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Phosphate solubilizing actinomycetes in the estuarine environment: An inventory

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Abstract: *Sediment samples were collected from different stations of the Vellar estuary for isolation of total actinomycetes and phosphate solubilizing actinomycetes. Phosphatase activity in the sediments was also investigated. Consistently, a higher number of actinomycetes, phosphate solubilizing actinomycetes and phosphatase activity were recorded from the clay sediments than the sandy sediments at all the stations. In all, 7 strains showed positive phosphatase activity. Among them, one strain PS-3 exhibited good activity and was further investigated for optimum phosphorus solubilization at different pH (6, 6.5, 7, 7.5 and 8) and incubation (1st day to 20th day) periods. The solubilizing activity was maximum at the pH 7 and an incubation period of 13 days was required for an appreciable quantity of phosphorus to be leached into the medium. Based on the chemotaxonomical and conventional methods of identification, the strain PS-3 has been tentatively identified as Streptomyces galbus. The present study indicates that phosphatase enzyme and S. galbus along with other actinomycetes species would play a major role in solubilizing the phosphate in the estuarine ecosystem and increasing the soluble phosphate concentration thereby enhancing the productivity.*

Key words: *Vellar estuary, Sediments, Actinomycetes, Phosphatase*
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Introduction

It is well known that the majority of phosphates in the sediments are present as insoluble organic and inorganic forms. The phosphate concentration in water depends upon various factors, of which the bottom deposits play a major role (Promod and Dhevendaran, 1987). Earlier studies have indicated the nature of phosphate exchange between the sediments and water, suggesting that sediments act as buffer on the phosphate concentration in the overlying water column (Pomeroy *et al.,* 1965; Promod and Dhevendaran, 1987). It is evident, therefore, the major portion of available phosphate is locked up as insoluble organic and inorganic phosphorous compounds in the sediments.

Microorganisms play an important role for transformation of phosphorous in water and sediments and the phosphate ions are reported to be strongly adsorbed by sediments with a high content of silt and clay (Seshadri *et al.,* 2002). The solubilization of phosphorus compounds may also be brought about by acids and enzymes of microbial origin (Alexander, 1961; Skujins, 1968). A few 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).

Among the different microbes, bacteria and yeasts are the potential candidates for dissolving the insoluble organic and inorganic phosphorus compounds and the occurrence and distribution of such organisms in the natural marine environment have been also reported (Ayyaakkannu and Chandramohan, 1970a; Naik *et al.,* 1982; De Souza *et al.*, 2000; Seshadri *et al.,* 2000; Seshadri *et al.,* 2002; Ogunwenmo *et al.*, 2005; Chukwu and Ogunmodede, 2005; Chukwu and Odunzeh, 2006). Till now, information on phosphorous solubilizing actinomycetes of the marine environment is scanty. Therefore, the present investigation was designed to study the phosphorous solubilizing actinomycetes in the sediments of the Vellar estuary and to identify the potential ones using chemotaxonomical and conventional methods of identification.

Materials and Methods

Isolation: Clay and sand sediment samples were collected from different stations (Station 1 - Seashore zone (sandy); Station 2 - Estuary mouth zone (sandy); Station 3 - Biological Station zone (clay) and Station 4 - Railway bridge zone (clay) of the Vellar estuary (Lat. 11°29'N, Long. 79°46'E), lying along the southeast coast of India. Sediment samples were collected by inserting a polyvinyl corer (10 cm dia.) into the sediments. The corer was sterilized with alcohol before sampling at each station. The central portion of the top 2 cm sediment sample was taken out with the help of a sterile spatula. This sample was then transferred to a sterile polythene bag and transported immediately to the laboratory. The sediment samples thus collected were air-dried aseptically. After a week, the sediment samples were incubated at 55°C for 5 minutes (Sivakumar *et al.,* 2005). Then, 10-fold serial dilutions of the sediment samples were prepared, using filtered and sterilized 50% seawater. One ml of the serially diluted samples was plated in the Kuster's Agar medium (Rathna Kala and Chandrika, 1995) for enumerating total actinomycetes population and Kuster's Agar medium supplemented with $CaHPO_4$ (5 g/l) for enumeration of phosphate solubilizing actinomycetes in triplicate and incubated at 37°C for 7 days. After 7 days, the colony forming units (CFU/g) were recorded. Phosphate solibilizers were isolated based on the halo zones produced around the colonies (Seshadri *et al.*, 2002). The size of the clear zone around the colonies was recorded as phosphate

solubilization and results were expressed as solubilization efficiency (Nguyen *et al.,* 1992). The well-developed, morphologically different colonies and phosphate solubilizing colonies were isolated and inoculated on appropriate agar plates for obtaining pure cultures and maintained in slants for further use.

Estimation of phosphatase activity: Phosphatase activity of the sediments was estimated by the method described by Kramer and Erdei (1958). The samples were incubated with phenyldisodium orthophosphate in appropriate salinities and the phosphatase activity was indirectly measured by the amount of phenol released. Total and adsorbed phosphates in various samples were also estimated adopting the method of Stickland and Parsons (1967).

Effect of pH and incubation period on phosphate solubilization: The strains which showed the maximum clearing zone in plate assay were further tested for optimum P-solubilization at different pH and incubation periods.

Effect of pH: Different pH concentrations *viz.,* 6, 6.5, 7, 7.5 and 8 of the 100 ml Pikovakaya's broth (Pikovakaya, 1948) were prepared using buffer solution. Single colony was inoculated and incubated at 37°C for 7 days.

Effect of incubation period: Single colony was inoculated in Pikovakaya's broth and incubated at different incubation periods (1st day to 20th day) to study the effect on phosphate solubilization. The fermentation was carried out in duplicate, keeping pH and temperature at 7 and 37°C respectively and average values were reported.

At the end of the incubation period, cultures were harvested and centrifuged at 5,000 rpm for 20 minutes and the filtrate was collected. The filtrate was used for phosphorus estimation by the paramolybdate blue method (Olsen and Sommers, 1982).

Taxonomic investigation: The genus level identification was made for the potential phosphate solubilizing strains using cell wall composition analysis studies (Lechevalier and Lechevalier, 1970). The species level identification of the strain was made by the methods described by the Shirling and Gottileb (1966), Nonamura (1974) and Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974).

Results and Discussion

Population density:

The total actinomycetes population density varied with different sampling stations (Table 1). The higher density was recorded at stations 3 ($1.15x10^3$ CFU/g) and 4 ($1.07 x10^3$ CFU/g) which are characterized by clay sediments. Comparatively lower density was recorded at stations 1 ($0.25 x10^3$ CFU/g) and 2 ($0.37x10^3$ CFU/g), characterized by sandy sediments. This confirms the earlier findings of Zobell (1938) and Ayyakkkannu and Chandramohan (1971) who have stated that clay sediments would harbour more microbial populations than the sandy sediments. This may be attributed to the presence of rich organic content in the clay sediments. Phosphate solubilizing actinomycetes were recorded from all the sampling stations, but the clay samples held richer populations than the sandy samples (Table 1). Ayyakkannu and Chandramohan (1971) have also recorded higher population density of phosphatase solubilizing bacteria in the marine clay sediments at Porto-Novo region, owing to the occurrence of higher microbial loads and rich phosphate content in the clay sediments.

Phosphatase activity:

Phosphatase activity of the sediments was recorded for all the sampling stations (Table 1). In general, clay sediments revealed higher phosphatase activity than the sandy sediments. The content of total phosphates were also higher in the clay sediments. This would indicate that the phosphatase activity present in the estuarine environment is mainly governed by the availability of total phosphate. This is substantiated by the fact that wherever the total phosphate content was found to be high, the phosphatase activity was also high (Table 1). Such correlation has been reported by Alexander (1961) and Ayyakkannu and Chandramohan (1971). It should be noted here that the sediments containing a large fraction of soil and clay are rich in phosphate (Pomeroy *et al.,* 1965). The present study confirms this.

Effect of pH and incubation period:

In the present study, 7 strains showed positive phosphate solubilizing activity. Among them, one strain was very potential. On plate assay, the strain showed larger zones of solubilization indicating an efficient solubilization of phosphate than the other strains (Table 2) and here, it was selected for determining the optimum phosphorous solubilization at different pH and incubation periods.

Optimum pH for better p-solubilization was 7 (Fig. 1). P-solubilization was less at pH 6 and 8. Similarly, bacteria, fungi and yeasts showed higher p-solubilization at pH 7-8, 6-7 and 6-6.5 respectively (Ayyakkannu and Chandramohan, 1970b; Naik *et al.,* 1982; Promod and Dhevendaran, 1987; Seshadri *et al.,* 2002). During the growth of the strain, a slight fall in pH of the culture medium from 7.5 to 6 was observed after incubation which could be due to the release of acidic substances produced by the strain during fermentation (Seshadri *et al.,* 2002).

In the present study, the optimum incubation period over the release of phosphate was 13 days (Fig. 2). Promod and Dhevendaran (1987), observed maximum solubilization of insoluble phosphate by *Pseudomonas* and *Vibrio* in 3 days while Goswami and Sen (1962) recorded maximum solubilization by fungi in 15 days. Production of halo zones on solid agar and release of phosphate in the medium could be ascribed to the release of organic acids *viz.* citric, glyoxalic, malic, ketobutyric, succinic, fumaric and tartaric by the microbes (Seshadri *et al.,* 2002).

Table - 1: Population density of total actinomycetes, phosphate solubilizing actinomycetes, phosphatase activity and total phosphate concentration at different station of the Vellar estuary

Station	Population density of total actinomycetes (CFU/g)	Phosphate solubilizing actinomycetes (CFU/g)	Phosphatase activity (µg phenol/g/h)	Total phosphate (mg/g)
1	0.25	0.09	1.045	0.08
2	0.37	0.12	1.19	0.13
3	1.15	0.59	5.92	0.15
4	1.07	0.51	5.53	0.19

Table - 2: Phosphate solubilization index for different actinomycetes strains on Pikovskaya's agar

Strain no.	Solubilization index
PS-3	41.66±2.3
PS-5	35.01±1.0
PS-6	29.58±1.3
PS-10	11.57±0.2
PS-11	39.08±1.3
PS-15	10.82±0.7
PS-17	25.23±1.1

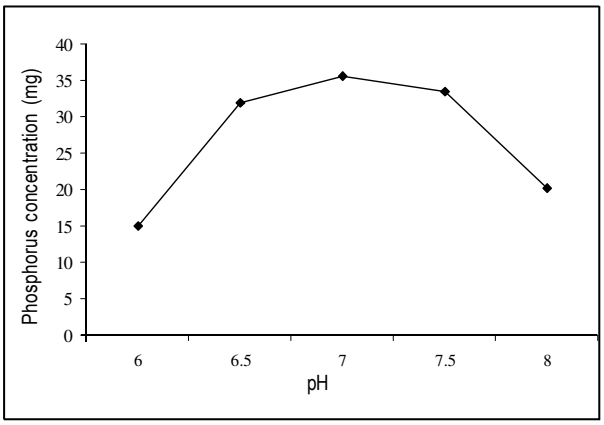

Fig. 1: Effect of pH on phosphorus solubilization

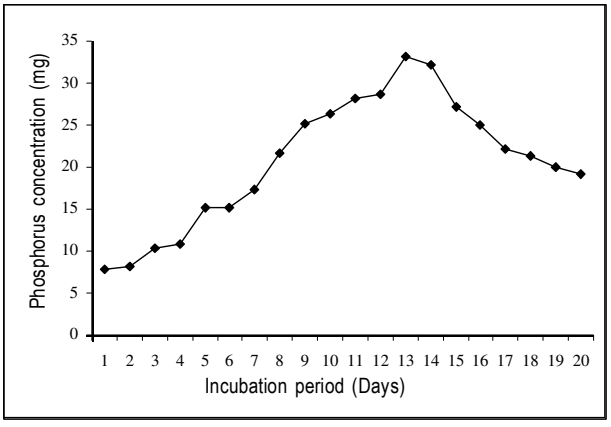

Fig. 2: Effect of incubation on phosphorus solubilization

Table - 3. Comparison between the strain PS-3 and *Streptomyces galbus*

Character studied (as per Nonomura key)	Strain PS-3	*S. galbus*
Colour of aerial mycelium	Grey yellow	Grey yellow
Melanoid pigment	+	+
Reverse side pigment	+	+
Soluble pigment	+	+
Spore chain	Spiral	Spiral
Spore surface	Smooth	Smooth
Carbon source assimilation		
Arabinose	±	±
Xylose	+	-
Inositol	+	+
Mannitol	+	+
Fructose	-	+
Rhamnose	+	-
Sucrose	-	-
Raffinose	±	±

Taxonomic investigation:

The strain possesses LL-DAP and it contains glycine in its cell wall. Presence of LL-DAP along with glycine indicates the cell wall type chemotype - I (Lechevalier and Lechevalier, 1970), which is the characteristic of the genera, *Streptomyces, Streptoverticillium, Chainia, Actinopycnidium, Actinosporangium, Elyptrosporangium, Microellbosporia, Sporichthya* and *Intrasporangium* (Lechevalier and Lechevalier, 1970).

The morphological, physiological and biochemical characteristics of the phosphorus solubilizing strain PS-3, tested in the present study, are given in Table 3. These characteristics were compared with those of the *Streptomyces* species given by Key of Nonomura (1974) and the Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974). The strain PS-3 showed variations only in a few characters when compared to the reference strain *Streptomyces galbus*, such as utilization of carbon compounds *viz.* xylose and rhamnose. Except this, all the other characters are similar to those of *S. galbus.* Hence, the strain PS-3 has been tentatively identified as *S. galbus*.

It is evident from the present study that phosphatase enzyme and *S. galbus* along with other actinomycetes species would play a major role in solubilizing the phosphate in the estuarine ecosystem and increasing the soluble phosphate concentration thereby enhancing the productivity of the aquatic system.

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