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Survey of halophilic bacterial diversity from Vedaranyam salt pans area in Nagapattinam district and applications

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Abstract
Halophilic bacteria grow in extreme conditions the hypersaline environments are vital of commercial significance. Halophilic microorganisms are that grow optimally within the presence of NaCl a minimum of zero 2M. The applications of halophilic bacterium embrace food and pharmaceutical industries, production of enzymes, polymers and varied cosmetic products the study was isolation and its characterization of probably necessary microorganisms from saltpans. during this preliminary investigation, the overall microorganism counts were studied from the salt sample at three-month intervals for one year. Totally seven organisms were known supported normal cultural, physiological characters. These strains were subjected to screening of potential enzymes such as; enzyme, protease, gelatinase, etc. The organisms *Bacillus firmus, Halophilic bacillus, Halobacillus salinus, Halobacillus salinarum, Halomonas aquamarina, Terribacillus halophilus* and *V. alginolyticus* showed positive for protease and amylase activity.

Keywords: Bacterial diversity, halophilic microorganisms, *Bacillus firmus,* enzymes

Introduction
Halophiles are the most promising source of enzymes stable in salts and organic solvents. Halophiles inhabit hypersaline environments where salinity often reaches saturation, and accumulate large concentrations of salts and/ or organic compatible solutes in their cells as an adaptation [1].
Bacillus species are gram-positive oxygen consuming or facultative anaerobic, sporulation bar molded microbes that are broadly spread in nature. Proteases are one of the biggest offering chemicals bookkeeping around 65% of the aggregate compound market overall [2]. Microorganisms from outrageous situations have pulled in extensive consideration as of late. This is basically because of the mystery that they hold about the sub-atomic upheaval of life and solidness of the macromolecules.
Halophiles are extremophile organisms that thrive in extreme environments with very high salt concentrations and inhabit hypersaline environments all over the biosphere [3].
Bacteria from seawater, sediment, marine invertebrates and seaweeds collected from different coastal areas of the China Sea. The antimicrobial activities of these bacteria were investigated [4].
Halophiles have outlined two distinctive versatile methodologies to adapt to the osmotic weight initiated by the high NaCl convergence of the typical situations they occupy [5]. Interestingly, direct halophiles gather the cytoplasm high measures of particular natural osmolytes, which work as Osmoprotectants, furnishing osmotic adjust without meddling with the ordinary digestion of the cell [6].
The fundamental guideline of probiotic is the use of microorganism capacity to break or unravel starch chains, protein, and fat as a result of the exceptional chemicals possessed by the organism to break the chains. Protease is a proteolytic chemical that catalyzes the severance of peptide bond into Oligopeptide and amino corrosive. The use of protease biotechnology can be found in cleanser industry, calfskin industry, added substance in sustenance industry, drug store, agribusiness, and fishery [7, 8].
Microorganism is the most potential asset of proteolytic protein. Hardly any strains of organism that can deliver protease are: *Bacillus licheniformis*, *Bacillus firmus*, *Bacillus alcalophilus*, *Bacillus subtilis, Bacillus thuringiensis* and *Pseudomonas* sp. [9]. Scarcely any business proteolytic proteins that were effectively cleansed are alcalase from *Bacillus* licheniformis, esterase from *Bacillus lentus*, bio feed expert from *Bacillus licheniformis* and subtilisin from *Bacillus alcalophilus* [7]. *Bacillus* sp. L21 bacterium and *Bacillus licheniformis* bacterium can deliver proteolytic compounds that are soluble base safe and halotolerant [10].
Halophiles living in alkaline environment, due to a wide diversity of environmental adaptation,

Phylogenetic diversity, unique salt tolerance mechanisms and potential applications. Bacteria, and probably the fungi, are furthermore responsible for the degradation of organic matter and the conversion of organic nitrogen to ammonia which can be used by the algae and perhaps brine shrimp and brine flies so common to hypersaline environments.

Materials and methods

Collection of Samples

The soil samples were collected from Vedaranyam saltpan of Nagapattinam district at three intervals four one year. All the soil samples were collected aseptically in sterile polythene bags and transferred to the lab for the further analysis [11].

Enumeration and Isolation of Halophilic bacterial isolates

The saltpan soil samples were collected from Vedaranyam. 10 gram of the soil sample was weighed and suspended in 100 ml of distilled water. The substance aliquot of 1 ml was serially diluted from 10^{-1} to 10^{-7}. The serial dilution 0.1 ml was taken and spread evenly over the Halophilic agar media Media (pH 8.0) [12] The plates were incubated at 37 ºC for 2-3 days. Growth was observed according to the color of the Colonies these procedures were carried out for every three months (January to December, 2017) intervals for one year. Different colonies grown on media were selected and purified for further investigation [13].

Phenotypic characterization

The cultures were purified on the same salt concentration and medium from which they were isolated. All tests were performed at that salt concentration until the salt/temperature studies were performed. Standard bacteriological tests for extracellular enzyme production were performed for gelatinase, amylase, and proteinase enzymes. Selected cultures were tested for other biochemical characteristics such as indole, Methyl red, Voges proscauer, citrate, urease, TSI and carbohydrate utilization [14, 15].

Results and Discussion

The present study was carried out isolation and characterization of halophilic bacterial isolates for this investigation at three months' interval for one year (2017). The soil samples were analyzed for the microbial diversity. The soil samples collected from Vedaranyam Salt pan in the different periods. It uncovers that the low salt fixations in the period because of the higher rain fall in the rainstorm time frame brought about the high thickness and assorted variety of halophilic microorganisms. The present study was identified of total of 7 representative bacterial isolates were obtained from the soil dilution plate during the whole period of soil sample collection. The following number of halophilic bacterium isolates selected from this study is described in Table 1.

Table 1: Enumeration of halophilic bacteria

S. No.	Soil Sample	Dilution	No. of colonies
1.	2017 March	10^{-1}	TNTC
		10^{-2}	130
		10^{-3}	118
		10^{-4}	96
		10^{-5}	56
		10^{-6}	30
		10^{-7}	18
2.	2017 June	10^{-1}	TNTC
		10^{-2}	TNTC
		10^{-3}	120
		10^{-4}	98
		10^{-5}	80
		10^{-6}	49
		10^{-7}	36
3.	2017 September	10^{-1}	152
		10^{-2}	130
		10^{-3}	110
		10^{-4}	88
		10^{-5}	71
		10^{-6}	30
		10^{-7}	15
4.	2017 December	10^{-1}	160
		10^{-2}	140
		10^{-3}	118
		10^{-4}	80
		10^{-5}	55
		10^{-6}	35
		10^{-7}	13

Phenotypic characterization

The colony morphology of all strains VE-1-VE-7 were circular, convex, pale yellow; smooth, circular, raised, orange-yellow; Smooth, irregular red colour colonies; Circular. Slightly irregular, convex colonies; Reddish, circular raised colonies; Dirty white, Transparent, flat colonies; Slightly yellow, circular Transparent, flat colonies; Slightly yellow, circular Transparent, flat colonies and yellowish, circular Transparent, convex colonies respectively. The summarization of the number of halophilic bacterium isolates selected from this study is described in Table 2.

Table 2: Identification and Characterization of isolated bacterial strains

Characteristics	VE1	VE2	VE3	VE4	VE5	VE6	VE7
Optimum pH	8.5	8.0	8.5	8.0	8.5	8.0	8.0
Optimum T^0	37^0C	37^0C	37^0C	37^0C	37^0C	37^0C	37^0C
				Growth at NaCl concentrations (%)			
5	+	+	+	+	+	+	+
10	++	++	++	++	++	++	++
15	+++	+++	+++	+++	+++	+++	+++
20	+	-	-	+	-	+	+
25	+	-	+	+	-	-	+
Pigments	+	+	+	+	+	+	+
Gram stain	Positive	Positive	Positive	Negative	Positive	Negative	Positive
Morphology	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Motility	+	_	+	-	+	-	-
Catalase	+	+	+	-	+	-	+
Oxidase	-	+	+	-	+	-	+
Indole	-	-	+	-	-	-	-
MR	+	-	-	-	-	-	-
VP	-	-	-	-	-	+	-
Citrate	+	-	-	-	-	+	-
Urease	-	-	-	-	-	-	-
Starch hydrolysis	-	-	-	-	+	-	+
Gelatin hydrolysis	+	+	+	+	+	+	+
Casein hydrolysis	+	+	+	+	+	+	+
				Carbohydrate fermentation			
Glucose	+	+	-	+	+	+	+
Sucrose	+	+	-	-	+	-	+
Lactose	+	+	-	-	+	-	+
Arabinose	-	-	-	-	-	-	+
Maltose	+	+	-	-	-	+	-
Mannose	-	-	-	-	-	-	-

Different colonies grown on media were selected and purified for further investigation. A total number of 36 colonies were selected for characterization and identification studies includes, 12 from March-2017, 11 from June-2017, 9 from September-2017 sample and 6 from December-2017 sample. Based on the cultural and phenotypic characteristics total of 7 strains were identified. The probably identified strains includes *Bacillus firmus*, *Halophilic bacillus*, *Halobacillus salinus*, *Halobacillus salinarum*, *Halomonas aquamarina*, *Terribacillus halophilus* and *V. alginolyticus* from VE1-VE7 respectively

Conclusion

The present study was aimed to investigate enumeration of halophilic bacteria and enzyme production by extreme halophilic bacteria. Many industries, such as leather, agricultural and food industries generate hypersaline wastewater. Such waste- water generally is alkaline and contains a high amount of organic content [1].

Effect of pH and NaCl concentrations on enzyme activity was also studied. The optimum pH for enzyme activity, pH 8.0, is in agreement with the optimum pH for the decolorization by *Halophilic bacillus*. Despite of the enzyme ability to decolorize methyl red in the presence of NaCl at concentrations up to 13% (w/v), it was shown that increasing the salt concentration significantly decreased the enzyme activity [16]. Halophilic and halotolerant bacteria have shown great potential in the production of enzymes with novel characteristics in the detergent, baking, dairy, and leather industries

References

1. Abdel-Hamed AR, Abo-Elmatty DM, Wiegel J, Mesbah NM. Biochemical characterization of a halophilic, alkalithermophilic protease from Alkalibacillus sp. NM-Da2. Extremophiles. 2016; 20(6):885-94.
2. Rao MB, Tanksale AM, Ghatge MS, Deshpande VV. Molecular and Biotechnological Aspects of Microbial Proteases†. Microbiology and molecular biology reviews. 1998, 62.
3. Todkar S, Todkar R, Kowale L, Karmarkar K, Kulkarni A. Isolation and Screening of Antibiotic producing Halophiles from Ratnagri coastal area, State of Maharahstra. Int. J Sci. Res Publ. 2012; 2(1):2250-3153.
4. Manikandan P, Tamizhazhagan V, Gnanasekaran A, Pk S. Purification, Characterization and In Vitro Antimicrobial Activity of Proteins from Marine Bacterium – Bacillus sp, 2017, 5(11).
5. Prakash, Upadhyay G, Gupta C, Pushpangadan P, Singh KK. Antioxidant and free radical scavenging activities of some promising wild edible fruits. International Food Research Journal, 2012, 19.
6. Dumorné K, Camacho-Córdova DI, Astorga-Eló M, Renganathan P. Extremozymes: A Potential Source for Industrial Applications. J Microbiol Biotechnol. 2017; 27(4):649-59.
7. GUPTA N. Isolation and Identification of Extracellular Alkaline Protease Producing Bacteria from Soil Sample and Enzyme Immobilization. researchgate.net. [cited 2018 Jun 26];
8. Khan MA, Ahmad N, Usman Zafar A, Nasir IA, Qadir MA. Isolation and screening of alkaline protease producing bacteria and physio-chemical characterization of the enzyme. African J Biotechnol. 2011; 10(33):6203-12.
9. Essghaier B, Dhieb C, Rebib H, Ayari S, Rezgui A, Boudabous A, *et al*. Plant Pathology & Microbiology Antimicrobial Behavior of Intracellular Proteins from

Two Moderately Halophilic Bacteria : Strain J31 of Terribacillus halophilus and Strain M3-23 of Virgibacillus Marismortui. 2014; 5(1):1-7.
10. Vishnuvardhan Reddy S, Thirumala M, Farooq M, Sasikala C, Venkata Ramana C. Marinococcus salis sp., nov., a moderately halophilic bacterium isolated from a salt marsh. Arch Microbiol. 2016; 198(10):1013-8.
11. Di Meglio L, Santos F, Gomariz M, Almansa C, López C, Antón J, *et al*. Seasonal dynamics of extremely halophilic microbial communities in three Argentinian salterns. FEMS Microbiol Ecol. 2016; 92(12):1-15.
12. Bergey DH, David H, Holt JG. Bergey's manual of determinative bacteriology. 9th ed. [cited 2018 Aug 8]. 1994, 787.
13. Saju KA, Babu MM, Murugan M, Raj ST. Survey on Halophilic microbial diversity of Kovalam Saltpans in Kanyakumari District and its industrial applications. J Appl Pharm Sci. 2011;
14. Agadagba SK, Bash E, Chitte RR, Deshmukh SV, Kanekar PP, Chutipongtanate S, *et al*. Isolation of Actinomycetes from Soil. J Microbiol Res. 2014; 3:136-40.
15. Arahal DR, Márquez MC, Volcani BE, Schleifer KH, Ventosa A. Reclassification of Bacillus Marismortui as Salibacillus Marismortui comb. Nov. Int. J Syst. Evol Microbiol. 2000; 5(4):1501-3.
16. Eslami M, Amoozegar MA, Asad S. Isolation, cloning and characterization of an azoreductase from the halophilic bacterium Halomonas elongata. Int. J Biol. Macromol. 2016; 85:111-6.