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Cadmium induced effect on growth and physiology in halophilic phosphobacteria

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Abstract: *The chosen phosphobacterial species are well tolerated to the wide range of salinity (20-40 ppt) and found to be pure marine forms. Effect of different concentrations of cadmium on the growth, phosphate solubilising activity and content of extracellular macromolecules in eight species of phosphate solubilising bacteria were carried-out in the present study at optimum level of salinity and found that these activities are found maximum at lower concentrations of cadmium and further reduction in the activities were noticed at higher concentrations. The maximum content of total sugar was noticed in S. aureus at 1 ppm level of cadmium. The content of protein was found maximum in B. megaterium and M. roseus without the addition of cadmium and also the phosphate solubilizing activity was found higher in all the chosen phosphobacterial strains with the addition of cadmium.*

Key words: *Cadmium, Extracellular macromolecules, Halophilic phosphate solubilising bacteria, Mangroves, Salinity*
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Introduction

Recent booming of industries preferably along the coastal area has affected greatly the man's physical environment. Besides drugs, antibiotics and radioactive substances, industrial wastes from such industries contain heavy metals, which are mutagenic, carcinogenic and teratogenic. Many of the heavy metals have deleterious effects on biological life (Blaudez *et al.,* 2000; Seeber *et al.,* 2002; Akermoun *et al.,* 2002). Recent discovery reveals that, the cadmium has joined with Pb and Hg posing extreme acute toxicity to biological systems (Volesky, 1990). The uptake of heavy metals, present in the industrial wastes, and the detoxification of metal ions by bacteria provide an additional mechanism of environmental protection. Microbes may play a large role in the biogeochemical cycling of toxic heavy metals also in cleaning up or remediating metal-contaminated environments (Shazia *et al.,* 2002; Zafer *et al.,* 2007; Arun Kumar and Achyuthan, 2007). Phosphobacteria or phosphate solubilizing bacteria are the most important microbes next to the nitrogen fixing microbes which plays a vital role in the phosphorous recycling in marine ecosystem. The heavy metal input through industrial wastes to the marine ecosystem would hamper the growth and activity of the phosphobacteria which in turn affect the health of the mangrove ecosystem both directly and indirectly. Hence it is of utmost importance to study the effect of cadmium on the growth, phosphate solublilising activity and extra-cellular macromolecular contents in halophilic phosphate solubilizing bacteria for the development of effective mangrove management policy.

Materials and Methods

0.5 ml of 24 hr old broth culture of identified phosphobacterial species *viz.*, *Bacillus megaterium*, *Micrococcus roseus*, *Bacillus subtilis*, *Proteus mirabilis*, *Staphylococcus aureus*, *Micrococcus luteus*, *Arthrobacter illicius* and *Enterobacter aerogenes* were maintained in our School of Marine Sciences, Alagappa University were inoculated into 4 ml of Pikovskaya's broth in 30 ml of screw cap tubes with various salinity regimes (0, 10, 20, 30, 35 and 40 g NaCl l^{-1}). All the test tubes were incubated in thermostat shaker at 37°C for 12 to 16 hr. The growth of the each bacterial species was assessed by measuring the optical density at 600 nm by using spectrophotometer (Shimadzu, Japan). Triplicates were maintained for each treatment.

Likewise the 24 hr old broth culture of identified bacterial species *viz.*, *Bacillus megaterium*, *Micrococcus roseus*, *Bacillus subtilis*, *Proteus mirabilis*, *Staphylococcus aureus*, *Micrococcus luteus*, *Arthrobacter illicius* and *Enterobacter aerogenes* were inoculated into Pikovskaya's broth with optimum salinity along with different concentration of heavy metal (cadmium) gradients (0, 0.1, 1, 10, 50 and 100 ppm). All the test tubes were incubated in a thermostat shaker at 37°C for 24 hr. After incubation, the optical density was taken at 600 nm by using spectrophotometer (Shimadzu, Japan). Remaining broth culture was subjected to centrifugation (6000 rpm) for 15 min and the supernatant was collected for the estimation of total sugars (Dubois *et al.,* 1956) and protein (Lowry *et al.,* 1951) by following standard procedures. Triplicates were maintained for each treatment.

To calculate the phosphate solubilizing activity, the bacterial culture grown in optimum salinity with different concentration of cadmium (cd) were centrifuged at 6000 rpm for 15 min. 1ml of the supernatant was taken and 10 ml of chloromolybdic acid (15.0 g of ammonium modybdate dissolved in 40 ml of distilled water and

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Table - 1: Effect of cadmium on the content of total sugars (μg l^{-1})

Name of the bacterial species	Concentration of Cd (ppm)					
	0	0.1	1	10	50	100
Bacillus megaterium	0.736	0.810	0.821	0.919	0.218	0.282
Micrococcus roseus	0.683	0.894	0.768	0.711	0.721	0.673
Bacillus subtilis	0.201	0.354	0.302	0.224	0.181	0.194
Proteus mirabilis	0.602	0.756	0.446	0.641	1.132	0.368
Staphylococcus aureus	0.807	0.508	0.120	0.938	0.744	0.823
Micrococcus luteus	0.204	0.224	0.296	0.141	0.226	0.235
Arthrobacter illicius	0.681	0.694	0.610	0.670	0.663	0.473
Enterbacter aerogenes	0.620	0.792	0.505	0.701	0.679	0.620

Values are found significance at 1% level between bacterial species and the concentration

Table - 2: Effect of cadmium on the content of extra cellular protein (mg g^{-1})

Name of the bacterial species	Concentration of Cd (ppm)					
	0	0.1	1	10	50	100
Bacillus megaterium	0.630	0.615	0.336	0.384	0.225	0.186
Micrococcus roseus	0.771	0.561	0.519	0.753	0.7160	0.249
Bacillus subtilis	0.723	1.194	0.593	0.432	0.480	0.543
Proteus mirabilis	0.261	0.354	0.624	0.762	1.005	0.981
Staphylococcus aureus	0.414	0.924	0.504	0.636	0.561	0.702
Micrococcus luteus	0.924	0.975	1.095	0.951	0.690	0.936
Arthrobacter illicius	0.396	0.585	0.444	0.408	0.561	0.567
Enterbacter aerogenes	0.441	0.345	0.546	0.534	0.522	0.612

Values are found significance at 1% level between bacterial species and the concentration

Table - 3: Effect of cadmium on the content of available phosphorus (μg l^{-1})

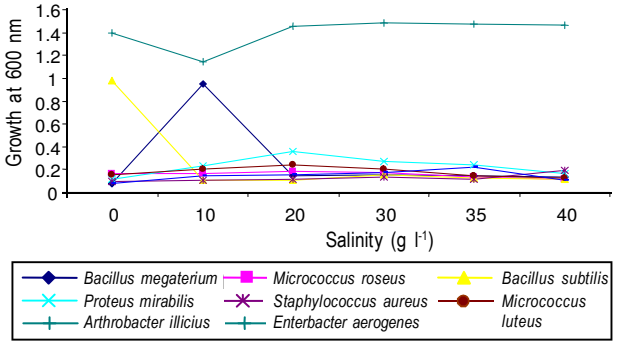
Name of the bacterial species	Concentration of Cd (ppm)					
	0	0.1	1	10	50	100
Bacillus megaterium	69	23	44	42	18	36
Micrococcus roseus	21	23	44	70	38	41
Bacillus subtilis	14	07	11	07	15	21
Proteus mirabilis	24	48	16	17	68	56
Staphylococcus aureus	68	22	23	29	70	27
Micrococcus luteus	27	15	35	20	21	39
Arthrobacter illicius	42	32	21	56	32	38
Enterbacter aerogenes	60	56	100	24	25	33

Values are found significance at 1% level between bacterial species and the concentration

added 342 ml of 12 N HCl and cooled; made up the volume to one liter with distilled water) was added. Further diluted the contents of the flask to 45 ml. To this, 0.25 ml of chlorostanous acid (2.5 g of $SnCl_2.2H_2O$ dissolved in 10 ml of conc. HCl and made up the volume to 100 ml with distilled water) was added and immediately made up to 50 ml by adding distilled water. The intensity of blue colour of the solution was measured at 600 nm by using spectrophotometer. Using the standard curve the phosphate solubilising activity of the test phosphobacteria was calculated by using the following formula: Content of available phosphorous (μg l^{-1}) = Standard value (16.66) X OD value of sample/total volume of sample X volume of sample taken for estimation (Mehta and Nautiyal, 2001). Triplicate analyses were made for each treatment. All the results were statistically analyzed for the significance by following the method of two way analysis of variance.

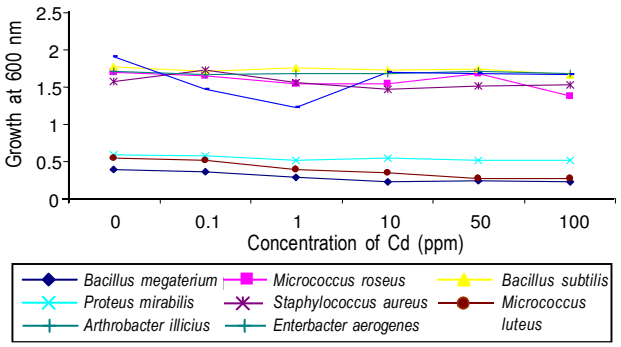
Results and Discussion

Mangroves provide unique ecological environment for diverse bacterial communities; the bacteria fill a number of niches and are fundamental to the functioning of these habitats. These bacteria largely control iron, phosphorous and sulfur dynamics and contribute to the soil and vegetation patterns (Sherman *et al.,* 1998). The present study has been initiated to find out the effect of heavy metal cadmium on the growth and phosphate solubilizing activity of chosen microbial isolates. The growth of *Micrococcus roseus, Proteus mirabilis* and *Micrococcus luteus* were found maximum at 20 ppt salinity. At 30 ppt salinity level, the growth of *Bacillus megaterium, B. subtilis* and *Arthrobacter illicius* were found higher. The growth of *Staphylococcus aureus* was increased with the increasing level of salinity and found better at 40 ppt salinity level. However, the growth of *Enterobacter aerogenes* was found better



Values are found significance at 1% level between bacterial species and the salinity levels

Fig. 1: Effect of salinity on the growth of phosphobacteria



Values are found significance at 1% level between bacterial species and different concentration of cadmium

Fig. 2: Effect of cadmium on the growth of phosphobacteria

at 35 ppt salinity (Fig. 1). However, the growth of the tested bacterial species was found reduced even at 0.1ppm concentration of cadmium (Fig. 2).

The present study observed that, the isolated phosphobacterial species from the mangrove ecosystem have wide range of saline tolerance. However, the growth and phosphate solubilising activity was decreased with the increasing concentration of heavy metals used in the present study, particularly at higher salinity levels the inhibition of growth and phosphate solubilising activity of isolated phosphobacterial species was more pronounced at higher salinity levels with lower concentrations of heavy metal. Onishi *et al.* (1984) reported that the reduction in the toxicity of cadmium was noticed in moderately halophilic *Pseudomonas* sp., when the sodium chloride concentration was increased from 1-3 M. It is obvious that, metal pollutants become more complex in marine ecosystem. Ravikumar *et al.* (2000) reported that, the higher pH levels are shown to enhance the toxicity of the heavy metals (Cd and Hg) whereas, the addition of NaCl is found to reduce the toxicity of Cd and Hg to the free-living nitrogen fixing *Azotobacter vinelandii* isolated from Pichavaram mangrove forest (South east coast of India). Zafer *et al.* (2007) reported the accumulation of heavy metals (Pb, Cd, Cu and Ni) in water, sediments and fishes and the heavy metal accumulation in certain marine animals was also reported (Arun Kumar and Achyuthan, 2007). The content of total sugar was found maximum in the cadmium treated cells at varied concentration levels. The maximum content of total sugar was noticed in *S. aureus* at 1 ppm level of cadmium (Table 1). The content of protein was found maximum in *B. megaterium* and *M. roseus* without the addition of cadmium. The other chosen phosphobacterial strains showed high level of extracelluar protein with the addition of cadmium in different concentrations. The halophilic phosphobacterial isolates *B. subtilis* treated with cadmium showed high level of extra cellular protein at 0.1 ppm level of cadmium (Table 2). And also the phosphate solubilizing activity was found higher in all the chosen phosphobacterial strains with the addition of cadmium (Table 3). Doyle *et al.* (1975). The present study also found that, the content of total sugars, protein and phosphate solubilising activity in chosen phosphobacterial isolates were found maximum in the heavy metal treated medium. The enhancements in the level of these biochemical constituents might play a role in the responses of cells to toxic heavy metals (Ravikumar *et al.*, 2002). Wu *et al.* (1995) reported that some common proteins are synthesized when the cyanobacteria is exposed to heavy metal stress. Ravikumar *et al.* (2007) reported that, the content of protein and total sugars were increased by the higher concentrations of heavy metals (Hg and Zn) whereas decreased trend was noticed in lower concentrations of heavy metals. It is concluded from the present study that, all the isolates from the mangrove environment are purely marine forms. The diversity and activity have been drastically inhibited with the discharge of industrial effluents in to the mangrove ecosystem and hence productivity of such ecosystem have expected to loss in near future.

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