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Bioprospecting of Halotolerant Marine Bacteria from the Kelambakkam and Marakkanam Salterns, India for Wastewater Treatment of Plant Growth Promotion

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Microbial life can be found over a wide range of extreme conditions like high salinity, pH, temperature, pressure, light intensity, oxygen and nutrients. Halotolerant or halophilic microorganisms, are able to live in saline environments, offer potential applications in various fields of Biotechnology. The halotolerant or halophilic microorganisms are important for maintenance of soil condition and nutrient recycling in saline environment. The environment is ideally placed at the inter-phase between the terrestrial and marine environment and support a rich and diverse group of microorganisms. In this study, attempts were made to isolate the halotolerant marine bacteria and screen them for enzymes to be used for the decolorization of textile dyes and tannery effluent. A total of 192 halotolerant marine bacteria were isolated from Kelambakkam and Marakanam Salterns and named as AMETH101 to AMETH292. The isolated strains were tested for their growth at various salt concentrations such as 0M, 0.5M, 2.5M and 5M. All were found to grow well at 0.5M NaCl concentration. These organisms were studied for their ability to produce enzymes like lipase, tannase and protease. Among these strains, 107 have exhibited lipase activity, 41 strains exhibited tannase activity and 130 strains have exhibited protease activity. Totally 192 strains were tested for IAA production using qualitative assay and found that 41 strains showed positive activity and the remaining strains showed negative activity. For morphological characterization, KOH string test and catalase tests were performed. The results indicated that among the 192 strains, 138 strains were gram positive and remaining were gram negative strains while all the strains were positive for catalase test. This preliminary study has demonstrated that bioprospecting of halotolerant marine bacteria may yield potential microorganisms to be used in various environmental and biotechnological applications. Isolated halotolerant bacteria are very good producers of extracellular enzymes and IAA. These halotolerant bacteria with the combined ability of producing bioremediation enzymes and plant growth promoting hormones enhances the potential uses of them for the treatment and reuse of industrial effluents for the irrigation of ornamental plants.

Keywords: Extremophiles, Halotolerant bacteria, Extracellular enzymes, Salterns, bioremediation

Halophiles are salt-loving organisms that inhabit hypersaline environments. They include prokaryotic and eukaryotic microorganisms with the capacity to balance the osmotic pressure of the environment and resist the denaturing effects of salts. Among halophilic microorganisms are a variety of heterotrophic and methanogenic archaea; photosynthetic, lithotrophic, and heterotrophic bacteria; and photosynthetic and heterotrophic eukaryotes (DasSarma and Arora, 2001). Most of

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the halophiles are considered as extremophiles. In comparison with the other extremophiles such as thermophilic and the alkaliphilic extremophiles, halophilic microorganisms have many biotechnological applications. Production of beta-carotene, ectoine and bacteriorhodopsin are the most successful biotechnological applications from halophiles. Salt-tolerant enzymes, treatment of saline and hypersaline wastewaters, and the production of exopolysaccharides, poly-beta-hydroxyalkanoate bioplastics and biofuel are more prospective applications from halophiles (Oren, 2010).

The halotolerance of many enzymes derived from halophilic bacteria can be exploited wherever enzymatic transformations are required to function under physical and chemical conditions, such as in the presence of organic solvents and extremes in temperature and salt content. Several halophilic hydrolases have been described, including amylases, lipases and proteases, and then used for biotechnological applications (Moreno *et al*., 2013). Enzymes from extremophilic microorganisms have evolved to function in a variety of extreme environments, and bioprospecting for these microorganisms has led to the discovery of new enzymes with high tolerance to non-natural conditions (Liszka *et al*., 2012). Halophilic Bacteria are metabolically more versatile than the Archaea, and their enzymatic activities are more diverse (Oren, 2010).

Bioremediation is the most effective management tool to manage the polluted environment and recover contaminated soil. Bioremediation uses bacteria, fungi, yeasts and algae to clean up contaminated soil and water. A large number of enzymes from bacteria, fungi, and plants have been reported to be involved in the biodegradation of toxic organic pollutants. The information on the mechanisms of bioremediation-related enzymes such as oxido-reductases and hydrolases have been extensively studied (Karigar and Rao, 2011). Hydrolases such as proteases and lipases and oxidoreductases such as tannase and laccase are having good prospects

in the field of enzyme mediated bioremediation of industrial effluents. Tannery and textile effluents are characterized to have high salinity (Ahmed *et al*., 2011). The salt tolerant enzymes produced by halophilic or halotolerant bacteria may have potential applications in the bioremediation of textile and tannery effluents. Moreover, the re-use of biologically treated effluents is also an another challenging area. Studies were undertaken to reuse the treated wastewater for irrigation purposes especially for the ornamental plants (Lubello *et al*., 2004). In this context, this preliminary study has been undertaken to isolate and screen halotolerant bacteria from two solar salterns of Tamil Nadu India for the production of hydorlases and oxidoreductaes with the ultimate aim of using them for bioremediation of industrial effluents. The selection process included the ability of potential halotolerant bacteria for the production of, indole acetic acid (IAA). This extra-cellularly produced plant growth hormone would add value to the treated effluent which can be reused for irrigation of ornamental plants.

MATERIALS AND METHODS

Collection of samples

The water and sediment samples were collected from Marakanam (around 12°14’53.9"N 79°56’10.1"E) and Kelambakkam Salterns (around 12°44’56.8"N 80°12’56.1"E) along the East Coast of Tamil Nadu, India. The surface water samples and Sediment soil samples were collected using sterilized plastic bottles, allowing enough air space in the bottles to facilitate thorough mixing and aseptically transferred in to sterile polythene bags and the samples were brought to the laboratory for bacteriological analysis.

Isolation of Halotolerant bacteria (HTB)

Ten gram of soil sample/ 10 ml of water sample were taken in sterile conical flasks and 90 ml of sterile distilled water was added to each flask. The flasks were kept in shaker for approximately 15 minutes at 100 rpm. This 10^{-1} dilution was serially diluted up to 10^{-3}dilution. Then 0.1 ml from 10^{-3} dilution was used to spread plate on Halophilic Agar medium and the plates were left for 7 days incubation at 37ºC. The halophilic agar medium containing (gm/L) Peptone - 5, Yeast extract - 3, $CaCl_2$- 0.1, Potassium chloride 5, $MgSO_4$ - 6, NaCl-30, Agar - 20.

Purification and storage of bacterial strains

After the incubation period,

morphologically different bacterial colonies were sub- cultured in Halophilic Agar (HA) plates. The colony characteristics were observed and noted.The purityof the isolated bacterial strains were tested by quadrant streaking and single colonies were again subcultured on the same medium. Then the purified bacterial strains were given a prefix of AMETH (indicating the University name and halophilic nature) followed by Arabian numericals in a series from 101.Then the strains were stored in aged double sterile sea water in 1.5 ml Eppendrof tubes at 4ºC until usage.

Biochemical characterization

Catalase and Oxidase test

A loop full of culture was taken and placed on a clean glass slide. A drop of 3% Hydrogen peroxide solution was placed on the bacterial culture and mixed well with sterile loop. Formation of air bubbles within 10 secs indicates positive result for catalase test. Oxidase discs (HiMedia, Mumbai) were used to screen the bacteria for oxidase production as per the manufacturer's instructions.

KOH string test

This is an alternative for Gram Staining especially when strain numbers to be tested are more. A clean glass slide was taken and one drop of 3 % KOH solution was kept in centre. Using a tooth pick the bacterial strain from the agar plate is placed on the KOH drop and thoroughly mixed. The formation of a sticky and thread like appearance within 45 seconds when the tooth pick lifted, indicates that the test bacterium is Gram negative and the formation of insoluble white precipitate indicates that it is Gram positive strain.

Salt tolerance spectrum of halotolerant bacterial (HTB) strains

Halophilic agar medium was prepared with different concentrations of NaCl (0, 0.5, 2.5 and 5 M NaCl) along with normal HA medium (control), sterilized and plated. All the isolated 192 halophilic bacterial strains were streaked individually in agar plates with different concentrations of NaCl. The plates were incubated at room temperature (35±2°C) for 7 days. The growth of bacteria in different salt concentrations was observed and a +++ scale was used to rate the cultures based on visual observation. The growth of bacterial strains at usual HA medium was taken as a reference and +++ indicates good growth, ++ average growth, + poor growth and- no growth.

Screening of HTB for extracellular hydrolases

All the HTB were subjected to screen for the production of extracellular enzymes such as hydrolases such as protease and lipase in a simple qualitative plate assay. The proteolytic activity of the organism was checked using nutrient agar medium prepared in seawater with casein (0.5%) as substrate. Test bacterial strains were streaked and incubated at room temperature for 7 days. Protease activity was visualized by the clear zone around bacterial patches, after the plates were flooded with saturated ammonium sulfate solution prepared in 0.1 N HCl. The lipolytic activity was checked using nutrient agar medium prepared in seawater with Tween 80 (0.5%) as substrate. Lipase activity was visualized as a zone of opalascence around the colonies after 7 days of incubation.

Screening of HTB strains for the production of oxidoreductase

Tannase was chosen as a candidature enzyme belonging to oxidoreductaes. The nutrient agar medium was prepared in aged seawater and a 0.5% tannic acid solution was also prepared separately and filter sterilized. After the sterilization, tannic acid was added with the molten medium just before pouring in petriplates. The halotolerant bacterial strains were streaked and the plates were kept for incubation room temperature for 7 days at darkness. Formation of a dark brown zone around the bacterial culture indicates the positive tannase activity.

Screening of HTB for IAA production

The bacterial strains which were characterized as Halotolerant were selected and screened for the production of IAA. The halotolerant strains were inoculated in sterilized Nutrient broth prepared in seawater supplemented with tryptophan (10 µg/mL) and incubated at 37ºC for 7 days in shaking conditions. After incubation period, fully grown bacterial broth cultures were centrifuged at 10,000 rpm for 10 minutes. To the supernatant (2 ml), two drops of orthophosphoric acid was added and incubated at room temperature for 10 minutes, followed by addition of 4 ml of Salkowski reagent (50 ml, of 35% sulphuric acid, 1 ml of 0.5M $FeCl_3$). Development of pink color indicates the positive result for IAA production and no color change indicates the negative result for IAA production (Ahmad *et al*., 2008).

RESULTS

Isolation, purification and storage Halotolerant bacteria (HTB)

A total of 192 pure cultures of halotolerant bacteria were isolated from different samples collected from Kelampakkam and Marakanam Salterns (Figure 1). All the bacteria were coded as AMETH101 to AMETH292 and preserved in refrigerated conditions.

Biochemical characterization

Three simple biochemical tests were done for the preliminary characterization of HTB to classify them. All the isolated HTB strains were found positive in catalase and oxidase tests indicating all are aerobic organisms. Further KOH string test was done to confirm their Gram nature. It has been found that 138 HTB strains were Gram positive and remaining 54 were Gram negative strains**.**

Salt tolerance spectrum of halotolerant bacterial (HTB) strains

Though all the HTB were isolated on high salt medium, they were further subjected to study their salt tolerance spectrum across different

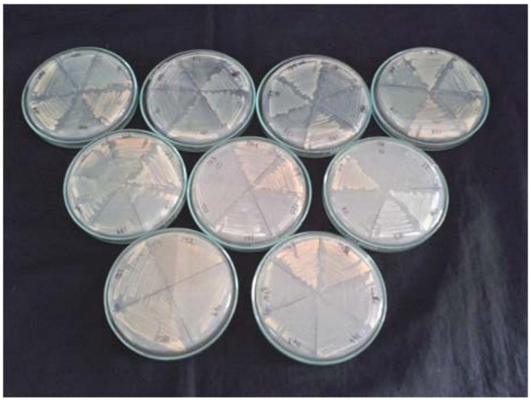
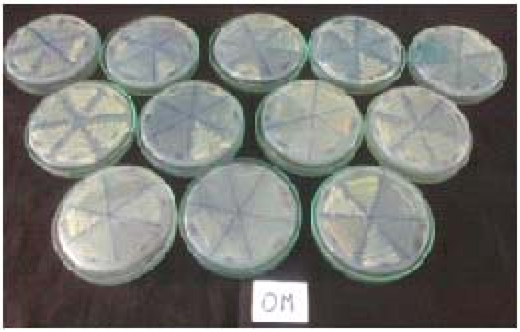
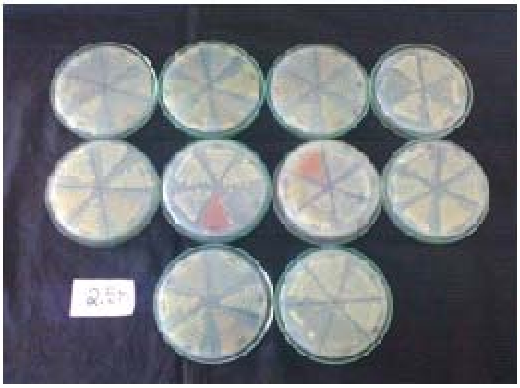


Fig. 1. Pure cultures of Halotolerant bacteria isolated from Kelambakkam and Marakkanam Salterns, India (Selected strains in HA medium)

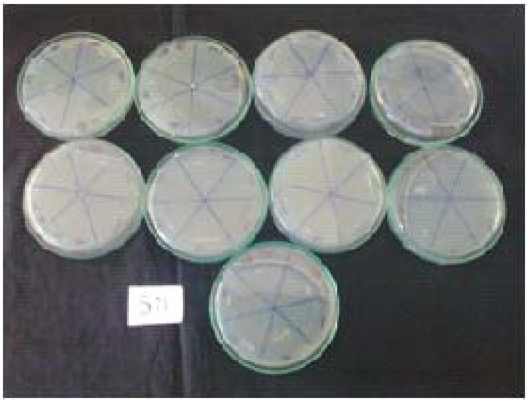


a) No NaCl

b) 0.5 M NaCl



c) 2.5 M NaCl



d) 5 M NaCl

Fig. 2. Salt tolerance spectrum of halotolerant bacterial (HTB) strains

concentrations of NaCl. A total of 177 strains were found to exhibit different degrees of growth in salt concentrations between 0-2.5M. However, none among the 192 strains exhibited growth at 5M. A total of 51 strains have grown well in the medium even without NaCl. A total of 55 strains have grown well in the concentrations up to 2.5 M NaCl (Figures 2 & 3). The results indicates that majority

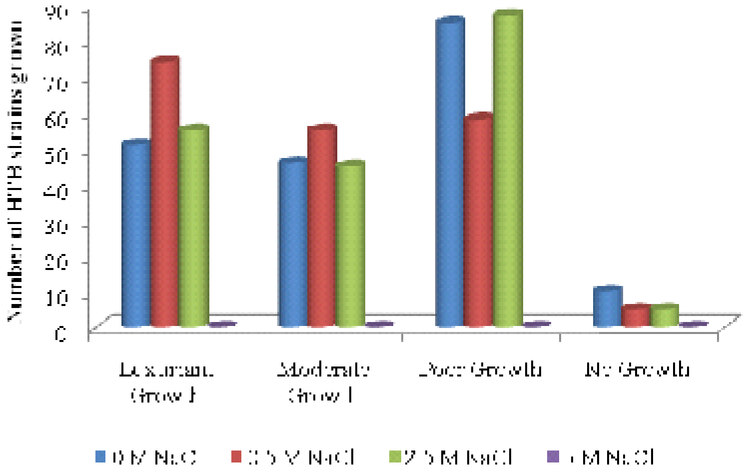


Fig. 3. Effects of bacterial inoculation under different concentrations of NaCl

a) Protease b) Lipase c) Tannase

Fig. 4. Screening of HTB for extracellular hydrolases and oxidoreductase

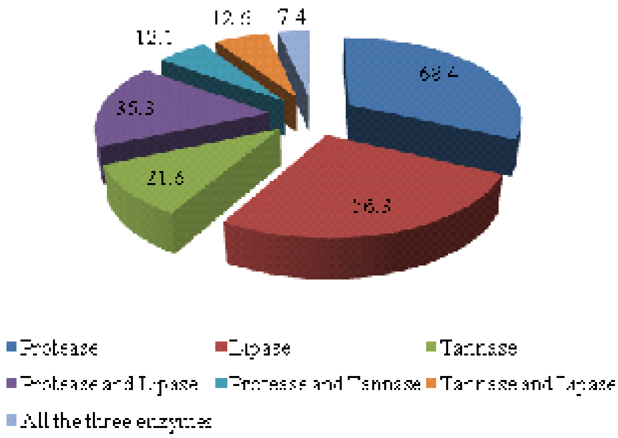


Fig. 5. Percentage of enzyme producing strains among the halotolerant marine bacteria isolated from Kelambakkam and Marakkanam Salterns, India

of the isolated bacteria were of halotolerant in nature and not halophilic. Only 10 strains out of 192 have failed to grow in 0M salt concentrations and need NaCl above 0.5M for their normal growth indicating their halophilic nature.

Screening of HTB for the production of extracellular enzymes and plant growth promoting hormones

Among the 192 HTB strains tested, 130 strains showed protease activity on casein agar plates (Figure 4a). Likewise, among 192 HTB strains, 107 strains exhibited lipase activity (Figure 4b). In case of tannase activity, only 41 strains exhibited tannase positive activity as they showed brown color change around their growth in tannic acid amended medium (Figure 4c). The summary of this experimental result is that 68.4, 56.3 and 21.6% of the strains produced Protease, Lipase and Tannase, respectively. Interestingly, 7.4% of the strains produced all the three enzymes indicating their ability to degrade organic and inorganic pollutants (Figure 5). Thirty nine strains were found to produce the plant growth promoting hormone, IAA (Figure 6).

Fig. 6. IAA production by one of the HTB strain AMETH122

DISCUSSION

Solar salterns are extreme hypersaline environments that are five to ten times saltier than seawater (150–300 g L-1 salt concentration) and typically contain high numbers of halophiles adapted to tolerate such extreme hypersalinity (Sabet *et al.*, 2009). In India, there are several coastal solar salterns along with the coastal line of the Bay of Bengal and Arabian Sea and inland solar salterns around Sambhar saltlake, from which sodium chloride is obtained for human consumption and industrial needs. Studies on characterization of such coastal and inland solar salterns are scarce and both the bacterial and archaeal diversity of these extreme saline environment remains poorly understood (Jose and Jebakumar, 2012). Unlike other salterns, most of the salterns in Tamil Nadu are fed by hypersaline spring water mixed with seawater and led to the ponds from bore wells. In addition, prokaryotic community development is restricted as salterns operate only during the arid part of the year (Manikandan *et al.*, 2009). In this context, this preliminary research work to isolate halotolerant bacteria from two different salterns of Tamil Nadu and screen them for the production of enzymes and plant growth promoting hormones with the ultimate aim of selecting these organisms for the bioremediation of industrial effluents.

The phylogenetic diversity of microorganisms living at high salt concentrations is surprising. Halophiles are found in each of the three domains: Archaea, Bacteria, and Eucarya. The metabolic diversity of halophiles is great as well: they include oxygenic and anoxygenic phototrophs, aerobic heterotrophs, fermenters, denitrifiers, sulfate reducers, and methanogens (Oren, 2002). Hence, bioprospecting of halophilic microorganisms is gaining momentum in recent years. Hypersaline environmental are always considered as a good source for the isolation of halophilic microorganisms. In the present study, sediment and water samples from two solar salterns along the East Coast of India Kelampakkam and Marakanam) were collected for the isolation and bioporspceting of halotolerant bacteria for environmental applications. A total of 192 halotolerant bacterial strains were isolated from Kaelambakkam Salterns, Chennai and they were named as AMETH101 to AMETH 292(Fig 1 and Fig 2). Several studies across the globe have concentrated on the salterns as a habitat for halophilic and halotolerant bacteria. Yeon *et al*. (2005) have isolated and cultured 64 moderately halophilic bacteria inhabiting solar saltern ponds in Taean-Gun, Chungnam Province, Korea. Thirty-five halophile cultures of both Bacteria and Archaea were isolated from the Exportadora de Sal saltworks in Guerrero Negro, Baja California, Mexico most of them belonging to the *Halorubrum*, *Haloarcula*, *Halomonas*, *Halovibrio*, *Salicola*, and *Salinibacter* (Sabet *et al*., 2009).

Morphological characterization of new isolates has always been found a much needed element of diversity and bioprospecting studies. Mageswari *et al*. (2012) have isolated 22 bacterial isolates and studied their distinct colony morphologies like pigmentation, texture, elevation, size and margin surface. In the present study, all the 192 halotolerant bacterial strains were positive for catalse and oxidase. Catalase and oxidase tests are always considered as confirmatory tests for categorizing a bacterium as aerobic (Shiba and Simidu, 1982). KOH string test results indicated that among the 192 strains, 138 strains were gram positive and remaining were gram negative strains. Besides molecular characterization, morphological characterizations were done for the halophiles isolated from solar salterns in Baja California, Mexico (Sabet *et al*., 2009). Various morphological (gram staining), biochemical and physiological (salinity) analysis were done to characterize the even halophilic bacterial strains isolated from sediment sample obtained from Dead Sea coast, Al Karak, Jordan (Tarawneh *et al*., 2008). Thus, morphological characterization of bacterial isolates is very essential especially in the perspective of bioprospecting.

The halophilic enzymes are considered a novel alternative for use as biocatalysts in different industries. Only a few industrial applications of halophilic enzymes such as the manufacturing of solar salt from seawater, fermented food, textile, pharmaceutical and leather industries have been reported (Oren, 2002; Delgado-García *et al*., 2012; Moreno *et al*., 2013). Several studies have been concentrated on the isolation, screening and characterization of various hydrolases and oxidoreductases from halophilic and halotolerant bacteria (DeFrank *et al*., 1993; Shivanand and Jayaraman, 2009; Khandeparker *et al*., 2011; Raval *et al*., 2013; Mesbah and Wiegel, 2014). In the present study, we screened a total of 192 halotolerant bacteria isolated from the two salterns of Tamil Nadu, India for the production of two hydrolases (protease and lipase) and one oxidoreductase (tannase). Our preliminary screenings have concluded that 67.7% of the strains produced protease, 55.7% Lipase and 21.3% strains have produced Tannase. Moreover, a total of 14 strains (7.3%) have produced all the three enzymes tested. The results were very much encouraging that few strains were capable of producing all the three enzymes tested because, they can be very well employed for the degradation of complex substances such as dyes.

The present study has been undertaken with the ultimate aim of identifying potential halotolerant bacterial strains which has the ability to degrade textile dyes and enhance the plant growth by the production of hydrolases and oxidoreductases along with plant growth promoting hormone IAA. Beneficial plant-associated bacteria play a key role in supporting and/or promoting plant growth and health. The phytohormone IAA production offers great promise for sustaining the increased crop productivity. Several halotolerant bacteria were previously characterized for the production of this important plant growth promoting hormone. A total of 140 halotolerant bacterial strains were isolated from Yellow Sea, near the city of Incheon in the Republic of Korea. All the strains were characterized for multiple plant growth promoting traits, such as the production of indole acetic acid (IAA), nitrogen fixation, phosphorus (P) and zinc (Zn) solubilization, thiosulfate (S_2O_3) oxidation, the production of ammonia (NH3) (Siddikee *et al*., 2010). Rhizosphere of a halotolerant plant *Suaeda fruticosa* from saline desert of Little Rann of Kutch, Gujarat (India) was explored for isolation of PGPR from the rare ecological niche having 4.33% salinity. Total 85 isolates from the rhizosphere belonging to different species were isolated. Out of 85 isolates, 23 could solubilize phosphate and 11 isolates produced IAA. Seven isolates showed both the traits of phosphate solubilization and IAA production (Goswami *et al*., 2014). Similarly, in the present study, a total 192 strains were screened for IAA production and found that 20.31% of the strains could produce IAA extracellularly. These strains further increase the scope for using them as plant growth promoting bacteria under salt influence soil conditions.

CONCLUSION

Halophilic or halotolerant marine bacteria are suitable candidates for various applications not alone in hypersaline environments but also in salt influenced environments such as coastal agricultural fields, saline sodic soils etc. The study has concluded that the isolated halotolerant marine bacteria were having the dual potential for degrading the organic and inorganic pollutants by producing hydrolases and oxidoreductases and enhancing plant growth promotion by producing indole acetic acid. Further characterizations would help to commercially exploit these organisms for industrial and agricultural applications in salt influenced environments.

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REFERENCES

1. Ahmed, M. K., Das, M. Islam, M. M., Akter, M. S., Islam, S., Al-Mansur, M. A., Physico-Chemical Properties of Tannery and Textile Effluents and Surface Water of River Buriganga and Karnatoli, Bangladesh. *World Applied Sciences Journal.*, 2011; **12:** 152-159.
2. Das Sarma, S., Arora, P., Halophiles, Encyclopedia of Life Sciences. London: *Nature Publishing Group.*, 2001; 1–9.
3. DeFrank, J. J., Beaudry, W. T., Cheng, T., Harvey, S. P., Stroup, A. N., Szafraniec, L. L., Screening of halophilic bacteria and Alteromonas species for organophosphorus hydrolyzing enzyme activity. *Chemico-Biological Interactions*., 1993; **87:** 141-148.
4. Delgado-García, M., Valdivia-Urdiales, B., Aguilar-González, C. N., Halophilic hydrolases as a new tool for the biotechnological industries. *Journal of the Science of Food and Agriculture.*, 2012; **92:** 2575–2580.
5. Goswami, D., Dhandhukia, P., Patel, P., Thakker, J. N., Screening of PGPR from saline desert of Kutch: Growth promotion in Arachis hypogea by *Bacillus licheniformis* A2. *Microbiological Research.*, 2014; **169:** 66-75.
6. Jose P. A., Jebakumar, S. R. D., Phylogenetic diversity of actinomycetes cultured from coastal multipond solar saltern in Tuticorin. *India Aquatic Biosystems.*, 2012; **8:** 23.
7. Karigar, CS., Rao, SS., Role of microbial enzymes in the bioremediation of pollutants: A review. *Enzyme res*., 2011; 1-11.
8. Khandeparker, R., Verma, P., Deobagkar, D., A novel halotolerant xylanase from marine isolate *Bacillus subtilis* cho40: gene cloning and sequencing. *New Biotechnology.* 2011; **28:** 814-821.
9. Liszka, M., Clark, M., Schneider, E., Clark, D.S. Nature versus nurture: developing enzymes that function under extreme conditions. *Ann Rev Chem Biomol Eng*., 2012; **3:** 77–102.
10. Lubello, C., Gori, R., Nicese, FP., Ferrini, F., Municipal-treated wastewater reuse for plant nurseries irrigation, *Water Res.,* 2004; **38:** 2939-47.
11. Mageswari, A. A., Subramanian, P. P., Chandrasekaran, S. S., Sivashanmugam, K., Babu, S., Gothandam, K.M., Optimization and immobilization of amylase obtained from halotolerant bacteria isolated from solar salterns. *Journal of Genetic Engineering and Biotechnology.*, 2012; **11:** 277-282.
12. Manikandan, M., Kannan, V., Pasic, L., Diversity of microorganisms in solar salterns of Tamil Nadu, India. *World Journal of Microbiology and Biotechnology.*, 2009; **25:** 1007-1017.
13. Mesbah, N. M., Wiegel, J., Halophilic alkali- and thermostable amylase from a novel polyextremophilic *Amphibacillus sp.* NM-Ra2. *International Journal of Biological Macromolecules.*, 2014; **70:** 222-229.
14. Moreno, M.L., Perez, D., Garcia, M. T., Mellado, E., Halophilic Bacteria as a Source of Novel Hydrolytic Enzymes. *Life.*, 2013; **3:** 38-51.
15. Oren, A., Diversity of halophilic microorganisms: environments, phylogeny, physiology, and applications. *Microbial. Ecol.*, 2002; **39**: 1–7.
16. Oren, A., Molecular ecology of extremely halophilic archaea and bacteria. FEMS. *J Ind Microbiol Biotechnol.*, 2002; **28:** 56-63.
17. Oren, A., Industrial and environmental

applications of halophilic microorganisms. *Environ Technol.,* 2010; **31:** 825-34.

18. Raval, V. H., Purohit, M. K., Singh, S. P., Diversity, population dynamics and biocatalytic potential of cultivable and non-cultivable bacterial communities of the saline ecosystems. *Marine Enzymes for Biocatalysis.,* 2013; 165-189.
19. Sabet, S., Diallo, L., Hays, L., Jung, W., Dillon, J. G., Characterization of halophiles isolated from solar salterns in Baja California, Mexico. *Extremophiles*., 2009; **13:** 643–656.
20. Shivanand, P., Jayaraman, G., Production of extracellular protease from halotolerant bacterium, *Bacillus aquimaris* strain VITP4 isolated from Kumta coast. *Process Biochemistry.,* 2009; **44:** 1088-1094.
21. Siddikee, M. A., Chauhan, P. S., Anandham, R., Han, G. H., Sa, T., Isolation, Characterization, and Use for Plant Growth Promotion Under Salt Stress, of ACC Deaminase-Producing Halotolerant Bacteria Derived from Coastal Soil. *J. Microbiol. Biotechnoly.,* 2010; **20:** 1577–1584.
22. Yeon, S., Jeong, W., Park, J., The Diversity of Culturable Organotrophic Bacteria from Local Solar Salterns. *The Journal of Microbiology.,* 2005; **43:** 1-10.