Marine bacteria: a potential bioresource for multiple applications

Ayona Jayadev, Lekshmi, M*. , Sreelekshmi, V., Lakshmi, S. Baby and Mary Franceena

Abstract - Marine microorganisms have developed distinctive metabolic and physiological capabilities to bloom in extreme habitats and produce novel metabolites which are not often present in microbes of terrestrial origin. They are a rich source of natural products with potential applications in drug discovery, environmental remediation, and the development of new resources for industrial processes. The present study aimed to isolate bacterial strains from marine water samples and screen their potential for various applications. A total of twelve bacterial strains were isolated and screened for enzyme production, Phosphate solubilization, EPS production and Azo dye decolorizing capacity. Most of the strains showed multiple potentialities. So these strains can be positively used for commercial applications.

Index terms- Azodye, Enzyme, Environmental remediation, EPS production, Marine microorganisms, Phosphate solubilization, Terrestrial

1 INTRODUCTION

Investigations on the natural products from the marine environment, including microorganisms have hastily increased over the past few years. They are considered highly valuable as it acts as an important source for industrially important molecules. Earlier reports depicts that 70% of the earth's surface is covered by oceans with rich microbial diversity and about 3.6x10-29 microorganisms were found in marine environments, including subsurface and harbor[1]. Marine environments are one of the most adverse environments owing to their varying nature of temperature, pH, salinity, sea surface temperature, currents, precipitation regimes and wind patterns. Due to the constant variation of environmental conditions, the microorganisms present in that environment are more properly tailored to the adverse conditions, hence, possessing complex characteristic features of adaptation.

The adaptation of marine organisms to a wide range of environmental conditions (temperature, salinity, tides, pressure, radiation, light) render them an enormous reservoir for biotechnological improvements [2]. Marine microbes act as are potent source of various industrially important bioactive compounds include enzymes, exopolysaccharides (EPS), biosurfactants, antibacterial, antiviral, anticancer compounds etc.

- *The authors are from P.G Department of Environmental Sciences, All saints' College, Thiruvananthapuram, Kerala.*
- **Corresponding author: Lekshmi, M. E-mail:lekshmi.envt@gmail.com*

Bacteria are remarkably adapted to diverse environmental conditions as they are present in almost all ecosystems. Considering that seawater typically contains 10-4 to 10-6 bacteria ml-1, and ocean currents can transport these organisms considerable distances, marine bacteria may encounter a far greater variety of habitats and environmental conditions than terrestrial bacteria [3]. The ability of marine bacteria to endure in and utilize the resources found in these various habitats will ultimately have a major effect upon their distribution in the sea. Despite a huge microbial diversity, there is a lack of laboratory cultures of the microbes that are most abundant in the environment that severely limits development of biodiscovery research. Therefore, the bacteria isolated from the marine environments are supposed to be better utilizing for wide applications.

Marine microorganisms were already proven to have many beneficial bioactivities in the production of industrial enzymes [4],[5], plant growth promotion potentials such as production of phytohormones and phosphate solubilisation [6], antifungal activity [7], biocontrol activity for plant disease control [8],[9], antibacterial and probiotic activity[10]. There are many reports which highlight the potential of marine bacterial strains for commercially important biotechnological applications in different fields such as human health, environmental health, sustainable food supply, sustainable energy supply and industrial applications. Marine bacteria secrete different compounds based on their habitat and their ecological functions. Reports highlighted that many heterotrophic bacteria are known to carry genetic and metabolic potentials to synthesize and control extracellular enzymes, which can degrade and modify a large variety of natural polymers in water basins [11]. In previous studies, a variety of microorganisms have been reported to produce amylase such as B. subtilis, B. licheniformis, B. brevis MTCC 7521,

Streptomyces erumpens MTCC 7317, Aspergillus awamori and Penicillium fellutanum [12],[13],[14] and [15]. Microorganisms known to produce L-asparaginase enzyme including Escherichia coli, Erwiniacarotovora [16], Vibrio [17], Aerobacter, Bacillus, Serratia, Xanthomonas, Photobacterium [18]. Lipase production from a variety of bacteria, fungi and actinomycetes has been reported by several workers [19]. Marine bacterial enzymes have several advantages for industrial utilization. They are found to be stable at room temperature over long periods. Researchers reported that the enzymes from theses microbes perhaps have characteristics properties such as heat tolerance, cryo-tolerance, pH tolerance, metal tolerance etc [20].

Phosphate solubilizing bacteria from sea sediments were reported to capable of accelerating the dissolution of apatite phosphate within the phosphorous cycle and interacting with carbon cycle. De Souza [21] reported the percentage occurrence of phosphate solubilizing bacteria from different niches viz. beaches, island, coasts and offshore and it was found that phosphate solubilizing bacterial population is higher in coastal areas than offshore areas.

Many marine bacteria produce exopolysaccharides (EPS) as a strategy for growth, adhering to solid surfaces, and to survive unfavorable conditions. Currently many research works focused on isolating new EPS producing bacteria from marine environments, particularly from extreme marine environments such as deep-sea hydrothermal vents characterized by high pressure and temperature and heavy metal presence.

The application of marine bacteria to environmental problems represents an area of potentially great importance, for both environmental and economic reasons. The advantage of using marine bacteria for bioremediation in situ is the direct use of organisms without any considerable genetic manipulation. Bioremediation approach becomes an acceptable, for utilizing the biological activity of organisms to degrade toxic chemicals in the environment. Many marine microbes are capable of degrading azo dyes, including bacteria [22], fungi [23], actinomycetes and algae [24]. A number of research groups investigated the capability of bacteria in the degradation of azo dyes. The bacterial reduction of the azo dye is typically nonspecific and bacterial decolourization is normally faster. Shertate and Thora [25] reported that as the enzyme system of marine microbes can function efficiently in a wide range of physico chemical conditions, these organisms can survive the harsh 6 saline conditions of the textile dye effluent and are very useful in dye degradation. In a view to the importance of marine bacterial strains for various applications, in the present study an attempt was made to isolate bacterial strains from marine water samples and screened their potential for multiple applications.

2 MATERIALS AND METHODS

2.1 Sample collection

Marine water samples for the isolation of bacterial strains were collected from Sangumugham, Vizhinjam and Veli coast, Thiruvananthapuram, Kerala. Location map is shown in Fig:1. The samples were collected in sterile bottles and were brought to laboratory maintaining a cold chain and refrigerated.

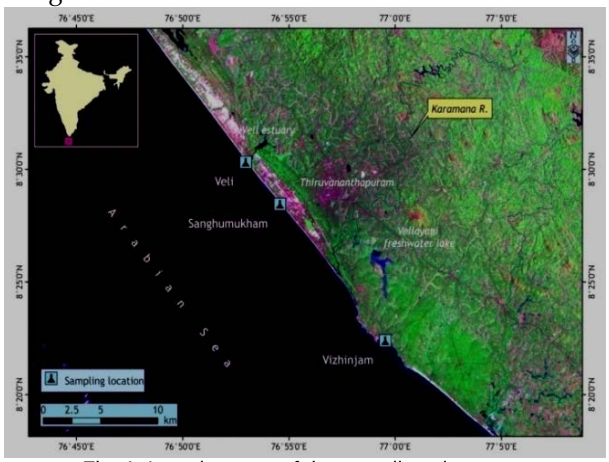


Fig. 1. Location map of the sampling site

2.2 Enrichment and Isolation of bacterial strains

For enrichment 1 ml of each selected sample was transferred to 100 ml of marine Zobell broth and incubated at 30°C for 2 days in shaker at 200rpm. Isolation of bacteria was done by the serial dilution and pour plate technique. A loopful of inoculum from the marine Zobell broth was streaked onto the marine Zobell agar and incubated at 30°C for 2 days. Single separated colonies were selected and the subcultures were maintained on marine Zobell slants at 4°C until further use.

2.3 Screening of bacterial isolates for enzyme production

The isolated bacterial strains were screened for the presence of different enzymes like amylases, L-asparaginases, lipases and proteases. All the isolated strains were inoculated on specific media by spot or streak inoculation method to screen the selected enzyme activities (amylases, L-asparaginases, cellulases, lipases and protease). The plates were incubated at 300 C for 7 days. The media as criteria for enzyme activities are given in Table: 1

TABLE: 1. METHODS FOR THE ENZYMATIC SCREENING OF BACTERIAL ISOLATES

Sl. No	Enzyme	Medium	Incubation (days)	Criteria for positive enzyme activity
1	Amylase	Starch agar media	4	Clear zones after

				flooding with iodine
2	L-asparagi nase	L-asparaginase -glucose agar media	4	Clear pink zones around the colonies
3	Lipase	Rhodamine B agar plate assay	7	Colonies showing fluorescenc e on UV irradiation
4	Protease	Skim milk agar	7	Clearing around the growth

2.4 Screening of Marine Bacteria for phosphate solubilization

For the screening procedure, quarter strength of Zobell marine agar was prepared and 1% of tricalcium phosphate was added before autoclaving the medium. This resulted in a milky white medium. The medium was poured on petriplates and made to soldify. After which the bacteria was patched in four corners of the plate and incubated for 7 days at room temperature [26]. A clear zone around the bacterial patches indicates the ability of the bacterium to solubilize phosphate in agar medium.

2.5 Screening of Marine Bacteria for EPS production

All 12 strains of marine bacteria were subjected for the screening. EPS producing bacteria was studied by modifying the protocol of Sayed [27].YMG agar medium was prepared by dissolving glucose: 2.5g, yeast extract: 0.75g, malt extract: 3g, peptone: 1.25g, monosodium glutamate 0.25g, sucrose: 7.5g along with 125mL sea water and 125mL distilled water, pH was adjusted to 7.0 and was poured in petriplates after sterilization. The bacterial strains to be tested for EPS production were streaked on the solidified medium. The plates were incubated at room temperature for 3 days, oozing out of gummy substances on the periphery of the bacterial colonies indicated the production of EPS.

2.6 Screening of bacterial isolates for tolerance of selected dyes

For primary screening all the isolated strains were tested for their tolerance to selected dye. Marine Zobell broth prepared in distilled water and sea water (1:1 ratio) is used for the test. All the 12 isolated bacterial strains were inoculated in 4 ml of broth added with 1 mL of chosen dyes (0.1% Congo red and 0.1 % of methyl red). Tubes were incubated for 48 hrs after which they were visually noted for decolourization if any. In addition, the growth of bacteria in the presence of dye was estimated during 24 and 48 hrs by taking absorbance of the culture broth at 600 nm in Elico UV Vis Spectrophotometer. Control was prepared with 4 mL of nutrient broth added with 1 mL of sterile distilled water.

Tolerance test was confirmed with the Plate assay. The bacterial isolates were plated by streaking the isolates in Basal Salt Medium (NaCl 1g,CaCl2.2H2O 0.1g,MgSO4 0.5g, KH2PO4 1g,Na2HPO4 1g,yeast extract 4g,agar 5g) added with 1 % of selected dye and incubated for 48 hrs and the clear zone around the bacterial colonies indicated the ability of the organism to tolerate azo dyes. Unspotted plate was used as the control.

3 RESULTS

3.1 Isolation and Enrichment of bacterial strains

Marine samples when inoculated in the enrichment media showed heavy growth of microorganisms after 2 days of incubation. Standard Plate Count was made to enumerate and isolate bacterial strains from enrichment. A total of 12 bacterial strains were isolated from the selected marine samples. 4 (Ab1, Ab2, Ab3 and Ab4) from Sangumugham. 4 (Bb1, Bb2, Bb3 and Bb4) from Vizhinjam and 4 (Tb1, Tb2, Tb3 and Tb4) from Veli, coast.

3.2 Screening of bacterial isolates for enzyme production

In primary screening, all the isolated strains were inoculated on specific media by spot inoculation method. The results are shown in Table: 2.

TABLE: 2: SCREENING OF BACTERIAL STRAINS FOR ENZYME PRODUCTION

Sl. No	Bacterial Strains	Amylase Activity	L-asparaginase Activity	Lipase Activity	Protease Activity
1	Ab1	+	-	+	-
2	Ab2	+	-	+	+
3	Ab3	-	-	+	-
4	Ab4	-	-	-	-
5	Bb1	+	-	-	+
6	Bb2	+	-	-	+
7	Bb3	+	-	-	-
8	Bb4	-	-	+	+
9	Tb1	-	-	-	-
10	Tb2	+	-	+	+
11	Tb3	+	-	+	-
12	Tb4	-	-	-	+

+ indicates the production of the enzyme while
– indicates no production

Out of the 12 strains 7 strains (Ab1, Ab2, Bb1, Bb2, Bb3, Tb2 and Tb3) showed amylase activity, 6 strains (Ab1, Ab2, Ab3, Bb4, Tb2 and Tb3) showed lipase activity and 6 strains (Ab2,Bb1, Bb2, Bb4, Tb2 and Tb4) showed protease activity respectively. Among the strains Ab2 and Tb2 showed all the selected enzyme activity where as Bb4 showed protease and lipase activity only. Most of the strains in the present study showed multiple enzyme activity. In the present study none of the strains showed L-asparaginase activity.

3.3 Screening of Marine Bacteria for phosphate solubilization

Among the 12 strains, two of them showed clear zone around their colony and the strains are Tb1 and Tb3 and the results are shown in Table: 3.

3.4 Screening of Marine Bacteria for EPS production

Out of 12 strains, 4 strains exhibit EPS production and the strains are Ab4, Tb1, Tb2, and Tb3. They produced gummy secretions either around the colony periphery or by whole colony in YMG medium. The results are shown in Table: 3 and Fig: 3

TABLE: 3. STRAINS SHOWING EPS PRODUCTION AND PHOSPHATE SOLUBILIZATION

Serial No.	Bacterial Strains	EPS production	Phosphate solubilisation
1	Ab1	-	-
2	Ab2	-	-
3	Ab3	-	-
4	Ab4	+	-
5	Bb1	-	-
6	Bb2	-	-
7	Bb3	-	-
8	Bb4	-	-
9	Tb1	+	+
10	Tb2	+	-
11	Tb3	+	+
12	Tb4	-	-

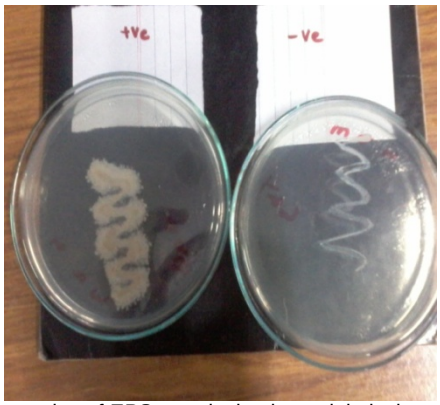


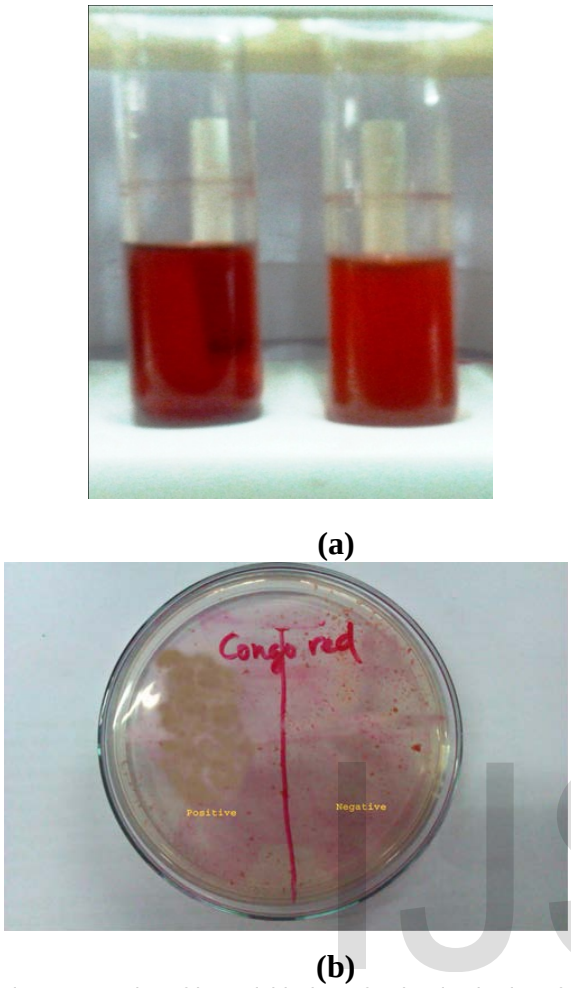
Fig: 2. Screening of EPS producing bacterial isolates

3.5 Screening of bacterial isolates for tolerance of selected dyes

In the present study the isolated strains were screened for the biodegradation potential of two selected azo dyes viz. Congo red and Methyl red and the results are presented here under. Screening was done to find the efficient bacterial strains capable of decolourising the selected dyes. All of the 12 bacterial strains isolated from the marine water samples were screened for their potential to tolerate congo red and methyl red by culturing the bacterial strains in Marine Zobell broth added with 0.1 % of selected dyes for 48 hrs and the growth of the isolates was observed at every 24 hrs interval by measuring the absorbance at 600 nm in spectrophotometer and the results are shown in Table: 4and 5. When grown with congo red, majority of the isolated strains showed medium growth in culture broth. Among the bacterial strains, Strain Ab2, Ab3, Bb2, Bb4, Tb3 and Tb4 showed maximum growth than the other strains. The tolerance test is confirmed by plate assay. Here also these six bacterial strains decolorize the tested dye considerably (Fig: 3).

TABLE: 4. TOLERANCE OF BACTERIAL STRAINS (ABSORBANCE AT 600 NM) TO 0 .1 % CONGO RED

Bacterial strains	24hrs	48hrs
Ab1	0.166	0.152
Ab2	5.55	5.65
Ab3	3.32	4.86
Ab4	0.152	0.322
Bb1	0.131	0.257
Bb2	3.3	2.96
Bb3	0.373	0.374
Bb4	1.16	2.82
Tb1	0.093	0.116
Tb2	0.277	0.287
Tb3	2.21	2.3
Tb4	1.84	1.96



(a)

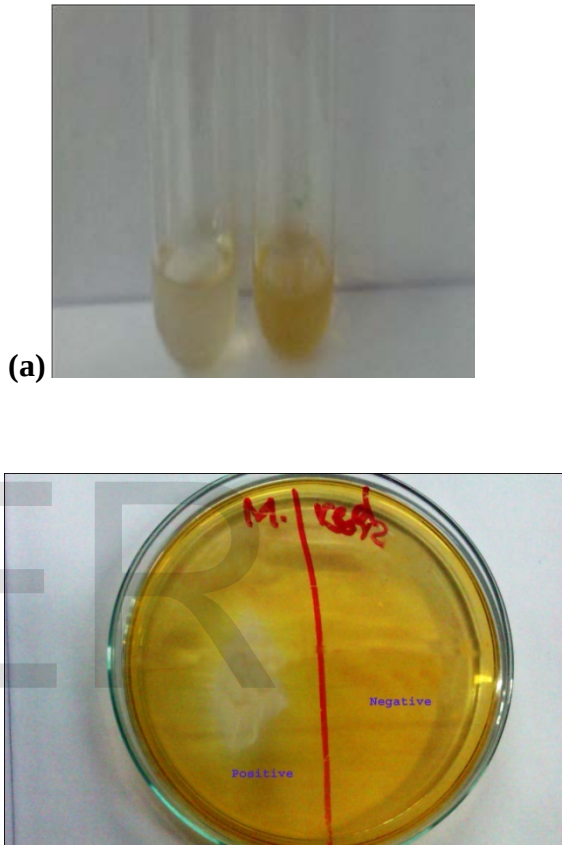
(b)

Fig: 3. Screening of bacterial isolates for decolourization of Congo red "(a)" Test tube assay, "(b)" Plate assay

In the case of culture medium added with 0.1 % methyl red all the isolated strains showed considerable growth and decolourisation and the results are shown in Table:5 and Fig:4. Among the strains, Ab2, AB4, Bb3, Bb4, Tb3 and Tb4 showed maximum growth and efficient decolourisation in basal salt media and plate assay. So these strains can be positively used for decolourisation assay for the dye methyl red. All the isolates showed maximum growth at 48 hours of incubation.

TABLE: 5. TOLERANCE OF BACTERIAL STRAINS (ABSORBANCE AT 600 NM) ADDED WITH 0.1% METHYL RED

Bacterial strains	24hrs	48hrs
Ab1	0.050	0.061
Ab2	0.570	2.28
Ab3	0.272	0.861
Ab4	0.860	1.081
Bb1	0.061	0.094
Bb2	0.101	0.452
Bb3	0.303	0.740
Bb4	0.824	1.73
Tb1	0.091	0.176
Tb2	0.056	0.090
Tb3	0.012	0.060
Tb4	0.230	0.784



(a)

(b)

Fig. 4. Screening of bacterial isolates for decolourization of Methyl red "(a)" Test tube assay, "(b)" Plate assay

4 DISCUSSION

Marine bacteria encompass multiple biotechnological applications. Marine bacteria can be a plausible source of new bioactive compounds for industrial, agricultural, environmental, pharmaceutical and medical uses [28].The secondary metabolites produced by marine organisms have more novel and unique structures owing to the complex living circumstance and diversity of species, and the bioactivities are much stronger than of terrestrial organisms [29]. Dionisi [30] isolated 26 marine bacteria from the marine water samples of Mumbai coastal area. He stated that the microorganisms from intertidal zones may be able to tolerate rapid and repeated fluctuations in environmental conditions including temperature, light and

salinity, and are exposed to wave action, ultraviolet radiation, as well as periods of drought. Hence, these microbes possess impending properties which can be exploited for biotechnological applications. The present study has been undertaken with the ultimate aim of identifying marine bacterial strains which has the potential to exhibit multiple applications in the field of growing industry. A total of twelve bacterial strains were collected from Sangumugham, Vizhinjam and Veli coast, Thiruvananthapuram, Kerala. All these strains showed considerable growth in the marine Zobell agar medium. All these strains were screened for their potential to produce amylase, L-asparaginase, lipase and protese enzymes. Except Ab4 and Tb1 almost all the strains showed more than one enzyme activity with slight variation. Ab4 and Tb1 failed to express the selected enzyme activity. In the present study none of the strains showed L-asparaginase activity. Strains Ab2 and Tb2 showed most of the selected enzyme activity. The enzyme production depends on the potential capability of each bacterium. Similar results were reported by Annika Durve [31]in the study on the marine bacteria for various applications. They reported that out of the 26 marine bacterial isolates, Isolate MBC2 produced most of the extracellular hydrolytic enzymes- amylase, cellulose, protease, lipase and Beta-galactosidase activity. The marine bacteria including marine actinomycetes exhibit diverse pattern in secreting extracellular enzymes .The nature of the sample from which the microbes isolated also play a crucial role in the production of secondary metabolites. Our research group has earlier reported in a study on marine actinomycetes, that the enzyme activity of the strains varied from isolate to isolate depending upon the cultural characteristics and physical conditions of each strain [32].

Microbial exopolysaccharides (EPS) are a structurally very diverse class of molecules. Extracellular polysacharides or exopolysacharides (EPS) are often found in the surroundings of the outer structures of prokaryotic as well as eukaryotic microbial cells.

Bacterial EPS are also complex mixture of macro molecular poly electrolytes including polysaccharides, proteins and nucleic acids, each comprising of variable molecular mass and structural properties. Exopolysacharides have major roles in different processes viz., formation of biofilm, protection of bacterial cell from desiccation, for maintaining primary cellular functions and antibacterial activity against predators, gelling ability, pollutant degradation kinetics, bioremediation activity and plasma substituting capacity[33,34].In the present study all the12 isolated strains were screened for their potential for the production of EPS. Out of 12 strains, 5 strains exhibit EPS production and the strains are Ab4, Tb1, Tb2, and Tb3.The EPS production depends on the metabolic condition of the microbes. Kumar [35] reported that polysaccharide synthesis and yields largely depends on environmental and nutritional conditions

Phosphate solubilizing bacteria living in both terrestrial and water ecosystem play a vital role in supplying phosphorus to plants. De Souza [21] reported the percentage occurrence of phosphate solubilizing bacteria from different niches viz. beaches, island, coasts and offshore and it was found that phosphate solubilizing bacterial population is higher in coastal areas than offshore areas. In the present study out of the 12 strains two strains showed phosphate solubilization activity.

Biological methods for the decolourisation of the textile dyes mainly involve the use of bacteria, fungi and plants .There are so many reports on the dye degrading ability of bacterial strains; works on the biodegradation of azo dyes by marine bacterial strains are very scarce. In the present study twelve strains six strains (Ab2, Ab3, Bb2, Bb4, Tb3 and Tb4) showed maximum tolerance to congo red and six strains (Ab2, AB4, Bb3, Bb4, Tb3 and Tb4) showed maximum tolerance to methyl red. So all these strains can be positively used for dye decolourisation.

In the present study all the isolated strains showed at least one of the selected activities studied. Among the strains Tb3 showed all the tested activities i.e. enzyme production, EPS production, phosphate solubilization and the ability to decolourise selected dyes. Most of the isolated strains showed more than two activities.

5 CONCLUSION

The present study clearly depict that the marine organisms have multiple potential for various applications. The promising results obtained from the present study can be regarded as a preliminary screening of the isolated strains for several purpose and these strains can be regarded as potential candidates for future prospects. Further investigations are needed to carry out in order to exploit these microbes for the large scale production of valuable metabolites.

ACKNOWLDGEMENT

The authors are grateful to University Grants Commission for the financial assistance.

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