Solubilization of inorganic phosphates by *Azospirillum halopraeferans*

Bacteria of the genus *Azospirillum* are widely distributed in the rhizosphere of tropical and subtropical grasses and sugarcane[1]. *Azospirillum halopraeferans* is reported to occur in the rhizoplane of plants growing in saline soil in Brazil[2]. Phosphates, widely distributed in nature in both organic and inorganic forms, are not readily available to plants in a bound state[3]. Many soil bacteria are reported to solubilize these insoluble phosphates through various processes[4–7]. A few reports have also indicated the P-solubilizing activity of some nitrogen fixers[8–11]. This paper reports the P-solubilizing capacity of three different strains of *A. halopraeferans*.

Type strains (LMG7107, LMG 7108 and LMG 7109) obtained from J. Dobereiner, EMBRAPA, Brazil were used in this study. Individual strains were grown on the Sperber's medium[12] and Pikovskaya's medium[13] in petri dishes for 3 days at 28 ± 2°C. The size of the clear zones around the colonies showing solubilization of phosphate on incubation was noted. The results are expressed as solubilization efficiency (E = Solubilization diameter (S)/Growth diameter (G) × 100) (ref. 14). For broth culture studies, single colonies grown on nutrient agar were inoculated into both the liquid media containing water insoluble P-sources. All the experiments were conducted in triplicate and the cultures were maintained for 16 days. Phosphate was determined by the paramolybdate blue method[15]. For analysis, the cultures were harvested on every alternate day, centrifuged at 10,000 rpm for 15 min and then subjected for estimation. pH of the medium was also recorded simultaneously.

While the strains showed weak zone of solubilization on Sperber's medium (Table 1, Figure 1), no activity was observed in Pikovskaya's medium in plate assay. However, the liquid environment offered encouraging results where the three strains showed good activity in both the broths employed. Morris and Allen[16], while studying the oxalate metabolism by micro-organisms echoed a conforming trend, where the organisms do not show clearing zone in plates and there was good calcium oxalate metabolism in liquid broth, attributed to simple cation dissociation. Periodical estimates of P in media revealed the potential of all the strains in releasing Pi from insoluble P sources. On Sperber's medium, the P concentration increased slowly, reached a peak on the tenth day and declined slowly on later days. Whereas in Pikovskaya's medium, the P concentration increased gradually achieving a peak on the sixth day. Among the strains, LMG 7109 was good in Sperber's medium and solubilized 122.54 µg P/ml, while LMG 7107 and LMG 7108 solubilized 84.61 and 47.19 µg P/ml, respectively (Figure 2). In Pikovskaya's medium, LMG 7109 showed maximum solubilization (1453.69 µg P/ml) on 14th day, whereas in LMG 7107 and LMG 7108 it was 1379.6 and 1452.04 µg P/ml on 6th day itself (Figure 3). Statistically no significant difference was observed in P-solubilization among strains in Sperber's medium except between 8 and 12 days (Figure 2), but in Pikovskaya's medium the strains differed significantly on all days (Figure 3).

In general, the bacterial activity was slow initially, which gradually increased

Table 1. Solubilization efficiency (E) of *A. halopraeferans* strains in plate assay

Strain	Sperber's medium*	Pikovskaya's medium
LMG 7107	151.14	Nil
LMG 7108	151.69	Nil
LMG 7109	150.56	Nil

*Results presented are average of three replicates.

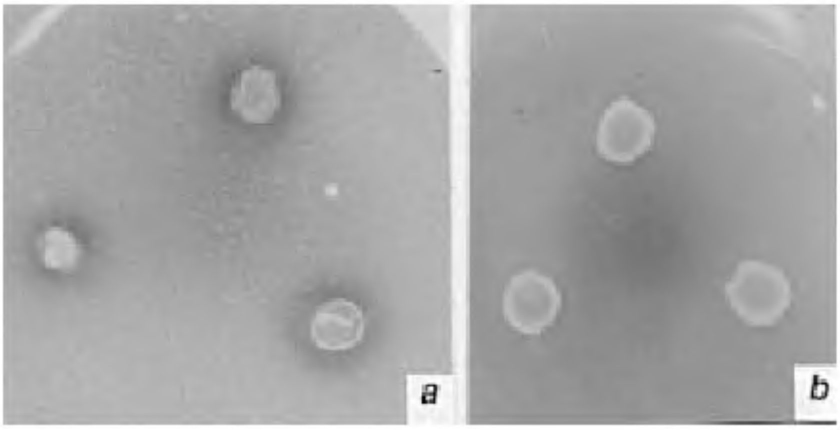

Figure 1. Plate assay for P-solubilization by *A. halopraeferans*. ***a***, Solubilization zone formed in Sperber's medium; ***b***, Colonies without any halo zone in Pikovskaya's medium.

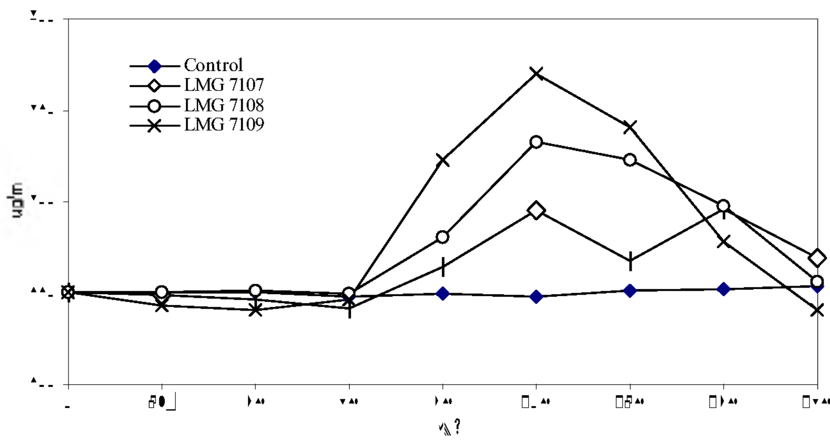

Figure 2. Change of P concentration in Sperber's medium.

in the middle and declined later. Decrease in P concentration during initial stages in Sperber's medium (Figure 2) can be attributed to the utilization of existing P for growth and development of the organism[6], in a later phase the bacteria would have started acting on the substrate for want of nutrients, thus releasing P from insoluble sources. Or, the cells after the initial shock could have utilized the available free P for metabolism and later to acclimatize to the given environment or due to substrate stress they would have solubilizing P. The better performance of bacteria in Pikovskaya's medium by all strains indicates the role played by the substrate to trigger the microbial action[7,17]. The P concentration in solution did not follow a sigmoid curve type, but some fluctuations and then increase in P concentration during later days in LMG 7108 and LMG 7109, which may be due to cell lysis and P precipitation brought about by organic metabolites[7,18,19].

There are several potential mechanisms reported for phosphate solubilization, that include modification of pH by secretion of organic acids and protons or cation dissociation[7,20–24]. In this study, pH values increased in Sperber's medium during early days and on incubation went down slowly (Figures 4 and 5). Maximum pH reduction recorded was 0.30 units in Sperber's medium and 0.53 units in Pikovskaya's medium. pH reduction was gradual, where it slowly went down and no revival was observed in later days. Results similar to this were observed by other workers[25,26], while studying cyanobacterium mediated P-solubilization.

A. halopraeferans, a non-glucose utilizing bacteria does not exhibit acidity in the presence of glucose[4]. Results obtained in the present study on glucose amended media indicate that acid production is not the only reason for P release into the media[7,27,28] and this can be related to the cation dissociation process[16]. From the above results, it can be concluded that an interesting phenomenon of P-solubilization by nitrogen fixing *A. halopraeferans* could be further studied to find out the mechanism of action or the involvement of genes. A study on the molecular mechanism would throw light on the *ps* (phosphate solubilizing) genes that could be incorporated in agriculture as *A. halopraeferans* offers traits for nitrogen fixation, phosphate solubilization and salinity tolerance.

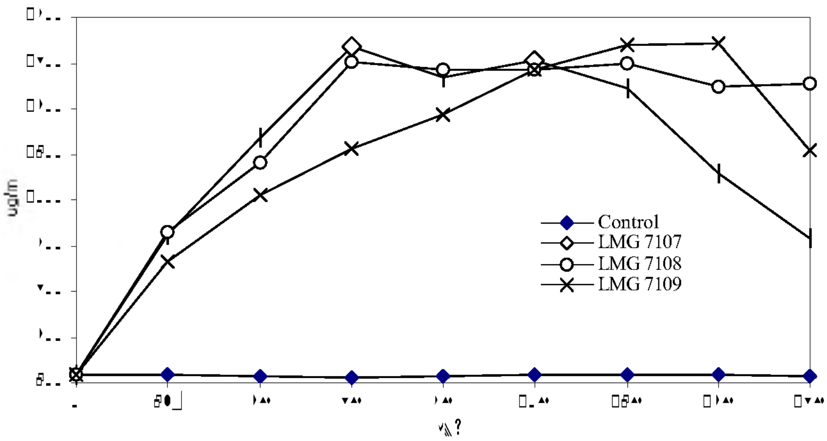

Figure 3. Change of P concentration in Pikovskaya's medium.

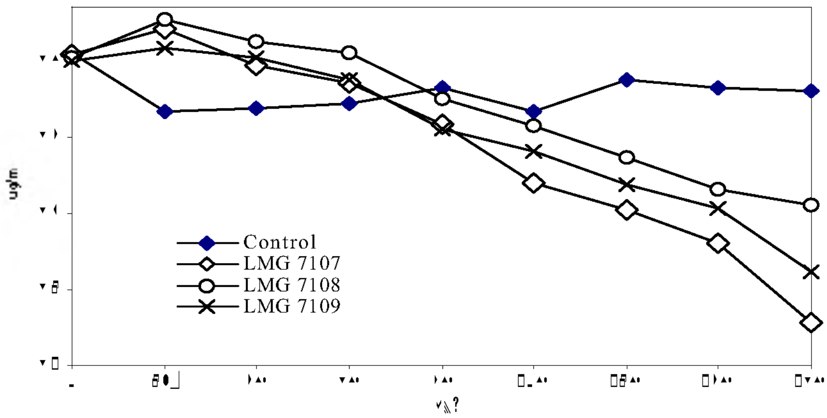

Figure 4. Change of pH in Sperber's medium.

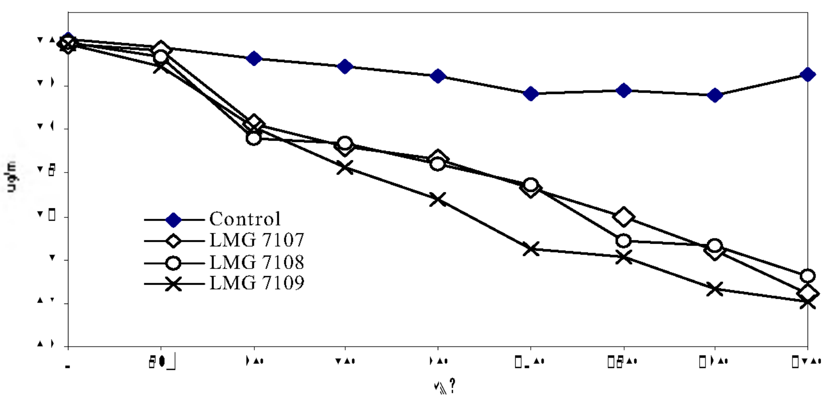

Figure 5. Change of pH in Pikovskaya's medium.

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Jellyfish ingress: A threat to the smooth operation of coastal power plants

Coastal areas are often preferred for setting up power stations due to the easy availability of sea water for condenser cooling. In such coastal power stations, there are instances of plant shutdown due to excessive accumulation of fouling debris inside the cooling circuits. In one of the power stations in UK, the quantity of fouling debris removed was about 40 tonnes per year and this was at times as high as 130 tonnes[1]. Recently, there have been a few instances, when jellyfish in large numbers entered the sea water cooling system of the Madras Atomic Power Station (MAPS) at Kalpakkam, causing plant shutdown[2]. While moderate ingress of jellyfish leads to a reduction in the plant efficiency, large arrivals may even lead to forced shut down of a power plant[2–4]. The present study deals with the ingress of jellyfish in MAPS cooling water system and its impact on the power plant operation.

MAPS consisting of two units, each of 235 MW(e) capacity is located at Kalpakkam (12°33′N and 80°11′E), 65 km south of Chennai (Figure 1) on the east coast of India. The power plant uses sea water as its condenser coolant. The sea water intake is located 420 m away from the shore and is connected through an approach jetty (Figure 1). Sea water enters the cooling water system through 16 windows (3.2 m height and 2 m width) located radially in the intake structure. From the intake point, water flows into the forebay by gravity. The sea water travels through the Travelling Water Screens (TWS) at the forebay before 12 pumps (6 for each unit) draw the sea water for condenser cooling as well as for cooling the process water heat exchangers. The TWS is made up of stainless steel mesh (20 mm pore size) with a platform to collect debris attached at an angle of 90° (Figure 1). The TWS moves vertically and takes about 12 min to complete a rotation.

Jellyfish arriving at the TWS were collected in the forebay during the period from January 1995 to December 1996. During collection, individuals arriving for a period of one hour, every alternate day were pooled. They were separated into different species and weighed using a common balance. From these data jellyfish landing was calculated and expressed as tonne/month.

Jellyfish-induced water blockage in the conduit was assessed by determining the water level difference between the forebay and the intake (head loss). The measurement was done from the platform at the respective places using a lead and line.

Three species of jellyfish were observed during the study period, namely *Crambionella stuhlmanni*, *C. buitendijki* and *Dactylometra quinquicirrha*. Among these three species, *D. quinquicirrha* was the dominant one. In 1995 and 1996, the percentages of each species of the jellyfish to the total jellyfish collected were: *D. quinquicirrha* 45 and 40%, *C. buitendijki* 34 and 36%, *C. stuhlmanni* 21