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DYNAMICS AND DIVERSITY OF PHOSPHATE MINERALIZING BACTERIA IN THE CORAL REEFS OF GULF OF MANNAR

Kannapiran E[1] and [2]J.Ravindran

1. Dept. of Zoology, DDE, Alagappa University, Karaikudi, Tamil Nadu -630 003, India
2. National Institute of Oceanography, Regional Centre, Council of Scientific and Industrial Research (CSIR), Dr. Salim Ali Road, PB. No. 1913, Kochi – 682 018, India

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Abstract

Phosphatase Producing Bacteria (PPB) and Inorganic Phosphate Solubilizing Bacteria (IPSB) are important to reef nutrition. These microbes and phosphate concentration play a significant role in the productivity of coral reef ecosystems. A study was conducted in Gulf of Mannar coral reef ecosystem to understand the diversity of these groups of bacteria and their competence in mineralizing the phosphate. The PPB isolates were identified under six genera i.e. *Bacillus, Pseudomonas, Micrococcus*, *Vibrio, Arthrobacter* and *Brevibacterium*. Likewise, the IPSB isolates were also identified that belong to six genera i.e. *Bacillus*, *Arthrobacter*, *Pseudomonas*, *Flavobacterium, Flavomonas* and *Micrococcus*. Among the PPB and IPSB strains, *Bacillus* represented more number of species than others. PPB exhibited maximum activity between pH 8 and 9 and the lowest was at pH 6. Among the phosphatase producers *Bacillus cereus* (546) showed maximum activity (0.333 mmol/l P) at pH 8. In general, the phosphatase activity in most of the *Bacillus* species varied with reference to different pH. The species *Bacillus megaterium* (573) showed highest phosphate solubilizing activity (0.906 mmol/l P) by producing 2-ketogluconic acid. The production of organic acids and phosphatase enzymes by these bacterial groups are responsible for the conversion of insoluble inorganic and organic phosphates into soluble forms which are available for the reef organisms.

Introduction

Coral reefs are known for their lofty productivity, although they reside in low nutrient oligotrophic water. Phosphorus is an essential element for growth and development of plants and animals which can influence the reef productivity. The concentration of organic phosphorus in the water column over the coral reef is usually high and it is even 5 times greater than that of the inorganic phosphorus [1, 2]. Bulk of phosphorus compounds in the coral reef environment is macromolecular and not readily accessible for absorption into the reef organisms. So, the organic phosphorus compounds are to be preconditioned by extracellular bacterial enzymes called 'phosphatases' for making them available to the nutrient cycles thereby to the corals and other reef organisms. Phosphatases are mainly of bacterial origin, and to a lesser extent they may originate from other reef organisms [3, 4]. Phosphatase activity is suggested as the possible factor in the decomposition of organic compounds and subsequent release of soluble inorganic phosphorus in to the marine environment [4].

The marine organisms acquire P as phosphate anions. However, phosphate anions are extremely reactive and may be immobilized through precipitation with cations such as $Ca^{2+,}$ Mg^{2+}, Fe^{3+} and Al^{3+}. In these forms, P is highly insoluble and unavailable to the organisms. As the results, the amount available to organism is usually a small proportion of this total. Several scientists have reported the ability of different bacterial species to solubilize insoluble inorganic phosphate compounds, such as tricalcium phosphate, dicalcium phosphate, hydroxyapatite, and rock phosphate [5]. The mechanism of phosphate solubilization by bacteria is normally due to the production of organic acids [6]. The abundance, occurrence and phosphate mineralizing activity of these groups of bacteria are influenced by environmental conditions [7, 8]. Coral reef waters are low in phosphate concentration and it seems reasonable to evaluate the ability of bacteria to mineralize the phosphates in water and sediments. Therefore, the present investigation was carried out to understand the diversity of these microbes and their efficiency in phosphate mineralization.

Materials and methods

This study was carried out from the Gulf of Mannar coral reef ecosystem (Fig.1). The Gulf of Mannar is situated in the southeast part of Indian peninsula. It extends from the Adam's bridge of Rameshwaram Island to Cape Comorin. There are 21 reef islands forming the 'archipelago' in this Gulf, lying between Rameshwaram and Tuticorin. These islands are surrounded by coral reefs in a discontinuous manner over a distance of about 140 km [9]**.** The reefs are mostly fringing reefs arising from the shallow seafloor and the depth is approximately 6 meters. This area is incredible for its richness and variety of flora and fauna and also sustains a good fishery industry. For the present investigation water, sediment and sediment deposited on dead corals were collected, every month

for a period of one year (January to December 2007) from the coral reef islands of Gulf of Mannar i.e. Manoli (Station 1) (9^o 14' N lat and 79^o 7'E long), Hare (Station 2) (9^o 12'N lat. and 79^o 5'E long) islands.

The Modified heterotrophic plate count agar medium (Consisted of: Peptone 3.0g, Potassium dihydrogen orthophosphate 0.2g, Magnesium sulphate 0.05g, Ferric chloride 0.001g, Soluble Casein 0.5g, Agar 15g and Distilled water 1000ml) was used for the enumeration of PPB from the collected samples [10]. After autoclaving, pH of the medium was adjusted to 7.2. Then 10ml of 0.2 micron filter sterilized 1% aqueous solution of phenolphthalene diphosphate (sodium salt) was added. The hydroxy apatite medium (Consisted of: Yeast extract 0.2g, Ammonium sulphate 0.5g, Magnesium sulphate 0.1g, Potassium chloride 0.2g, Dextrose 10g, Soil extract 200ml, Distilled water 800ml, Trace element 1ml, Agar 18g and pH 7.2±0.1) [11] was used for the enumeration of Inorganic Phosphate Solubilizing Bacteria (IPSB). After sterilization 60ml of 10% calcium chloride and 40ml of 10% Potassium dihydrogen phosphate were added to the above medium. Sterile 1N Sodium hydroxide was also added until the formation of white insoluble calcium phosphate. The pour plate method was followed for the enumeration and isolation of both groups of bacteria.

The inoculated plates were incubated at 28 ± 1^oC in an inverted position. The phosphatase positive colonies appeared pink colour when they are exposed to ammonia vapour. The IPSB colonies formed a clear halo zone around them. The respective bacterial colonies were counted and expressed as colony forming units (CFU) per ml of water and gram of sediment. The representative colonies based on their morphology were purified and identified up to species level based on the fatty acid profile of the bacteria by using Microbial Identification System (MIS) (Hewlett Packard 5890, USA) [12] .

Phosphatase activity

The phosphatase activity (mineralization) was measured by using para-nitrophenyl phosphate (p-NPP) as dissolved organic substrate. When p-NPP loses its phosphate and becomes para-nitrophenol (p-NP), the solution turns yellow. Therefore, the activity of the enzyme is measured by following the colour development of the solution.

The identified strains were tested for their ability to produce phosphatase enzyme at different pH ranging from acidic to alkaline level (6-9). The bacterial strains were cultured in nutrient broth for 72 hours. One millilitre of the broth was then transferred to 15ml test tubes containing 5ml pre-sterilized basal medium, to which 2ml of filter sterilized acetate and tris buffer was added respectively to maintain the acidic and alkaline pH. To this 0.5ml filter-sterilized solution of the substrate p-nitrophenyl phosphate (0.005 M) was added. A corresponding tube was prepared with

0.5ml of substrate and 1ml of distilled water was added in place of the bacterial culture and maintained as control. The tubes were incubated for 72 hours at 28^{o}C (±1^{o}C). After incubation, 1ml of 0.2N Sodium carbonate was added; centrifuged at 3000 rpm for 15 minutes and p-nitrophenyl phosphate metabolite was measured at 418nm using a spectrophotometer. The values of the control tubes were subtracted from the experimental ones to calculate the amount of nitrophenol liberated. One mole of nitrophenol is equivalent to 1 atom of phosphorus. The activity was expressed as mmol/l P. All experiments, regardless of the microorganism or organic acid determination were repeated twice. Results presented are the means of all replicates from both repetitions. The inorganic phosphate and total phosphate concentration were estimated in the water sample collected from the study area [13].

Phosphate solubilizing activity

The identified strains were tested for their ability to dissolve precipitated inorganic phosphate. The bacterial strains grown on solid agar medium containing 1.5% nutrient agar were inoculated into nutrient broth and incubated for 72 hours at room temperature (28±2^{o}C). One millilitre of the broth was then transferred to 3ml basal medium containing the following ingredients: Yeast extract 2g, Dextrose 10g, Peptone 2.5g, Distilled water 1000ml and pH 8±0.2. To each tube containing one culture, 0.5ml of 1% suspension of calcium hydroxy apatite was added under constant stirring. Tubes containing 0.5ml 1% suspension of calcium hydroxy apatite without bacterial culture was maintained as control. A separate control was maintained for each of the experimental culture and the cultures were incubated for 72 hours at room temperature (28±1^{o}C).

At the termination of experiment, both the experimental and control cultures were centrifuged at 3000 rpm for 15 minutes. The reactive phosphate was determined colorimetrically [13]. The difference in phosphate concentration of the experimental and control tubes was taken as the amount of phosphate liberated by the cultures from the hydroxyl apatite. The phosphate solubilization activity was expressed as mmol/l P. The organic acid produced by *Bacillus megaterium* was extracted by using Amberlite XAD-2 resins and identified as 2-ketogluconic acid. Since, this strain showed maximum phosphate solubilizing activity.

Results and Discussion

The biological decomposition of organic phosphorus in the marine environment is considered as the result of bacterial action and rate of decomposition of organic phosphorus by bacteria depend on the density of the phosphatase producing bacterial population [14]. This has been demonstrated in coastal waters [15], as well as in coral reef environment [16]. Higher bacterial population ($45x10^{4}$ CFU ml^{-1} and $54x10^{6}$ $CFUg^{-1}$) was reported in Pitchavaram mangrove environment [17]. In the present study, phosphatase producing bacterial population density ranged

from 3.5 to 98.4x10^4 CFU ml^{-1} in water, 5.5 to 87x10^5 CFU g^{-1} in sediments and 5.5 to 51 x 10^5 CFU g^{-1} in sediment deposited on dead corals at station 1(Manoli island); from 3.5 to 82.5x10^4 CFU^{-1} in water, from 5 to 63 x 10^5 CFU g^{-1} in sediments and 3 to 39.5 x10^5 CFU g^{-1} in sediments deposited on dead corals at station 2 (Hare island). When compared to the PPB population density from the east coast of India the present study showed slightly higher PPB population [17, 18]. Further, IPSB population density in coral reef islands ranged from 10.4 to 38.5 x10^4CFU ml^{-1} in water, 7.5 to 56.5 x10^5 CFU g^{-1} in sediments, 8.5 to 76.5X10^5 g^{-1} in sediments deposited on dead corals at station 1; from 8.3 to 33.5x10^4 CFU m^{-1} in water, from 6 to 49.5 x 10^5 CFU g^{-1} in sediments, 6 to 48x10^5 CFU g^{-1} in sediment deposited on dead corals at station 2. The fluctuations in population density of phosphatase producing bacteria and inorganic phosphate solubilizing bacteria are depicted in Fig. 1& 2.

Higher PPB and IPSB population density was observed during monsoon season in water as a result of high nutrient concentration in the study area due to land run-off (Fig. 3). The monsoonal rain enriches the coastal environment with essential nutrients**.** In Gulf of Mannar, strong winds and waves during monsoon season resuspended the bottom sediments along with dissolved and particulate nutrients throughout the water column. Subsequently, bacteria rapidly grew and increased their population density by utilizing the added nutrients. Similar observation was also reported from the Great Barrier Reef of Australia. The major source of nutrients to the Great Barrier Reef lagoon and reef productivity is from sediment resuspension [19].

There is a significant positive correlation between the inorganic phosphate concentration and density of phosphatase producing bacterial population ($r = 0.843$ at station 1 and $r = 0.874$ at station 2), indicating the release of soluble inorganic phosphorus is primarily controlled by microbial populations. It was interesting to note that the pH remained alkaline throughout the study period (Fig. 4). During monsoon season, the pH was low alkaline and in summer season, it was high alkaline. The higher alkaline pH was due to the removal of CO_2 by the photosynthesizing coral community. The lower alkaline pH was due to the dilution of seawater by freshwater inflow during monsoon season. The higher alkaline pH facilitates calcium carbonate precipitation and subsequent formation of interstitial lime paste; most essential for the coral development [20].

In sediment higher PPB and IPSB populations were recorded during post monsoon due to large amount of dissolved and particulate nutrients introduced into the study area through land run-off during monsoon season. Consequently the major portion of these nutrients gradually settled to the bottom sediments, thereby accounting for the higher nutrient contents during post monsoon season. Further, bacterial population is established in the upper few millimetres of oxidized sediments. The long term effect of this population is expected to accumulate and hold an additional

pool of phosphorus in the sediments. If the sediments are not disturbed, this pool stands isolated from the water. If the surface sediments are scoured by tides, waves or rain, the microorganisms and phosphate will be dispersed to the overlying water column. Addition of these essential nutrients stimulated bacterial action [19]. In many reef waters, corals on the reef flats support bacterial growth by extruding mucus. In this study also, higher bacterial number was observed at Manoli Island (station 1) due to the release of mucus from a variety of corals than at Hare Island (station 2) where no such coral diversity and distribution was noticed.

Bacterial phosphatases are the enzymes that promote the degradation of organic phosphorus compounds into orthophosphates and it has crucial role in the nutrient dynamics of the coral reef and many associated aquatic ecosystems. Phosphatase activity primarily depends on the type and concentration of natural substrates and other physiochemical factors such as temperature, ionic strength, pH and metal ions [21, 22]. The phosphatase production starts when the phosphate pool is depleted. Therefore, it has been suggested that the phosphatase activity in a water body is an index of phosphorus nutritional status of the system [5, 23]. In the present study also, higher phosphatase producing bacterial population density and higher amount of total phosphate in the water column was observed during monsoon season.

The PPB isolates were identified under 6 genera of *Bacillus* (6 species), *Pseudomonas* (3 species), *Micrococcus* (3 species) and one species each of *Vibrio, Arthrobacter* and *Brevibacterium*. In general, among PPB isolates, *Bacillus* was represented with more number of species followed by *Pseudomonas, Arthrobacter, Micrococcus, Flavobacterium, Flavomonas, Vibrio* and *Brevibacterium* (Table 1). All the strains were found to decompose the organic phosphorus. Of all the species tested, *Bacillus* species showed the maximum activity. The present findings are in conformity with earlier report [24].

Previous studies have reported that bacterial strains had the ability to decompose organic phosphorus by producing phosphatase enzyme at different pH (6 to 9), quite a few groups displayed varying phosphatase activity over the tested pH range. Different groups of bacteria showed maximum hydrolyzing activity with phosphatase either at alkaline or acidic pH and hence two categories viz. alkaline and acidic phosphatases have been recognized [25]. In this study, phosphatase activity of different isolates showed maximum activity in different alkaline pH condition (Fig. 5). Strong phosphatase activity was recorded in pH 8 and 9. Majority of the *Bacillus* species showed high phosphatase activity but the proportion of the phosphatase activity varied with varying species and with varying pH. *Bacillus cereus* (546) isolated from the sediment sample showed the highest activity (0.333 mmol/l P) whereas the lowest activity was shown by *Brevibacterium acetylicum* (91) (0.068 mmol/l P).

The insoluble inorganic phosphate solubilizing bacterial (IPSB) isolates were identified under following genera: *Bacillus* (7 species), *Arthrobacter* (3 species), *Pseudomonas (*2 species) and one species each of *Flavobacterium, Flavomonasand* and *Micrococcus* (Table 1). Inorganic phosphate solubilizing bacteria isolated from the coral reef environment were tested for their ability to solubilize insoluble inorganic phosphate. *Bacillus* species are capable of solubilizing the insoluble inorganic phosphate at a range of (0.635 – 0.906 mmol/l P). Next to *Bacillus*, species of *Pseudomonas* showed moderate activity. (0.625-0.635 mmol/l P) *Flavobacterium, Micrococcus* and *Arthrobacter* showed medium activity (0.453-0.513 mmol/l P). *Flavomonas* (98) showed meagre activity (0.3 mmol/l P) (Fig. 6).

Mechanisms of phosphate solubilization in nature can vary due to the cumulative action of many factors such as pH, oxygen concentration, wind velocity, wave action, water flow, organic matter content, carbohydrate level, etc [26]**.** In the present study, all the strains showed activity under aerobic culture conditions but the proportion of solubilizing activity varied with the varying species. The strain of *Bacillus megaterium* (573) isolated from the sediment samples showed very high activity (0.906 mmol/l P). It is clear that the activity of some bacterial strains *in vitro* is very promising in solubilizing the insoluble phosphates (Fig.6). In the present study, organic acid produced by *Bacillus megaterium* was identified as 2-ketogluconic acid. This would help in chelating the cations, thus preventing the precipitation of phosphate ions. The earlier workers also reported that oxalic, succinic and 2-ketogluconic acids are produced by the isolates of *Bacillus* and *Streptococcus* [30, 6, 8, 26].

Concluding Remarks

In conclusion, we demonstrated bacterial phosphatase and phosphate-solubilizing activity associated with coral reef ecosystem. This is the first report on PPB and IPSB associated with coral reefs of Gulf of Mannar and their phosphate-mineralizing potential. In summary, these microbes mineralized the organic and insoluble inorganic phosphate by producing phosphatase and organic acid thus facilitates the reef community to acquire soluble phosphorus, which is indispensable for the reef development where low concentrations of phosphorus causes various limitations.

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References

[1] Atkinson, M.J., 1981. Phosphate flux as a measure of net coral reef flat productivity. Proc. Fourth. Inter. Coral Reef Symp., Manila, 1, 417-418.

[2] Sorokin, 1992. Phosphorus metabolism in coral reef communities: exchange between the water column and sediment biotopes. Hydrobiol., 242, 105-114.

[3] Atkinson, M.J., 1987a. Alkaline phosphatase activity of coral reef benthos. Coral Reefs, 6(2), 59-62.

[4] Atkinson, M.J., 1987b. Rates of phosphate uptake by coral reef fiat communities. Limnol. Oceanogr., 32(2), 426-435.

[5] Mudryk, Z.J., 2004. Decomposition of organic and solubilisation of inorganic phosphorus compounds by bacteria isolated from a marine sandy beach. Mar. Biol., 145, 1227-1234.

[6] Chen, Y.P., Rekha, P.D., Arun, A.B., Shen, F.T., Lai, W.A., Young, C.C., 2006. Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. Appl. Soil Ecol., 34, 33-41.

[7] Kobori, H., Taga, 1980. Extracellular alkaline phosphatase from marine bacteria: purification and properties of extracellular phosphatase from a marine *Pseudomonas sp*. Can. J. Microbiol., 26, 833-838.

[8] Wright, A.L., Reddy, K.R., 2001. Phosphorus loading effects on extracellular enzyme activity in everglades wetland soils. Soil. Sci. Soc. Am. J*., 65*, 588-595.

[9] Kannapiran, E., Kannan, L., Purushothaman, A., Thangarajdou, T., 2008. Physico-chemical and microbial characteristics of the coral reef environment of the Gulf of Mannar marine biosphere reserve, India. J. Env. Biol. 29(2), 215-222.

[10] Barber, M., Kuper, S.W.A., 1951. Identification of *Staphylococcus pyrogens* by the phosphatase reaction. J. Path. Bact., 63, 65-69.

[11] Ayyakkannu, K., Chandramohan, D., 1971. Occurrence and distribution of phosphate solubilizing bacteria and phosphatase in marine sediments of Portonovo. Mar. Biol., 11, 201-205.

[12] Gunasekaran, P., Ajaykumar, R., Prabakaran, S., 1991. Identification of bacterial strains by Microbial Identification System. In: Modern Approaches in Bioprocess Technology of Enzymes and Fuels. Madurai Kamaraj University, Madurai, p, 60.

[13] Strickland, J.D.H., Parsons, T.R., 1972. A practical handbook of seawater analysis. Bull. Fish Res. Bd., Canada, No.167, p 310.

[14] Kobori, H., Taga, N., Simidu, U., 1979. Properties and generic composition of phospatase producing bacteria in coastal and Oceanic sea water. Bull. Jpn. Soc. Fish., 45(11), 1429-1433.

[15] Venkateswaran, K., Natarajan, R., 1984. Role of phosphatase in mineralization of organic phosphorus in Portonovo coastal waters. Indian J. Mar. Sci., 13, 85-87.

[16] Dunlap, W.C., 1985. Measurement of extracellular phosphatase activity in reef waters of the Central Great Barrier Reef, Australia. Proc. Fifth Int. Coral Reef Symp., Tahiti, 3, 451-456.

[17] Venkateswaran, K., Natarajan, R., 1983. Seasonal distribution of phosphate solubilizing bacteria and phosphatase producing bacteria in Porto Novo waters. Ind. J. Mar. Sci., 12, 213-217.

[18] Seshadri, S., Ignacimuthu, S., 2002. Variations in heterotrophic and phosphate solubilising bacteria from Chennai, southeast coast of India. Ind. J. Mar. Sci., 31 (1), 69-72.

[19] Chongprasith, P., 1992. Nutrient release and nitrogen transformation resulting from resuspension of Great Barrier Reef shelf sediments, James Cook University, Townsville, p. 274.

[20] Vacelet, E., 1984. Microbiology in coral reefs. Oceanis, 10(1), 85-97.

[21] Mc Comb RB., Bowers GN, Posen S. 1979. Alkaline phosphatases. Plenum Press, New York, p, 986.

[22] Srividya, S., Soumya, S., Pooja, K., 2009. Influence of environmental factors and salinity on phosphate solubilization by a newly isolated *Aspergillus niger* F7 from agricultural soil. Afr. J. Biotechnol., 8 (9), 1864-1870.

[23] Rodriguez, H., Fraga, R., Gonzalez, T., Bashan, Y., 2006. Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. Plant. Soil., 287, 15–21.

[24] Niewolak, S., Korycka, A., 1976. Solubilization of basic clay by microorganisms in fertilized lakes. Pol. Arch. Hydrobiol., 13, 25-52.

[25] Jansson, M., Olssonl, H., Pettersson, K., 1988. Phosphatases: Origin, characteristics and Function in lakes. Hydrobiologia, 170, 157-175.

[26] Fleischer, S., Bengtsson M, Johansson, G., 1988. Mechanism and aerobic Fe (III) – P solubilisation of the sediment water Interface. Verh. Internat. Verein. Limnol., 23: 1825-1829.

1. FIGURES AND TABLE CAPTION PAGE

Fig.1. PPB density in Water (W), Sediments (S) and Dead coral sediments (DCS) recorded from January to December 2007 at station 1 and 2

Fig.2. IPSB density in Water (W), Sediments (S) and Dead coral sediments (DCS) recorded from January to December 2007 at station 1and 2

Fig.3. Inorganic phosphate and total phosphate profile of the study area

Fig.4. pH profile of the study area

Fig.5. Phosphatase activity of PPB isolated from the Coral reef islands of Gulf of Mannar

Fig.6. Phosphate Solubilizing activity of IPSB from the Coral reef islands of Gulf of Mannar

Table 1. Species composition of Phosphate Solubilizing and Phosphatase producing bacterial strains

Phosphate Solubilizing Bacteria		Phosphatase Producing bacteria	
Strain No.	**Species**	**Strain No.**	**Species**
98	*Flavomonas oryzihabitans*	546	*Bacillus cereus*
570	*Arthrobacter ilicis*	149	*B. sphaericus*
21	*Artrobacter viscosus*	549	*B. fredenreichi*
95	*Arthrobacter ramosus*	153	*B. sphaericus*
55	*Micrococcus agilis*	204	*B. sphaericus*
634	*Flavobacterium aquatile*	354	*B cereus*
536	*Pseudomonas mesophilica*	361	*Psedomonas mendocina*
532	*Psedomonas pseudoalcaligenses*	624	*P. stutzeri*
93	*B. filicolonicus*	601	*P. caryophylli*
548	*B. cereus*	146	*Micrococcus varians*
76	*B. sphaericus*	135	*M. luteus*
34	*B. laterosporus*	196	*M. luteus*
611	*B. circulans*	543	*Arthrobacter aurescens*
584	*B. cereus*	228	*Vibrio furnissi*
573	*Bacillus megaterium*	91	*Breevibacterium acetylicum*

Figures:

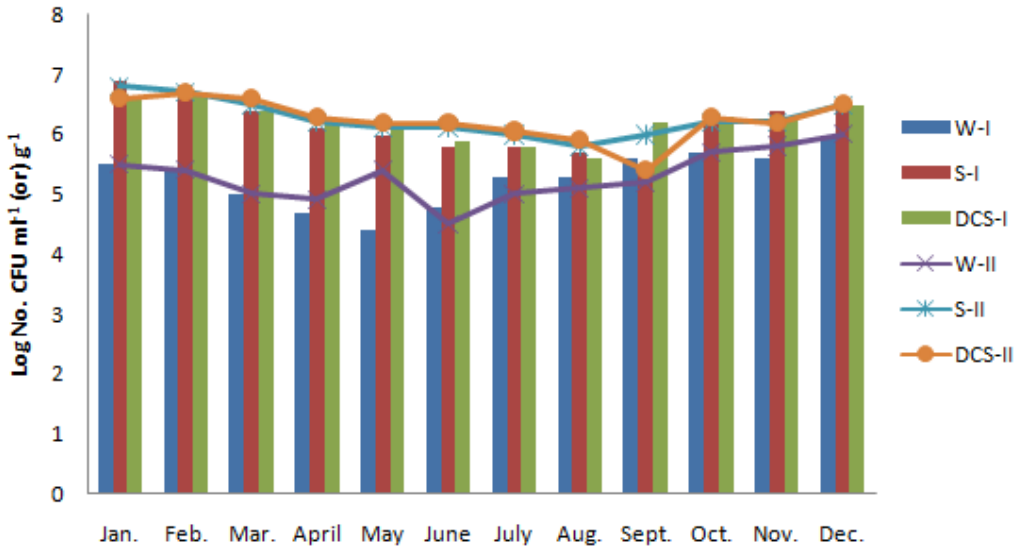

Fig. 1

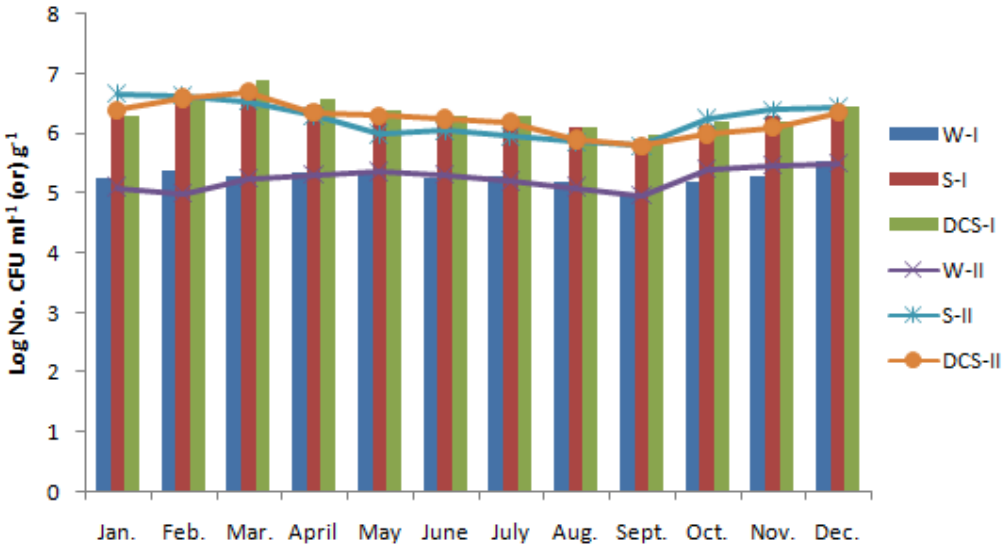

Fig. 2


µM P
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
J F M A M J J A S O N D
TP-I
TP-II
IP -I
IP-II

Fig. 3

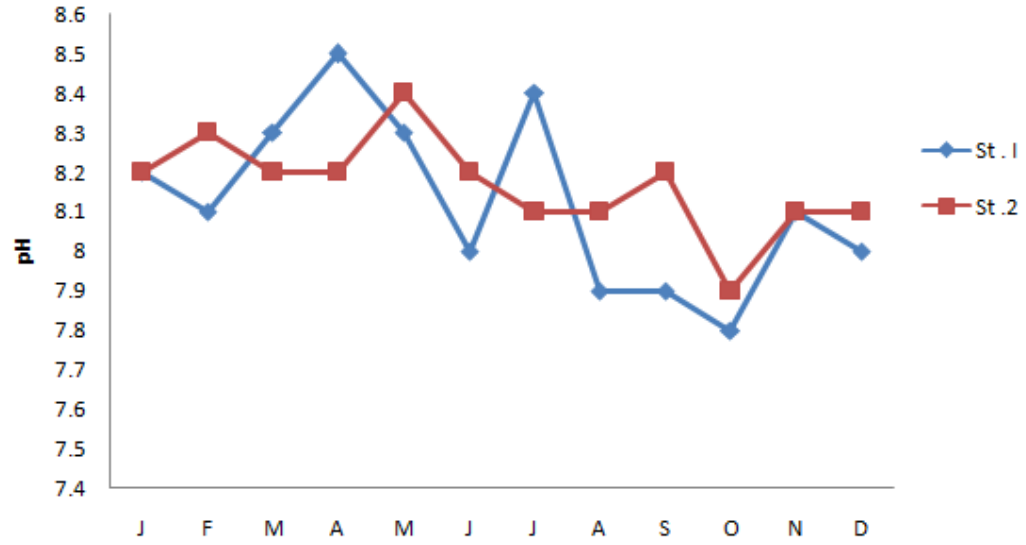
pH
8.6
8.5
8.4
8.3
8.2
8.1
8
7.9
7.8
7.7
7.6
7.5
7.4
J F M A M J J A S O N D
St . I
St .2

Fig. 4

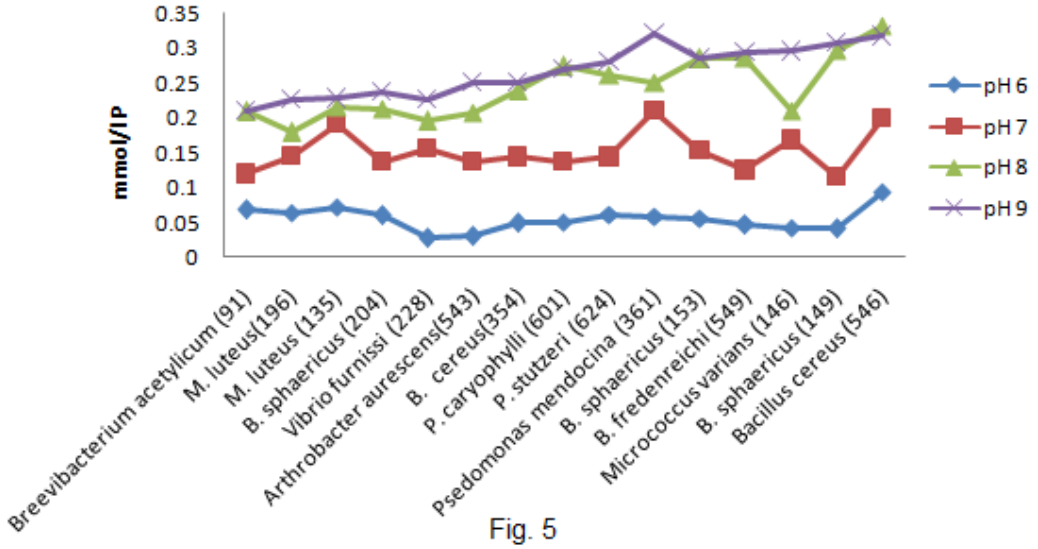
mmol/l P
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
pH 6
pH 7
pH 8
pH 9
Breevibacterium acetylicum (91)
M. luteus(196)
M. luteus (135)
B. sphaericus (204)
Vibrio furnissi (228)
Arthrobacter aurescens(543)
B. cereus(354)
P. caryophylli (601)
P. stutzeri (624)
Psedomonas mendocina (361)
B. sphaericus (153)
B. fredenreichi(549)
Micrococcus varians (146)
B. sphaericus (149)
Bacillus cereus (546)

Fig. 5

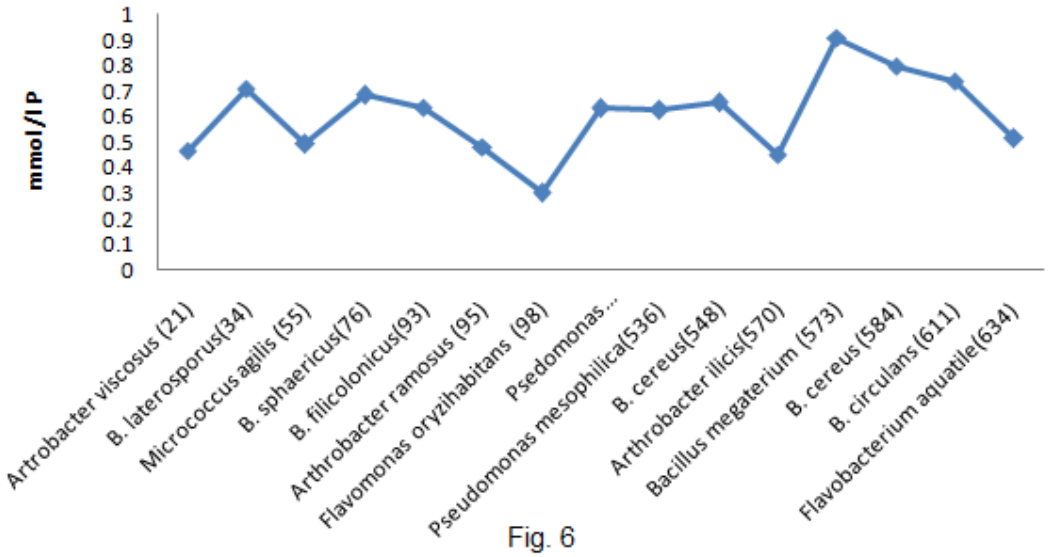
mmol/l P
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Artrobacter viscosus (21)
B. laterosporus(34)
Micrococcus agilis (55)
B. sphaericus(76)
B. filicolonicus(93)
Arthrobacter ramosus (95)
Flavomonas oryzihabitans (98)
Psedomonas...
Pseudomonas mesophilica(536)
B. cereus(548)
Arthrobacter ilicis(570)
Bacillus megaterium (573)
B. cereus (584)
B. circulans (611)
Flavobacterium aquatile(634)

Fig. 6