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Exploration of the biocatalytic potential of Halophilic bacteria isolated from Coastal Regions of Gujarat

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ABSTRACT
For decades marine environments have been explored for microbial diversity and diverse biomolecules including novel biocatalysts. The present study was aimed in quest of mining marine samples (soil and water) for the isolation of unexplored bacterial cultures and utilizing biocatalytic potential under a multitude of physicochemical adversities. A total of 115 morphologically distinct bacterial isolates were obtained from sites namely Khambhat, Dhuvaran, Bhavnagar, Tithal, and Diu deploying enrichment culture technique using Zobell marine broth (pH-9) supplemented with varying NaCl (5-20% w/v) concentration. All the isolates were characterized initially by their cultural and morphological characteristics. The biocatalysis potential was assessed on selective media by calculating the halo formation around the colonies after the utilization of specific substrates. Out of all isolates, 55 bacteria were able to synthesize protease. Amylase secretion was detected among 27 isolates, followed by 24 bacteria that were found to be lipase as well as laccase producers respectively. Eight isolates were able to synthesize xylanase followed by 06 organisms that secrete cellulase. Another important enzyme L-glutaminase biosynthesis was checked and 35 isolates gave positive results. The most promising cultures will be selected for biochemical profiling and further studies. All these enzymes have suitable applications in food, textile, detergent, medicine, healthcare, and allied areas and will be explored for the same.
Keywords: *Halophiles, Hydrolytic enzymes, Protease, Amylase, L-glutaminase, Xylanase*

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INTRODUCTION

Microorganisms serve as a gold mine and are mostly preferred for industrial fermentation as they grow rapidly, required minimum space for cultivation, and are easy to manipulate its genetic nature. Isolating a microbial strain that can synthesize the enzyme in great quantities is the first step in the establishment of a technology for the industrial production of enzymes [1]. Bacteria are frequently employed to produce extracellular alkaline proteases at a commercial level across all types of microorganisms [2]. Alkaliphilic, halophilic, and other extreme forms of active enzymes are said to be abundant in the marine ecosystem and have many uses in various industrial operations. Environmental, commercial, and fundamental bioenergetics interests are sparked by marine bacteria that thrive in the pH range of 8.0 to 11.0 and in the NaCl concentration range of 0.3-5.0 M. These commercial uses have led to the isolation of halo-alkaliphilic bacteria from a range of natural and artificial habitats. Additionally, these organisms were obtained from non-alkaline environments including neutral and acidic soils, indicating that they may be rather common [3,4]. Numerous halo-alkaliphiles can adjust their environment, which is one of their most significant and notable features. To optimize the external pH for development, they can alkalinize neutral media or acidify extremely alkaline medium. The curiosity in halo-alkaliphiles' physiological adaptation to high salt and pH as well as its prospective usefulness in biotechnological applications has caused them to gain more attention recently, despite earlier being thought of only as oddities [2-5]. The most vital phases in the commercial manufacturing of enzymes are the screening of efficient protease-producing microbial strains and the selection of the best candidate organism. Similarly, industries need enzymes that can work under a variety of physico-chemical conditions, thereby selecting enzymes that act effectively under the desired

conditions is essential. The main impetus behind the lookup for new enzyme sources has been the need for proteolytic enzymes with better activity and stability at extreme salt, pH, and temperatures [2-6]. The work relates to isolate and screen halotolerant bacteria that produce extracellular alkaline protease and biochemical characterization of isolate in view of isolating some unexplored bacterial isolate.

MATERIALS AND METHODS

Samples collection

For the isolation of the haloalkaliphilic bacteria, the marine water and soil samples were collected from the various sites along the coast of Gujarat (Dhuvaran, Khambhat, Tithal, Somnath, Diu, Nadabet, and Bhavnagar) (Fig. 1). The samples were collected in the sterile sampling bottles; the pH and temperature of all the samples were measured manually at the time of the sample collection, and processed for the isolation in the laboratory. The samples were stored at 4°C until further processing.

Physical and chemical analysis of the samples

Before proceeding with the isolation, the marine soil and water samples were subjected to physio-chemical analysis, such as electric conductivity and pH for both type of samples, while, organic carbon, P, K, S, Na, and N determinate for soil samples and calcium, magnesium and chloride was analysed for water samples. All the parameters were analysed as per the method described by BIS (Bureau of Indian Standards). The details of sample characterization are listed in below table 1.

Enrichment and isolation

The haloalkaliphilic bacteria were isolated by taking 1.0 gm of the soil sample into the 100ml of the enrichment medium using modified Schlegel and Jannasch enrichment culture techniques [7] in Zobell marine broth (ZMB) and nutrient broth with varying concentrations of NaCl (5%, 10%, and 15% (w/v)) at pH 9. Similarly, 10ml of water samples was taken and mixed it with enrich medium (As per above mention for soil) for the enrichment. The pH of the medium was adjusted by adding separately autoclaved Na_2CO_3 (20%w/v). After inoculation, the flasks were incubated on an environmental shaker at 37°C with regular monitoring of the turbidity of the enrichment media. After 72h of growth, samples were processed by serial dilution technique up to 10^{-7}. A 100µl of each dilution was spread onto ZM agar and nutrient agar (3%w/v) plates and incubated at 37°C for 48h to obtain isolated colonies. After 48h of incubation, based on colony characteristics, various isolated colonies were selected and pure cultures were obtained by subsequent streaking on the nutrient agar plate.

Maintenance and preservation

The pure cultures were preserved on the nutrient agar media (5-15% w/v NaCl; and pH 9) and stored at 4°C. After screening for the extracellular proteases, the isolates were preserved on a gelatin agar medium. The cultures were subsequently transferred on fresh nutrient agar every 3 months intervals. Regular maintenance of these cultures in slants and glycerol stocks was carried out.

Screening for extracellular enzymes

Actively growing cultures of different isolates were prepared on the nutrient agar plate (0-20% NaCl; pH 9) and used as inoculum (A_{600}>1.0) for the primary screening of hydrolytic enzymes. The cultures were inoculated in the form of regular spot inoculation on respective selective enzyme screening- medium (Detailed medium name and composition are described below in Table 2). The pH of the medium was adjusted to 9 by adding separately autoclaved 20% Na_2CO_3 (w/v). The plates were incubated for 48-72h at 37°C and reagent/solution was added to confirm the positive isolates for enzyme secretion. The clear zone surrounding the colony indicated the secretion of extracellular hydrolytic enzymes [8-9].

Characterization of the organisms

Colony characteristics, cell morphology, and Gram reaction

The pure cultures of the isolated bacteria were streaked on the nutrient agar plate with the corresponding enrichment conditions of the NaCl (5-15%, w/v) and pH 9, and their colony characteristics were observed. The cell morphology, cell arrangement, and the Gram reaction were followed in the activated culture in nutrients at the corresponding enrichment conditions.

Biochemical characterization

The isolates were studied for their biochemical and metabolic activities. The biochemical tests included the production of catalase, oxidase, H_2S, ammonia, indole, hydrolysis of urea, reduction of nitrate and litmus; fermentation of the sugars such as glucose, fructose, sucrose, maltose, lactose, and xylose. All the biochemical media and their test reagents were prepared as mentioned by Cappuccino and Sherman [10]. Owing to the halophilic nature of the organisms, all the biochemical media were supplemented with 5% (w/v) NaCl. The individual isolate was inoculated to the respective biochemical medium and incubated at 37°C for 24h and results were subsequently observed.

RESULTS AND DISCUSSION

Sites for samples collection

The haloalkaliphilic bacteria were isolated from the four seawater samples collected from the Dhuvaran region (pH 8.5) (22°13'52.9"N 72°45'39.3" E); from the Khambhat region (pH 8.9) (22°18'0"N 72°37'12" E), from Somnath (pH 8.5) (20°53'16.9"N 70°24'5.0" E), from Tithal (pH 8.3) (20.588°N 72.901°E), from Bhavnagar (pH 9.0) (21°46'12"N 72°09' 0" E) and another from the Diu region (pH 8.0) (20°42'36"N 70°58'48"E). The temperature was below 37°C at the time of sample collection. The salinity and pH of the samples varied from 2.5- 4% and 8.0-8.9, respectively. All four samples were colorless.

Physical and chemical analysis of the samples

The salinity and alkalinity of the collected seawater/soil samples were nearly equal in all samples, but the values of the E.C., O.C., P, K, S, N, *Ca, *Mg, *Cl, and Na concentrations varied. The seawater/soil samples collected from other regions were harder compared to water collected from the Khambhat. The results of the chemical and physical analysis of the seawater resembled the literature values for the seawater as described in the previous chapter Table 1. Multiple research is indicating that the type of soil and its micro/macro constituents play an important role in the growth of microorganisms [4]. Similarly, the presence of nutrients in the respective habitat is also responsible for the occurrence of selective microbes over there and the biosynthesis of primary and secondary metabolites by it [11-12].

Enrichment and isolation of the organisms

Isolation of haloalkaliphilic bacteria was carried out from the samples collected from the Dhuvaran (Soil-pH 8.5), Khambhat (Soil-pH 8.9, Water-pH 9.2), Somnath (Soil-pH 8.5), Tithal (Soil-pH 8.3, Water-pH 8.6), Bhavnagar (Soil-pH 9.0), Nadabet (Soil- pH 8.5, Water-pH 9.2) and Diu (Soil-pH 8.0) at about 1-2 feet deep seawater. The pH of the samples indicated the alkaline nature of the habitat. Figure 2 and Table 3 describe, by using enrichment techniques on the nutrient medium (5-15% w/v NaCl, pH 9), 115 haloalkaliphilic bacteria were selected based on their colony characteristics and cell morphology.

Concerning salt, nearly all the isolates were able to grow in the range of 0-20 (w/v) NaCl at pH 9. However, the optimum pH for growth was 9 and the optimum salt concentration ranged between 2.5-10% w/v for most of the bacteria. This indicated that the organisms were moderately halophilic bacteria. Numerous bacteria were isolated from the different coastal regions of Gujarat such as *Geomicrobium halophile, Oceanobacillus oncorhynchi, Oceanobacillus khimchii* [3] and *Halomonas pacifica, H. stenophila, Bacillus haynesii, B. licheniformis* and *Oceanobacillus aidingensis* [13].

Characterization of isolates

Colony characterization

The pure culture of all the isolates was maintained on a nutrient agar plate supplemented by salt (NaCl) (Fig 3). The isolates were primarily screened based on the colony appearance on the nutrient agar medium. The size of the colonies was in the range of 1-4mm, with a round/oval shape and entire margin and transparent/opaque with smooth/rough texture. Several colonies are found to be pigmented (yellow, cream, pink, red, orange, white) as well as with flat/raised elevation (Table 4).

Cell morphology and Gram reaction

The isolates varied in their cell morphology, cell arrangement, Gram reaction, spore formation ability, and motility. Among all the isolates, 78 were observed to be Gram-positive while 37 were Gram-negative bacteria. The morphology of the majority of the isolates is related to small rod/coccid while some are big thick rod-shaped. The arrangement was mostly single, chain, and in pairs with few in clusters according to their Gram reaction (Fig 4). A total of 12 bacteria were able to produce terminal spores. The motility testing indicates a total of 26 bacteria were found to be motile while others are non-motile. Similarly, Sanjay and his co-workers [14] isolated a total of 20 different types of bacteria, among them 3 were pigmented and 17 were non-pigmented isolates, 7 Gram-negative cocci, 2 Gram-positive cocci, 4 Gram-negative bacilli, and 7 Gram-positive bacilli were identified based on the form of the cells and Gram's staining. The results of the motility tests showed that 18 isolates were motile and only 2 isolates were non-motile.

Screening for the extracellular enzymes

The isolates were screened for the secretion of different extracellular enzymes; proteases, amylases, xylanase, cellulase, lipase, glutaminase, laccase, tannase, urease, cellulases, catalase, oxidase, and amidase (Table 5). The detailed results of the screening of hydrolytic enzymes was described in Table 5. Overall, among all isolates, 55 isolates were able to synthesize protease, 27 were able to secrete amylase, 24 isolates produced lipase and laccase, 35 isolates produced glutaminase, while cellulase and xylanase were produced by only 6 and 8 isolates, respectively. The extracellular enzyme secretion by bacterial isolate is shown in Figure 3.1. Enzymes have previously been utilized in several industrial goods and processes, as a result, their demand is increasing. More than a few research has examined the function of hydrolytic enzymes

produced by marine bacteria in a variety of harsh environments that open their path for industrial applications [3-4, 15-17].

Biochemical characterization

The biochemical and metabolic activities of the selected five potent isolates were studied for further characterization. Nearly all the isolates showed positive catalase, oxidase, and ammonia production test with varying extents and negative tests for Indole and H_2S production. All the isolates were Nitrate positive except Tw-0.5-9-2 and D-5-9-4. For Triple Sugar Iron (TSI) Test K-15-9-6 did not show any reaction. The biochemical test and its results are depicted in Table 6. The sugar utilization test indicates glucose, fructose, lactose, and sucrose are the sugar that is frequently utilized by the organism for growth. However, the efficacy of sugar utility is varying concerning isolate metabolism. The isolates also screen for the production of plant growth-promoting substances and the degradation of industrial textile dye. The details of the biochemical profiling of all five isolates was described in Table 6.

Fig. 1 Sample collection from Gujarat coastal region

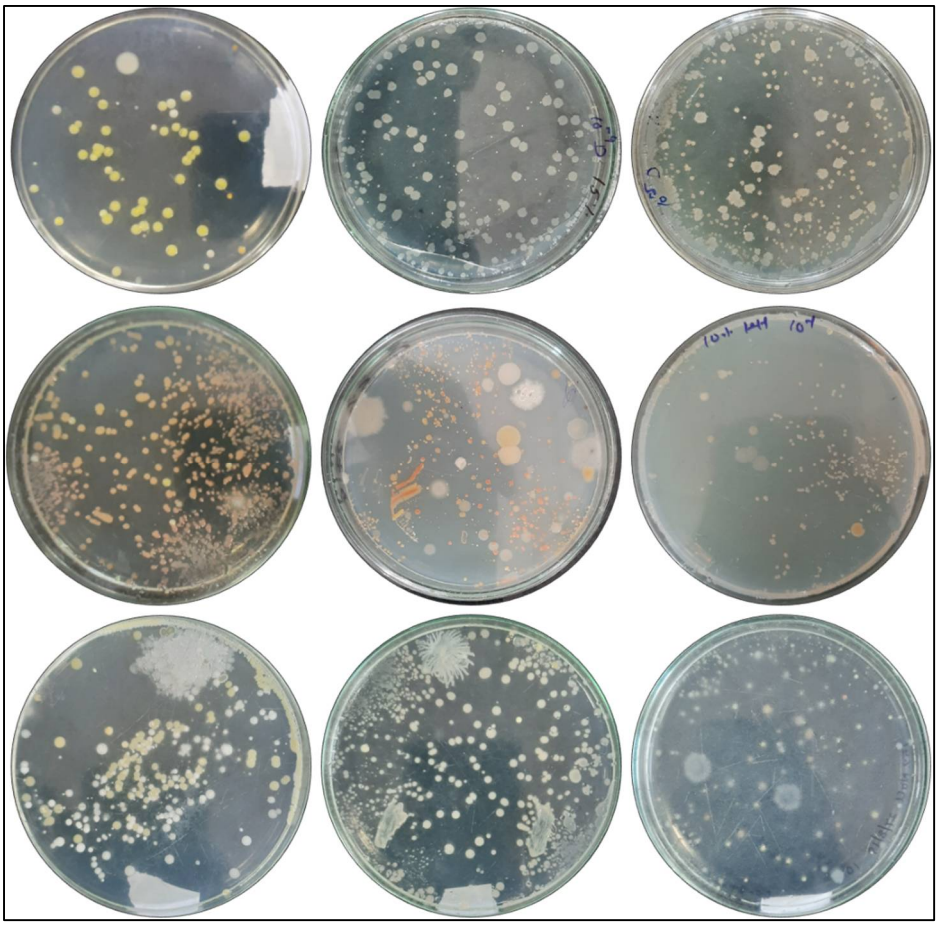

Fig. 2 Isolation of salt tolerant bacteria from Gujarat coastal region

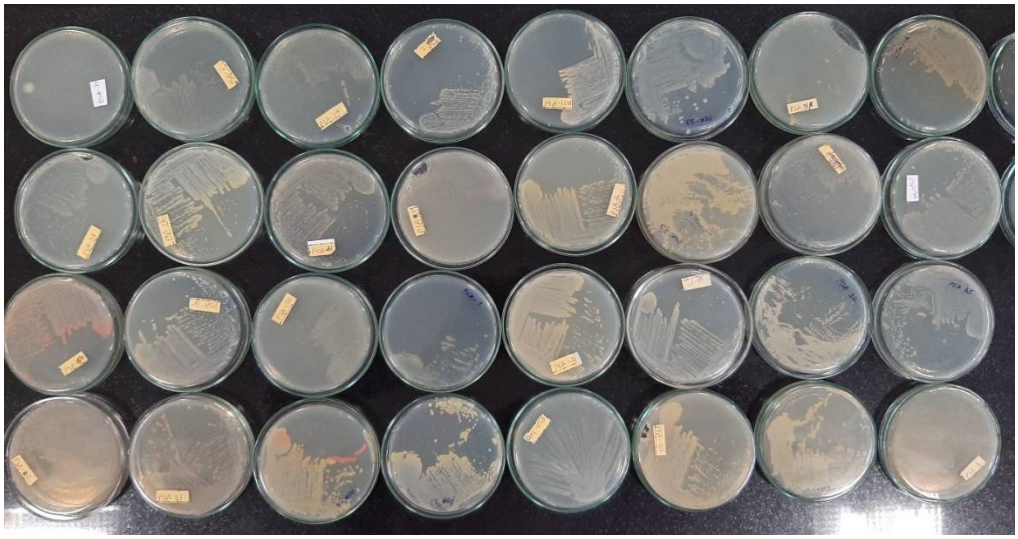

Fig. 3 Pure culture plates of the isolates

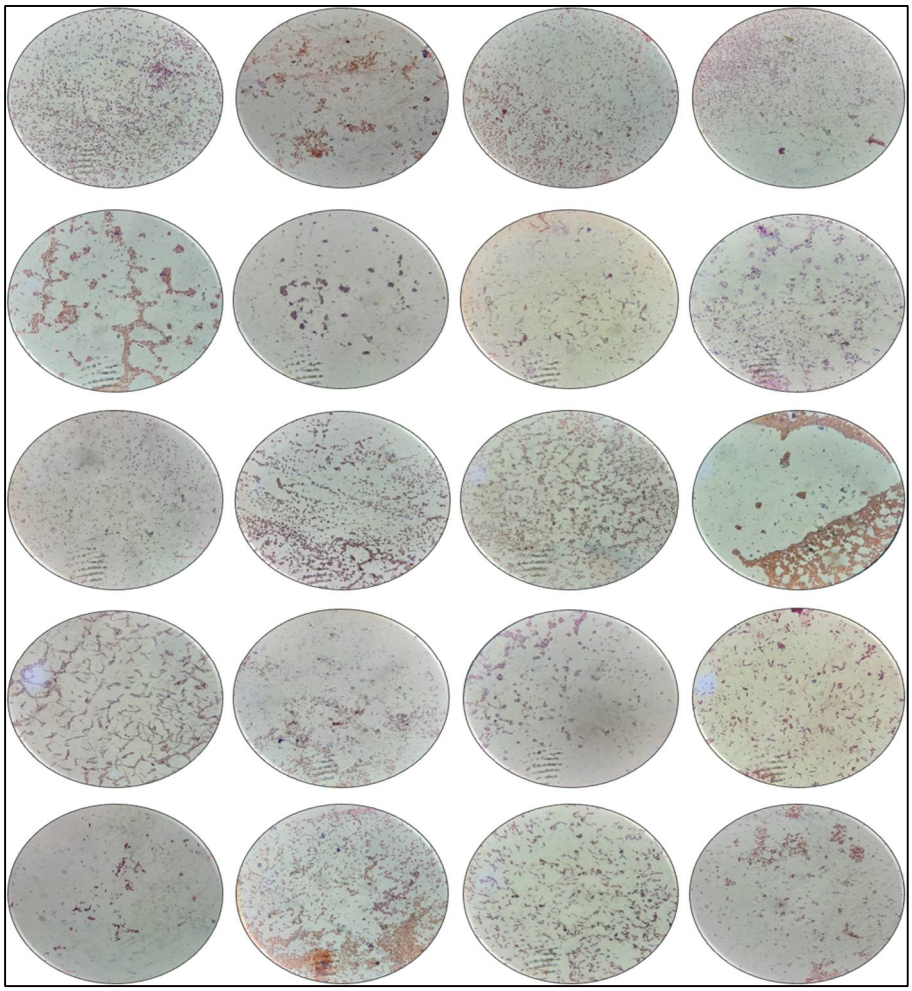

Fig. 4 Gram staining of the isolates

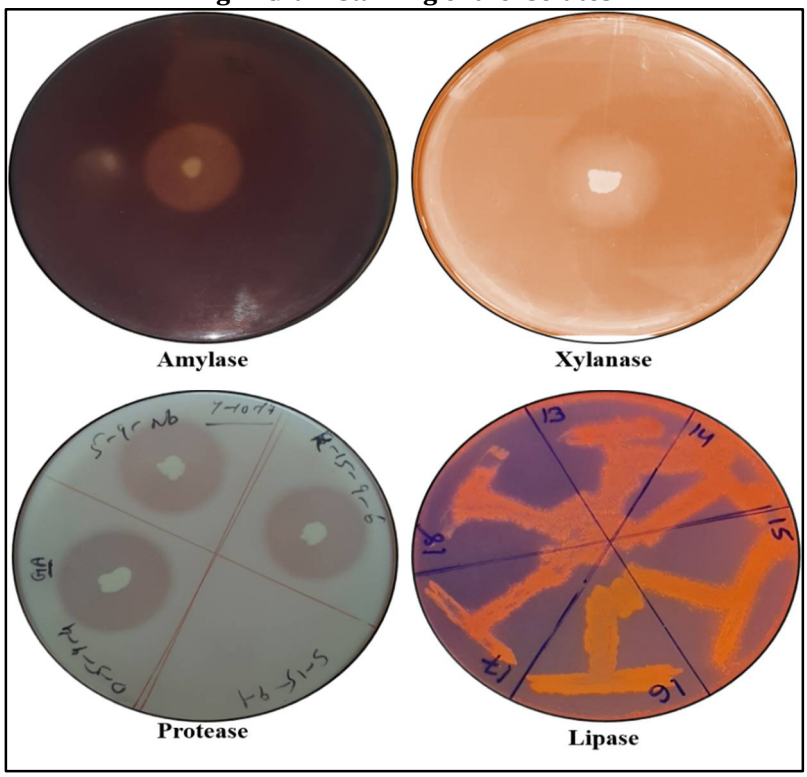

Fig. 5 Screening of hydrolytic enzymes

Table 1 Physical and chemical analysis of seawater samples

Parameter	Method name	Collection site								
		Tithal (T)		Dhuvaran (D)	Nadabet (N)		Khambhat (K)		Diu (Du)	Somnath (S)
		Soil	Water	Soil	Soil	Water	Soil	Water	Water	Water
*E.C. (Ds/m)	EC meter	3.338	48.49	2.037	31.97	118.9	19.00	3.027	55.65	56.25
pH	pH meter	8.80	7.03	8.87	7.59	6.79	8.29	7.96	7.72	7.38
*Ca (ppm)	Versenate EDTA Method	-	1000	-	-	1600	-	90	900	900
*Mg (ppm)	Versenate Method	-	1320	-	-	2340	-	150	1800	1680
*Cl (ppm)	Silver Nitrate Method	-	28400	-	-	74550	-	21300	35500	35500
*O.C (%)	Walkley & Black	0.12	-	0.21	0.42	-	0.48	-	-	-
*P (ppm)	Olsen's Method	18.34	-	7.62	9.88	-	157.90	-	-	-
*K (ppm)	Flame Photometer	805	-	610	560	-	1095	-	-	-
*S (ppm)	Turbidity	66.85	-	63.10	538.9	-	441.7	-	-	-
*N (ppm)	Alkaline $KMnO_4$ Method	20.83	-	12.37	12.56	-	2.76	-	-	-
*Na (ppm)	Flame Photometer	18500	-	27750	55000	-	47000	-	-	-

*Tests were performed as per the BIS (Bureau of Indian Standards) IS: 3025

Table 2 List of medium for screening of hydrolytic enzymes

Enzyme	Screening media	Reagent used for test	Interpretation
Amylase	Starch agar medium	Iodine solution 0.01N	Zone of hydrolysis indicates positive results
Xylanase	Xylene agar medium	1% Congo red	Zone of hydrolysis indicates positive results
Protease	Gelatine agar medium	Frazier's reagent	Zone of hydrolysis indicates positive results
Lipase	Rhodamine agar medium	-	In UV light, fluorescence indicates positive results
Cellulase	Cellulose-supplemented nutrient agar medium	1% Congo red 1N NaOH	Zone of hydrolysis indicates positive results
Glutaminase	Glutamine salt medium with phenol red	-	Colorless to pink color change indicates positive results
Laccase	Tannic acid-supplemented nutrient agar medium	-	Colorless to black/brown color change indicates positive results

Table 3 Isolation of bacteria from Gujarat coastal region

Site	Location	Enrichment of sample	Total isolates
Dhuvaran	22°13'52.9"N 72°45'39.3"E	Zobell marine medium (5%, 10%, 15%, 20% w/v NaCl)	22
Khambhat	22°18'0"N 72°37'12"E		37
Somnath	20°53'16.9"N 70°24'5.0"E		12
Diu	20°42'36"N 70°58'48"E		06
Tithal	20.588°N 72.901°E		03
Bhavnagar	21° 46' 12" N 72° 09' 0" E		35
Total			115

Table 4 Cultural characteristics of isolates

Isolate	Colony character						
	Size	Shape	Margin	Elevation	Texture	Opacity	Pigment
S-15-10-1	Medium	Oval	Entire	Flat	Mucoid	Opaque	Off white
S-15-9-1	Medium	Irregular	Undulate	Raised	Mucoid	Opaque	Cream
S-10-9-2	Medium	Round	Entire	Flat	Mucoid	Transparent	Colourless
S-5-9-1	Small	Round	Entire	Convex	Mucoid	Opaque	Light Pink
S-5-9-2	Medium	Irregular	Undulate	Raised	Mucoid	Opaque	Cream
S-5-9-3	Medium	Round	Entire	Raised	Mucoid	Opaque	Red
S-15-10-2	Medium	Round	Entire	Flat	Mucoid	Opaque	Off white
S-15-10-3	Small	Round	Undulate	Raised	Mucoid	Opaque	Off white
S-10-9-1	Medium	Irregular	Entire	Convex	Mucoid	Opaque	Off white
DU-15-9-4	Medium	Round	Entire	Flat	Mucoid	Opaque	Off white
DU-15-9-3	Small	Irregular	Lobate	Raised	Mucoid	Opaque	Off white
DU-15-9-1	Medium	Round	Entire	Raised	Smooth	Opaque	Off white
B-15-9-11	Medium	Round	Entire	Convex	Mucoid	Opaque	Off white
B-15-9-12	Medium	Irregular	Lobate	Convex	Mucoid	Opaque	Off white
B-15-9-7	Medium	Round	Entire	Raised	Mucoid	Opaque	Off white
B-10-9-9	Small	Round	Entire	Convex	Smooth	Opaque	Off white
B-10-9-7	Small	Round	Undulate	Raised	Mucoid	Opaque	Cream
B-5-9-3	Medium	Oval	Entire	Raised	Mucoid	Opaque	Lemon Yellow
D-15-9-3	Small	Round	Entire	Convex	Mucoid	Opaque	Cream
D-15-9-2	Medium	Round	Lobate	Flat	Mucoid	Transparent	Cream
D-15-9-5(2)	Medium	Irregular	Entire	Flat	Mucoid	Transparent	Cream
D-5-9-4	Medium	Oval	Entire	Raised	Mucoid	Opaque	Lemon Yellow
D-5-9-3	Medium	Irregular	Lobate	Raised	Mucoid	Opaque	Lemon Yellow
D-15-9-4	Medium	Round	Entire	Flat	Mucoid	Transparent	Cream
D-5-9-1	Small	Round	Undulate	Flat	Mucoid	Transparent	Pink
D-15-9*1	Medium	Round	Entire	Raised	Mucoid	Transparent	Lemon Yellow
D-5-9-4(2)	Medium	irregular	Lobate	Raised	Mucoid	Opaque	Yellow
D-10-9-4	Small	Oval	Entire	Raised	Mucoid	Opaque	Orange
D-15-9-5(1)	Medium	Round	Lobate	Convex	Mucoid	Opaque	Cream
K-15-9-9	Small	Oval	Entire	Raised	Mucoid	Opaque	Red
K-15-9-4	Medium	Irregular	Lobate	Convex	Mucoid	Opaque	Cream
K-15-9*2	Small	Oval	Entire	Raised	Mucoid	Opaque	Orange
K-15-9-2(1)3	Medium	Round	Entire	Raised	Mucoid	Opaque	Cream
K-15-9-7	Small	Oval	Entire	Raised	Mucoid	Opaque	Cream
K-15-9-10	Medium	Irregular	Lobate	Raised	Mucoid	Opaque	Cream
K-20-9-4	Medium	Round	Entire	Flat	Mucoid	Transparent	Cream
K-15-9-2	Medium	Irregular	Lobate	Raised	Mucoid	Opaque	Cream
K-15-9-3	Medium	Round	Entire	Flat	Mucoid	Transparent	Off white
K-15-9-6	Small	Oval	Entire	Raised	Mucoid	Opaque	Yellow

K-20-9-1	Medium	Oval	Lobate	Raised	Mucoid	Opaque	Off white
K-15-10-2	Small	Round	Entire	Flat	Smooth	Transparent	Yellow
K-10-9-3	Medium	Round	Lobate	Raised	Mucoid	Opaque	Orange-red
K-5-9-2	Medium	Round	Entire	Flat	Smooth	Transparent	Lemon yellow
K-20-9-3	Medium	Round	Entire	Flat	Mucoid	Transparent	Pink
K-15-9-5	Small	Round	Lobate	Raised	Smooth	Opaque	Orange
K-10-9-2	Medium	Irregular	Undulate	Convex	Mucoid	Opaque	Pink
K-10-9-1	Small	Oval	Entire	Raised	Mucoid	Opaque	Orange
K-10-9-2(1)	Medium	Round	Entire	Flat	Smooth	Transparent	Golden yellow
K-20-9-K2	Small	Round	Entire	Raised	Smooth	Opaque	Cream
K-15-10-1(9)	Medium	Irregular	Lobate	Convex	Mucoid	Opaque	Golden yellow
K-20-9-K1	Small	Oval	Entire	Raised	Mucoid	Opaque	Cream
K-20-9*1(4)	Small	Oval	Undulate	Raised	Mucoid	Opaque	Off white
K-20-9*1	Medium	Round	Entire	Flat	Mucoid	Transparent	Off white
K-20-9-K3	Medium	Round	Lobate	Flat	Smooth		Yellow
K-15-9*10	Small	Irregular	Entire	Raised	Smooth	Opaque	Yellow
K-10-9-4	Small	Round	Entire	Convex	Mucoid	Opaque	Off white
Tw-0.5-9-1	Medium	Round	Lobate	Raised	Mucoid	Opaque	Off white
Tw-0.5-9-2	Big	Round	Entire	Raised	Smooth	Opaque	Cream
Tw-0.5-9-3	Medium	Irregular	Entire	Raised	Mucoid	Opaque	Off white

Table 5 Hydrolytic enzymes screening of selected isolates

Hydrolytic enzymes screening of selected isolates								
Site	**Isolate**	**Protease**	**Amylase**	**Glutaminase**	**Lipase**	**Xylanase**	**Cellulase**	**Laccase**
Somnath	S-15-10-1	+	+	+	-	-	-	-
	S-15-9-1	+	+	+	-	-	-	-
	S-10-9-2	+	+	+	-	-	-	-
	S-5-9-1	+	+	+	+	+	-	-
	S-5-9-2	+	+	+	+	-	-	+
	S-5-9-3	+	+	+	+	-	-	-
	S-15-10-2	+	-	+	+	-	-	+
	S-15-10-3	+	-	+	+	-	-	+
	S-10-9-1	+	-	+	-	-	-	+
Diu	DU-15-9-4	+	+	+	-	-	-	-
	DU-15-9-3	+	-	+	-	-	-	-
	DU-15-9-1	+	+	+	-	-	-	-
Bhavnagar	Bh-15-9-11	+	-	+	-	-	-	-
	Bh-5-9-3	+	+	+	+	+	-	+
	Bh-15-9-7	+	-	+	+	-	--	-
	Bh-10-9-9	+	-	+	-	-	-	-

	Bh-10-9-7	+	+	+	-	+	-	-
Dhuvaran	D-15-9-3	+	+	+	+	-	+	-
	D-15-9-2	+	+	+	+	-	+	-
	D-15-9-5(2)	+	+	+	+	+	+	-
	D-5-9-4	+	+	+	+	+	-	+
	D-5-9-3	+	+	+	+	-	-	-
	D-15-9-4	+	+	+	+	-	-	+
	D-5-9-1	+	-	+	-	-	-	+
	D-15-9*1	+	-	-	-	-	-	-
	D-5-9-4(2)	+	-	-	-	-	-	-
	D-10-9-4	+	-	-	+	-	-	+
	D-15-9-5(1)	+	-	+	+	+	-	-
Khambhat	K-15-9-9	+	+	+	-	-	-	+
	K-15-9-4	+	+	-	+	-	-	-
	K-15-9*2	+	+	-	+	-	-	-
	K-15-9-2(1)3	+	+	-	-	-	-	-
	K-15-9-7	+	+	+	-	-	-	-
	K-15-9-10	+	+	+	+	+	-	+
	K-20-9-4	+	+	+	-	+	-	+
	K-15-9-2	+	+	-	+	-	-	+
	K-15-9-3	+	+	+	+	-	-	-
	K-15-9-6	+	+	+	+	+	+	+
	K-20-9-1	+	+	+	-	+	-	-
	K-15-10-2	+	+	+	-	-	-	+
	K-10-9-3	+	+	+	+	-	-	-
	K-5-9-2	+	-	-	-	-	-	+
	K-20-9-3	+	-	-	-	-	-	--
	K-15-9-5	+	-	+	-	-	-	+
	K-10-9-2	+	-	+	-	-	-	+
	K-10-9-1	+	-	-	+	-	-	-
	K-10-9-2(1)	+	-	-	+	-	-	+
	K-20-9-K2	+	-	+	-	-	-	-
	K-15-10-1(9)	+	-	-	-	-	-	-
	K-20-9-K1	+	-	-	-	-	-	-
	K-20-9*1(4)	+	-	-	-	-	-	+
	K-20-9*1	+	-	-	-	-	-	+
	K-20-9-K3	+	-	-	-	-	-	+
	K-15-9*10	+	-	-	-	-	+	+
	K-10-9-4	+	-	-	-	-	+	+
Tithal	Tw-0.5-9-1	+	+	-	+	-	+	-
	Tw-0.5-9-2	+	+	-	+	+	+	-
	Tw-0.5-9-.3	+	+	-	+	-	+	-

Table 6 Biochemical characteristics of the isolates

Isolates	K-15-9-6	D-5-9-4	B-5-9-3	Tw-0.5-9-2	K-10-9-3
Sugar utilization					
D-Xylose	-	-	+	+	+
D-Sorbitol	-	+	+	+	+
D-Glucose	+	+	+	+	+
Fructose	+	+	+	+	+
Saccharose/Sucrose	+	+	+	+	+
D-Trehalose	+	+	+	+	+
D-Mannitol	-	+	-	-	-
D-Mannose	-	-	+	-	+
Lactose	+	+	+	+	-
D-Galactose	-	+	-	+	-
D-Ribose	-	+	-	+	+
D-Maltose	-	+	+	+	+
D-Raffinose	-	+	+	+	-
Biochemical test					
Methyl red (M-R) test	-	-	+	+	-
Voges-Proskauer's (V-P) test	-	-	+	+	+
Huge and Leifson's (O-F) test	-	+	-	-	-
Citrate utilization test	+	-	-	-	+
Phenylamine deaminase test	-	+	+	+	-
Nitrate reduction test	+	-	+	-	+
Triple sugar iron (TSI) test	-	+	+	+	+
Motility	+	+	+	+	-
Screening of PGP traits					
Indole acetic acid	+	+	-	+	-
Gibberellic acid	+	+	+	+	+
Ammonia production	+	+	-	-	+
HCN production	-	-	+	+	-
Zink solubilization	+	-	-	-	-
Phosphate solubilization	-	+	-	+	+
EPS production	-	+	+	-	+
Geosmin production	-	-	+	+	+
Dye degradation					
Azo dye	+	+	-	+	-
Triaryl methane dye	+	-	-	+	+
Polyphenolic dye	+	+	+	+	+

+, Positive; -, Negative

CONCLUSION

Based on the results of the current study, it can be concluded that the marine coastal region is a distinct habitat for salt-tolerant bacteria and that it is best for the biosynthesis of hydrolytic enzymes. Proteases are synthesized by 55 bacteria out of the total isolates, followed by amylase by 27 bacteria, laccase, and lipase by 24 bacteria, xylanase by 8 isolates, cellulase by 6 isolates, and L-glutaminase by 35 isolates. This research is an effort towards isolating different microorganisms with the ability to biosynthesize hydrolytic enzymes. These enzymes may meet a wide range of industrial needs, including those in the food, pharmaceutical, dairy, detergent, leather, and textile industries, among many others.

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Competing Interests

The authors have declared that no competing interest exists.

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