22. These types of phosphate solubilizing efficiency have been reported by many authors (Ponmurugan and Gopi, 2006; Sahu *et al.*, 2007).

**Table 2: Phosphorous solubilizing ability and phosphatase activity of PSB *in vitro* condition**

| Isolates | Available P (µg P $ml^{-1}$) | Phosphatase activity (µmoles/g/h) |
|---|---|---|
| *Enterobacter* sp TPSB20 | 159.84 | 19.92 |
| *Bacillus* sp TPSR21 | 189.06 | 27.12 |
| *Pseudomonas* sp TPSB22 | 182.92 | 25.38 |
| *Flavobacterium* sp TPSB23 | 201.38 | 31.24 |
| *Pseudomonas* sp TPSB24 | 167.74 | 21.65 |

Similarly *Flavobacterium* sp TPSB23 showed highest phosphatase activity of 31.24 µmoles/g/h followed by *Bacillus* sp TPSR21 (27.12 µmoles/g/h). However, there was a positive correlation (r=0.996) between qualitative phosphate solubilizing efficiency and phosphatase activity. This might be due to the availability of higher amount of phosphate in the medium and the ability of the strains to solubilize (Sahu *et al.*, 2007).

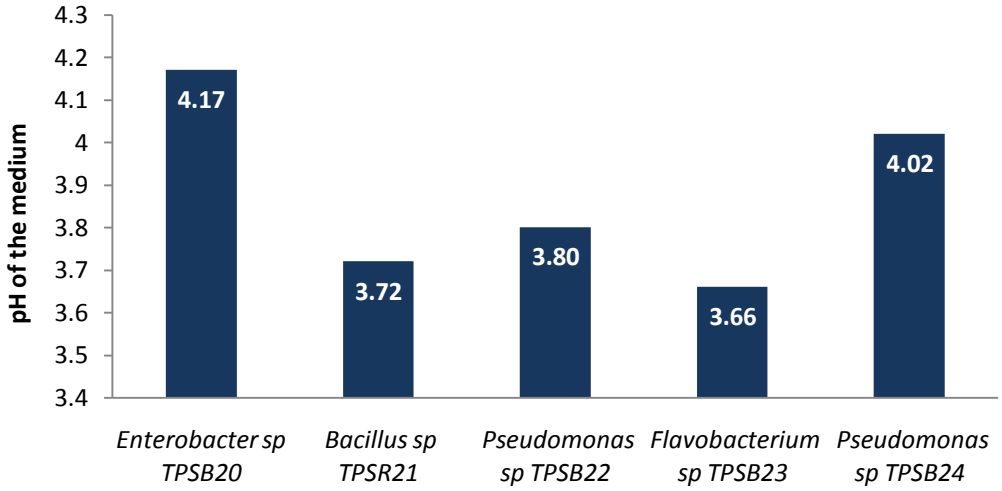


**Figure 3: Change of pH of the liquid medium after incubation**

Maximum drop in pH from an initial pH of 7.0±0.2 after 120 h was associated with higher levels of phosphate solubilization. *Flavobacterium* sp TPSB23 showed maximum drop of pH from initial where pH was decreased to 3.66 (Figure 4). The decrease in pH clearly indicates the production of organic acid and phosphatase, which is considered to be responsible for phosphate solubilization activity (Perez *et al.*, (2007).

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