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Microbial biodiversity along Purnagad rocky shore, Ratnagiri

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ABSTRACT *Analysis of intertidal zone sea water samples collected during winter along the Purnagad coast, was carried out. Physicochemical parameters were analysed on spot while microbiological analysis were carried out in the laboratory. Forty three different types of colonies were isolated from ten spots, covering an area of 10 Kms. Five efficient phosphate solubilisers bacterial strains were selected out of the total isolates, based on their ability to form clear zone on Pikovskaya's agar medium and their phosphate solubilising activities were calculated. Pigmented colonies were isolated from water samples of most of the spots. Spot P7 showed presence of abundant and varied pigmented bacterial cultures. Three spots showed presence of bacteriophages.*

Introduction:
The search of new microorganisms aids in understanding the ecosystem and provides opportunities to discover new compounds of commercial importance. Research into marine microorganisms and their metabolites has thus become a major task in the search for novel pharmaceuticals. Hence marine microorganisms are attractive to researchers because they can potentially produce compounds with unique biological properties.

Until now, marine Streptomyces, Pseudomonas, Pseudoalteromonas, Bacillus, Vibrio and Cytophaga isolated from seawater, sediments, algae, and marine invertebrates are known to produce bioactive agents. They are able to produce indole derivatives (quinines and violacein), alkaloids (prodiginines and tambjamines), polyenes, macrolides, peptides, and terpenoids (Soliev et al., 2011). Application of marine bacteria in nanotechnology has also been described (Sharma et al., 2012).

Phosphorus (P) deficiency is a major constraint for crop production. Plants absorb inorganic form of P which acts as an essential element for plant growth and development. The level of inorganic P is very low in the soil and the available P is in insoluble form. The phenomena of fixation and precipitation of P in soil is generally highly dependent on pH and soil type. Thus, in acid soils, phosphorus is fixed by free oxides and hydroxides of aluminum and iron, while in alkaline soils it is fixed by calcium. Bacteria, fungi and actinomyces present in the soil convert insoluble P into soluble form for plant growth. Among these, bacteria are most predominant. The beneficial effects of phosphate solubilizing bacteria on crops have been well documented (Richardson et al., 2001).

Although the most abundant marine biological entities, bacteriophages are uncharacterized because few of their hosts have been cultivated. There is nearly no information on the distribution and abundance of specific natural marine phage-host systems (PHS) and their genetic relationship to other phages in the marine environment.

Marine phages are the main mortality factor for marine bacterioplankton. Most marine virus particles are believed to infect bacteria and it has been estimated that 10 to 20% of planktonic marine bacteria are lysed by viruses (phages) per day (Suttle, 1994). The use of phages to control infections in aquatic environment, such as fish diseases, seems to be particularly promising. In fact, the potential use of phage therapy to control disease in aquaculture systems has been demonstrated in some studies (Imbeault et al., 2006; Karunasagar et al., 2007; Nakai et al., 1999; Vinod et al., 2006)

In the present investigation, we report for the first time the bacterial biodiversity as well as the abundance of bacteriophages from rocky shore of Purnagad.

Materials and methods:
Sampling site
The coastal belt of Ratnagiri has rocky as well as sandy beach. There are number of invaginations in the main land which are called the creeks. The stretch being abundant in mangroves form a very rich ecosystem. The site of present study i.e. Purnagad, has a sandy stretch followed by rocky patch of about 10 Kms, lies at 73.3 °N 16.8 °E (Fig. 1).

Sampling
Water sample were collected at every 1 km distance from the mid-intertidal zone, making a total of 10 spots. Water samples were collected in sterile Eppendorf tubes (1.5 ml capacity). Collected samples were stored in refrigerator till use.

Physicochemical parameter
Temperature, pH, salinity and Dissolved oxygen of sea water samples were analysed at the site, using thermometer, hand pH meter and salinometer .

Bacteriological analysis
The sea water samples were serially diluted using sterile saline and spread plated on nutrient agar media (Hi media, India) supplemented with 2.5% NaCl. The plates were

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incubated at R.T. for 24 hrs. The colonies obtained were characterized.

Isolation and screening of phosphate solubilisers

The isolates obtained on nutrient agar were spotted on Pikovaskaya's agar (PA) medium. Pikovskaya's medium contains Tricalcium phosphate (0.5 g Yeast extract, 10 g Dextrose, 5 g $Ca_3(PO_4)_2$, 0.5 g $(NH_4)_2SO_4$, 0.20 g KCl, 0.1 g $MgSO_4.7H_2O$, 0.0001 g $MnSO_4.H_2O$, 0.0001 g $FeSO_4.7H_2O$, 18 g Agar in 1000 mL distilled water) (Pikovskaya, 1948). All plates were incubated for 24 - 48 hrs at R.T.

Phosphate solubilising efficiency

Isolates obtained on PA were purified on NA media plates by quadrant streak method. The purified cultures were then spot inoculated on PA. Plates were incubated at R.T. and zones of clearance were measured after 24 and 48 hrs incubation. The phosphate solubilising activity was calculated using the formula (Kukreja et al., 2010):

$$\text{Phosphate solubilising efficiency} = \frac{\text{Zone of clearance}}{\text{Zone of growth}} \times 100$$

Results:

The rocky shore of Purnagad was selected for the present study. Large number of Oysters, Zooanthids, Barnacles, sponges, ornamental fish, Sorgassum (brown algae), Green Colreppa, Ulva and other algal species were abundantly found along the shore. The temperature of the water ranged between 27 °C to 32 °C (Table I). Highest temperature was recorded at four spots, 1, 2, 6 and 9 while the least temperature was recorded at site 3. Salinity of the sea water was between 20 to 50, lowest being at spot 5 and highest at spot 10. The pH also showed a wide variation ranging 7.6 and 9.2. The lowest pH value was recorded at spot 3 and highest at spot 10 (Table 1).

The colonies obtained on nutrient agar were selected based on their distinct colony characters, which were noted, and the isolates purified and maintained on nutrient agar slants. The cream coloured colonies were of four types i.e large mucoid opaque undulate margin, large mucoid watery undulate, small flat circular mucoid opaque and transparent. Large and small brownish coloured opaque mucoid colonies were found at spots P1, P4, P5, P6, P7 and P9. Pinpoint yellow colonies were found at most of the spots.

Plates of water sample from spots P1, P4, P5 and P7 showed presence of small plaque like clearings. The plaques were more in number on the plate from site P4 (Fig. 2). Salt precipitation was observed at spot P7 and P9. Ten different types of pigmented colonies were isolated from the water sample of spot P7 (Fig. 3). The colours ranged from milky-white to yellow to orange to red to bluish- white to moss green. Similarly the salt precipitation on plating on nutrient agar showed presence of five different pigmented colonies.

On Pikovaskaya's media, the colonies were colourless to white with clear zones around the colonies. Out of the total 43 isolates, only 5 isolates showed phosphate solubilising activity. The isolates showing clear zones were selected and maintained on Pikovaskaya's agar slants. The zones of clearance and growth of selected isolates upon spot inoculation on PA were measured and the phosphate solubilising efficiency was calculated (Table 2). Isolates P3-3 showed the best solubilising activity on Pikovaskaya's agar media.

Discussion:

Presence of large numbers of species reflects the richness and biodiversity of the region. Barnacles, Zooanthids, oysters, shellfish were observed all along the rocky shore. The algal species were seen covering the rocks exposed to waves. Sea anemones and sponges were also observed and documented (Unpublished data). The present study is the first report from Purnagad rocky shore.

The pH of the water at almost all the sites except P3-3 was alkaline in nature. The salinity was also on the higher side. Highest salinity being at spot 10. High salinity may be due to the exposure of water pools to sunlight. While the low salinity at spots P3 and P5 can be explained due to the mixing of freshwater in the saline pools. Water springs were observed at several places, in the rocky walls of the overhanging hills along the rocky stretch. Mixing of fresh water in the seawater resulted in the lowering of the salinity due to dilution factor. Most of the spots being exposed to strong sunlight may be contribute to increased salinity and thus alkalinity.

Growth on NA and PA shows very low number of the isolates having ability to solubilise phosphate. Similar results have been observed by Venkateswaran and Natarajan (1984) and De Souza et al. (2000). Venkateswaran and Natarajan (1984) reported 10-15% of the total viable counts to be capable of solubilising inorganic phosphate. The variation in numbers depends on the bacterial abundance and the nature of the samples. Around the islands and coastal areas the number of PSB was higher as compared to those in the sandy beaches and offshore areas. It is probable that the offshore organisms are poor solubilizers of inorganic phosphate because these organisms are generally low in the uptake of carbon and therefore, do not change the pH of the medium drastically. De Souza et al. (2000) has reported five of the 88 phosphate solubilizing isolates to have higher phosphatase activity.

The isolate P3-3 showed the highest phosphate solubilising efficiency of 240. Kukreja, et al. (2010) reported a highest efficiency of 202 among Pseudomonas sp. Among the mangrove isolates, as high as 533 solubilising efficiency has been noted at Sakhartar creek, Ratnagiri (Unpublished data). The undisturbed, nutritionally rich habitat of the mangroves thus houses bacteria of immense potential as seen in the isolates. These bacteria are therefore promising candidates as plant growth promoting bacteria especially in the saline fields.

A large portion of soluble inorganic phosphate applied to soil as chemical fertilizer is immobilized rapidly after application due to phosphate fixation by aluminum, calcium, iron, magnesium and soil colloids (Rodriguez and Fraga, 1999) and becomes unavailable to plants (Singh and Kapoor, 1994). Therefore, P is often a limiting nutrient in agricultural soils. In particular, P-solubilizing micro-organisms (bacteria or fungi) are able to solubilize unavailable soil P and increase the yield of crops.

Although several phosphate solubilizing bacteria occur in soil, usually their numbers are not high enough to compete with other bacteria commonly established in the rhizosphere. Thus, the amount of P liberated by them is generally not sufficient for a substantial increase in situ plant growth. Therefore, inoculation of plants by a target microorganism at a much higher concentration than that normally found in soil is necessary to take advantage of the property of phosphate solubilization for plant yield en-

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hancement (Rodriguez and Frega, 1999).

Exposure to sun is related to pigmentation in bacteria. The halophiles bacteria found in salterns where temperatures go up to 45 – 50 °C, with high salinities, are mostly pigmented having the pigment bilirubrin and bilirhodopsin. Numerous pigmented bacterial isolates were obtained from site P7 and also from salt collected for site P7 and P9. These sites being at raised levels, the sea water in the pools are exposed to strong sunlight during the low tide. Hence, the abundance of pigmented cultures at these sites.

Pigmentation in marine and halophilic cultures is a common feature. A total of 14 pigmented cultures were isolated from the intertidal zone of Purnagad rocky shore. Lee et al. (2006) has described the isolation of yellow coloured colonies for sea water in the South Sea of Korea. Jayanth et al. (2002) have reported the isolation of 162 pigmented bacteria from Tuticurin coast of Tamil Nadu. The proportion of pigmented bacteria as against total bacterial load was found to be 6% and 62 of the isolates showed antagonistic activity. Thus the marine isolates can also bioprospected for novel antagonistic compounds (Austin, 1989; Soliev et al., 2011).

Incidence of bacteriophages at most of the sampling sites is in concurrence with observations and studies of other workers. Further work on the isolation, characterization and identification of these phages needs to be carried out. This is the first step towards studying the diverse microbial flora of the Konkan especially the Ratnagiri coastline of Maharashtra along with its bioprospecting studies.

Table 1: Physical parameters of the sea water along Purnagad rocky beach

Spot	Temperature (°C)	pH	Salinity
1	32.0	8.8	35
2	32.0	8.3	37
3	27.0	7.6	23
4	28.0	8.1	36
5	28.5	8.2	20
6	28.0	8.5	37
7	29.0	8.5	36
8	29.0	8.7	40
9	30.0	8.9	36
10	30.0	9.2	50

Table 2: Phosphate solubilising activity of the isolates from Purnagad rocky beach

Isolate number	Phosphate solubilising efficiency (E)
P3-3	240
P8-1	120
P7-2	200
P7-3	150
P9-1	166

Figure 1: Sampling site at Purnagad beach, Ratnagiri.

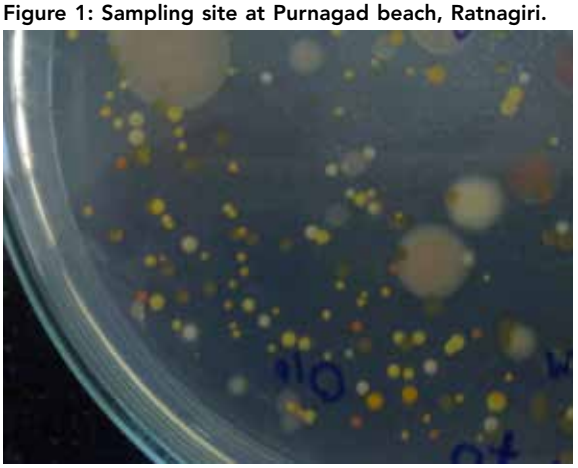

Figure 2: Pigmented bacterial colonies isolated on nutrient agar media

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