

Marine Halotolerant Bacteria from Saurashtra Coast: Characterization and Potential Applications in Food Enzyme Production and Nano-biotechnological Processes

Deviben Chhatrodiya^{1,2} and Jignesh H Kamdar^{1*}

¹Department of Microbiology, School of Science, RK University, Gujarat, India

²J M Science College & P.G. Center, Veraval, Gujarat, India

*Correspondence to:

Jignesh H Kamdar
Department of Microbiology,
School of Science,
RK University,
Gujarat, India.
E-mail: jignesh.kamdar@rku.ac.in

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Abstract

Marine environments, particularly coastal regions, harbor a rich and under-explored diversity of halotolerant bacteria with potential biotechnological applications. The present study aims to isolate, identify, and characterize halotolerant bacterial strains from the coastal areas of Veraval and Kodinar in Saurashtra, Gujarat, to assess their physiological traits and potential uses. This study explores the isolation, diversity, and physiological characterization of marine halotolerant bacteria from the coastal areas of Veraval and Kodinar in Saurashtra, Gujarat. A total of five soil samples were collected from distinct locations in these coastal regions, and 20 bacterial isolates were obtained. From these, 14 halotolerant strains were selected based on their ability to grow in high salt concentrations (2 - 10% NaCl). These isolates were comprehensively characterized for various physiological traits, including biofilm formation, exopolysaccharide (EPS) production, antibiotic sensitivity, antagonistic activity against pathogens, coexistence, metal resistance, hemolytic activity, enzyme production (amylase, protease, and lipase), and nitrate reduction. Additionally, the isolates were optimized for growth under varying conditions, such as pH, temperature, carbon source, nitrogen source, and salt concentration. The findings of this study highlight the remarkable diversity and potential applications of these halotolerant bacteria in sustainable agriculture, environmental management, and biotechnology. The isolates exhibited a range of beneficial traits, suggesting their potential use in promoting plant growth, mitigating soil pollution, and developing novel biofertilizers and biopesticides. This research contributes to the understanding of marine microbial diversity and its applications, providing valuable insights for future studies and potential industrial applications. The study's results have significant implications for the development of sustainable agricultural practices and environmental management strategies. Further research on these halotolerant bacteria could lead to the discovery of novel enzymes, bioactive compounds, and other valuable products.

Keywords

Environmental management, Halotolerant bacteria, Physiological characterization, Plant growth promotion, Sustainable agriculture

Introduction

Microorganisms that thrive in extreme environments have drawn significant scientific interest due to their potential applications in biotechnology and environmental sustainability. Among them, halotolerant bacteria microbes capable of surviving in high-salinity conditions without requiring salt for growth play a crucial role in various ecological and industrial processes [1]. Unlike halophiles, which depend on high salt concentrations for growth, halotolerant bacteria can endure osmotic stress and maintain cellular functions in both saline and non-saline environments, making them highly versatile [2]. Halotolerant bacteria are

widely distributed in nature, inhabiting diverse ecosystems such as salt flats, marine environments, saline soils, hypersaline lakes, and even extreme habitats like deep-sea hydrothermal vents [3]. Their ability to resist osmotic stress is attributed to various adaptive mechanisms, including the production of compatible solutes (e.g., trehalose, glycine betaine) and specialized membrane transport systems that regulate ionic balance [4]. These unique physiological adaptations allow them to survive in fluctuating salinity conditions, making them an essential part of microbial ecology and nutrient cycling [5]. From an industrial perspective, halotolerant bacteria offer valuable applications in bioremediation, agriculture, and biotechnology. For instance, they are employed in the degradation of pollutants in saline environments, enhancing plant growth in salt-affected soils, and producing extremozymes enzymes that remain functional under extreme conditions [6]. The discovery of novel halotolerant strains has also led to advancements in pharmaceuticals, where salt-resistant enzymes are used in drug formulations and bioactive compound synthesis [7]. Despite their potential, challenges remain in fully harnessing halotolerant bacteria for commercial and environmental applications. Further research is required to explore their genetic diversity, optimize their industrial use, and develop bioengineering strategies for enhancing their resilience [8]. This study aims to provide an in-depth analysis of the classification, physiological adaptations, and emerging applications of halotolerant bacteria, highlighting recent advances in this rapidly growing field.

Halotolerant bacteria, including *Staphylococcus*, *Bacillus*, *Vibrio*, *Halomonas*, *Pseudomonas*, *Corynebacterium*, and *Deinococcus* species, thrive in high-salt environments and have diverse applications. These bacteria are used in industrial biotechnology for enzyme production and bioplastics [9], agriculture for salt-tolerant biofertilizers and biocontrol [10], and environmental bioremediation for wastewater treatment and oil spill cleanup [11]. They also have medical applications in antibiotic production and probiotics. Additionally, their resilience makes them valuable in space and extreme environment research [12]. Halotolerant bacteria have diverse applications across various industries, including industrial biotechnology for enzyme production and bioplastics [9], agriculture for salt-tolerant biofertilizers and biocontrol [13], and environmental bioremediation for wastewater treatment and oil spill clean-up. They also have medical applications in antibiotic production and probiotics [14]. Additionally, halotolerant bacteria are used in marine biotechnology for fermented food production and salt-tolerant probiotics for aquaculture, and in space research for astrobiology and biotechnology in extreme environments [15]. The purpose of this research is to investigate isolation, diversity, and physiological characterization of marine halotolerant bacteria from the coastal regions of Veraval and Kodinar of Saurashtra, Gujarat. This study emphasizes under-characterized halotolerant strains of the particular coastal belt of Saurashtra, studying not just their tolerance to salinity but also their multifunctional characteristics with biotechnological value, the study aims to contribute toward the knowledge on marine microbial diversity and evaluate the biotechnological possibilities of the isolates for agricultural, industrial, and environmental applications.

Hypothesis

Marine halotolerant bacteria from the Saurashtra coast possess diverse physiological adaptations and functional traits that make them suitable candidates for biotechnological applications, particularly in stress-resilient agriculture and environmental management.

Materials and Method

Soil sample collection and characterization

Soil samples were collected in a systematic way from five different coastal locations in the districts of Veraval and Kodinar, Gujarat, India. The sites for sampling were Adari (20°57'33.6" N, 70°17'58.4" E), Malshram (20°45'34.1" N, 70°46'41.4" E), Devli (20°46'36.9" N, 70°43'00.5" E), Gorakh Madhi (20°54'23.7" N, 70°32'18.4" E), and Chhara (20°44'49.7" N, 70°44'32.0" E). The geographical coordinates of the selected locations were obtained with a global positioning system (GPS). To ensure sample integrity, the soil samples were aseptically retrieved in sterile zipper bags and sterile containers and taken to the laboratory immediately for additional analysis [1]. The retrieved samples were then tested for different physicochemical attributes, such as pH, electrical conductivity (EC), available phosphorus (mg/kg), potassium (mg/kg), and total organic carbon (%), using standard procedures.

Isolation and primary screening of halotolerant bacteria

Sample were not serially diluted and plated on nutrient media with different concentrations of NaCl (2%, 4%, 6%, 8%, and 10% w/v). Different colonies are chosen and purified by repeatedly streaking over the same salt-enriched media during a 24 - 72 h incubation period at 30 °C [1]. In order to find the salinity range optimal for the growth of the isolates, the isolates were inoculated into NaCl concentrations ranging from 2% - 10% in broth media and incubated at 30 °C for 24 - 72 h [16]. Colonies showing growth on high-salt conditions were purified next.

Morphological and biochemical characterization

Morphological characteristics

Colony morphology was determined by streaking isolates on nutrient agar plates that were enriched with NaCl (2% - 10%) and incubated at 30 °C for 24 - 72 h. The shape, margin, elevation, and pigmentation were observed [17]. Gram staining was conducted with routine crystal violet, iodine, ethanol, and safranin staining for the discrimination of Gram-positive and Gram-negative bacteria.

Analysis of biochemical properties

Biochemical characterization, including methyl red test, Voges-Proskauer test, citrate utilization test, indole production test, catalase test, and sugar utilization test were performed according to standard protocols [18].

Screening for enzyme production

The bacterial isolates showed different enzymatic activities reflected by clear hydrolysis zones.

Casein hydrolysis test

The Casein hydrolysis test was used to assess the ability of bacterial isolates to produce extracellular protease enzymes. The isolates were inoculated on a casein agar medium composed of skim milk powder (28 g/L), tryptone (5.0 g/L), yeast extract (2.5 g/L), glucose (1.0 g/L), and sodium chloride (50 g/L). The plates were incubated at 37 °C for 36 h. After incubation, a clear zone appeared around the colonies indicating casein degradation due to protease activity [19].

Starch hydrolysis test

Starch hydrolysis test was conducted to determine the ability of organisms capable of hydrolyzing starch into maltose through the activity of extracellular enzyme α -amylase enzyme. 25 ml of medium containing nutrient agar with starch was poured into the plate and cultures were spotted on a plate and incubated at 37 °C for 24 h. After incubation, the plate was flooded with Lugol's iodine. Positive isolates showed a zone of clearance surrounding the colony [20].

Gelatin hydrolysis test:

The test was conducted in nutrient gelatin broth. The main purpose of this test was to detect the ability of an organism to produce the enzyme gelatinase. The gelatin tube was inoculated with the given organism by stabbing the media multiple times using inoculating wire. The tube was incubated at room temperature (25 °C) for 24 - 48 h. Liquefaction upon refrigeration showed a positive test [21].

Lipid hydrolysis test

The test was performed to detect the presence of enzyme lipase. Nutrient agar was prepared and after the autoclave 1% lipid source (any oil) was added and plate were prepared. The isolates were spotted on the plate and incubated at 37 °C for 24 - 72 h. A zone of clearance or color change surrounding growth of organism indicated positive result [22].

Functional characterization of isolates

Biofilm formation

To qualitatively assess the biofilm formation ability of marine bacteria using Congo red agar (CRA). CRA plate was prepared by supplementing nutrient agar plate with 0.08% CR and 5% sucrose. The agar plates were streaked with isolates and incubated at 37 °C for 24 h. The formation of dark and rough colonies indicated biofilm production, while no color change or red colonies indicated negative results [23].

EPS production

EPS production was determined by CRA plate and KOH string test. CRA plate was prepared with nutrient agar with (0.08% w/v CR). Isolates were streaked on the plate and incubated at 37 °C for 24 h. After incubation a colony was picked up and mixed with a KOH solution. The formation of string indicated positive result [24].

Effect of heavy metals concentration on bacteria growth

Inhibition zone assay was conducted to screen the heavy metals resistance patterns of the isolated bacterial strain against different heavy metal salts using agar disc diffusion

method. Sterile nutrient agar plates were prepared, and the bacterial cultures were spread evenly on plates. The number of plates prepared corresponded to the number of heavy metal salts used in the study [25].

Antibiotic sensitivity test for the organism

Antibacterial susceptibility assay was conducted using the disc diffusion method. Sterile nutrient agar plates were prepared and inoculated with the test of bacterial cultures. Thereafter, the antibiotic discs were placed onto the inoculated plates, which were incubated at 37 °C overnight. The antibacterial activity of each antibiotic was expressed as the mean diameter (in mm) of the zone of inhibition produced after incubation [26].

Antagonistic activity

The antagonist activity of isolates against pathogenic bacteria and fungi was assessed using the agar diffusion method. Briefly, isolates were applied to lawns of pathogenic bacteria (e.g., *Escherichia coli*, *Pseudomonas*) or fungi (e.g., *Mucor*, *Aspergillus niger*) on agar plates. After incubation, the diameter of inhibition zones was measured to determine the antagonist activity of the isolates [27].

Co-existence test

The compatibility and potential constructive interaction between bacteria were determined. The bacteria were grown under their respective growth conditions for 24 h, and then samples were streaked perpendicularly to each other on the surface of nutrient agar plate. After a 24 h incubation period, plates were examined for possible antagonistic effects such as inhibition zones [28].

Physiological characterization

Optimization of carbon source

The growth patterns of isolates were studied using 1% glucose, lactose, sucrose, and dextrose as carbon sources, while keeping other parameters constant. Isolates were inoculated into the broth and incubated at 37 °C for 24 h. Then cell density was determined by spectrophotometer at 620 nm [29].

Optimization of nitrogen source

Nutrient broth was prepared with 1% different nitrogen sources including urea, peptone, yeast extract, ammonium sulphate. Isolates were inoculated into the broth and incubated at 37 °C for 24 h. Then cell density was determined by spectrophotometer at 620 nm [29].

Optimization of temperatures

Nutrient broth was inoculated with isolates and incubated at different temperatures (28 °C, 37 °C, 45 °C, and 55 °C) for 24 h. Then cell density was determined by measuring the optical density at 620 nm using a spectrophotometer [30].

Optimization of pH

The nutrient broth was adjusted to different pH (3 - 12) using 1 N NaOH and 0.1 M HCl. The isolates were inoculated into the broth and incubated at 37 °C for 24 h. After incubation, cell density was determined by measuring the optical density at 620 nm. using a spectrophotometer [30].

Optimization of NaCl concentration

Nutrient broth was prepared with different NaCl concentration (1% - 10 %). Isolates were inoculated into the broth and incubated at 37 °C for 24 h. Cell density was then determined by measuring the optical density at 620 nm using a spectrophotometer [31].

Result

Soil sampling and characterization

Soil samples were collected from five coastal sites in Veraval and Kodinar, Gujarat: Adari, Malshram, Devli, Gorakh Madhi, and Chhara. The locations were recorded using GPS. The physicochemical analysis revealed all soils were mildly to moderately alkaline (pH 7.4 - 8.9) with varied EC, indicating differing salinity levels (Table 1). These characteristics are consistent with typical saline coastal soils and influence the microbial diversity present (Figure 1).

Isolation and primary screening of halotolerant bacteria

A total of 36 morphologically distinct bacterial colonies were isolated using nutrient agar supplemented with 2% NaCl (Figure 2). Serial dilution and spread plate techniques facilitated the isolation. Growth was evaluated by increasing NaCl concentrations (2 - 10%). Most isolates exhibited good growth up to 6% NaCl, indicating halotolerance, while only a few, such as isolate 11, maintained growth at 10%, demonstrating high salt resistance. These findings confirm that the isolates are adapted to the saline conditions of coastal soil.

Morphological and biochemical characterization

Morphological characteristics

The colonies appeared after 1 - 2 days of incubation at 37 °C. Displayed characteristic textures, including dry, moist, or powdery. Pigmentation varied, ranging from white, yellow, to light orange and off white (Table 2). All isolates were sub-cultured and maintained on slants for further characterization. Gram staining confirmed that all were Gram-positive, indicative of robust peptidoglycan-rich cell walls (Figure 3).

Biochemical characteristics

Biochemical testing confirmed catalase and dehydrogenase activity in most isolates, and diverse metabolic capabilities for sugar utilization. These features further support their environmental adaptability and potential for industrial application.

Screening for enzyme production

Casein hydrolysis test

Out of 14 halotolerant bacterial isolates, 12 exhibited casein hydrolysis, as indicated by a clear zone around their colonies (Table 3). Two isolates did not show any zone of hydrolysis, indicating a lack of caseinolytic activity (Figure 4).

Starch hydrolysis test

Out of 14 halotolerant bacterial isolates, 11 exhibited starch hydrolysis, as indicated by a clear zone around their colonies after iodine staining. Three isolates did not show any zone of hydrolysis (Figure 5).

Gelatin hydrolysis test

Out of 14 halotolerant bacterial isolates, 11 exhibited gelatin hydrolysis, as indicated by liquefaction of the gelatin medium. Three isolates did not show any liquefaction, indicating a lack of gelatinolytic activity (Figure 6).

Lipid hydrolysis test

Out of 14 halotolerant bacterial isolates, 10 exhibited lipid hydrolysis, as indicated by the formation of a clear zone or precipitation around their colonies. Four isolates did not show any zone of hydrolysis (Figure 7).

Functional characterization of isolates

Biofilm formation

CRA, the black/brown coloration indicates a **positive** biofilm producer, as it enhances bacterial survival and colonization in the host gut and the environment (Table 4). Out of 14 isolates S_1Z_1 , S_3T_7 , S_3Z_5 , S_5Z_7 and S_1LZ_3 gives biofilm positive results while rest of biofilm negative result (Figure 8).

EPS production

S_1Z_1 , S_1Z_7 , S_1LZ_3 and S_3T_7 can produce EPS, meaning they have a higher ability to form biofilms and resist environmental stress, antibiotics, and host immune responses. S_4WZ_3 , S_6OZ_2 do not produce EPS, suggesting they have a weaker ability to form biofilms and may rely on planktonic (free-floating) growth instead (Figure 9).

Effect of heavy metals concentration on bacteria growth

The heavy metal tolerance test revealed that several halotolerant isolates demonstrated varying degrees of resistance to nickel, lead, copper, and cobalt at 50 mM and 100 mM concentrations. Notably, isolates S_3T_7 and S_1Z_7 exhibited consistent and strong tolerance to all tested metals. In contrast, iso-

Table 1: The different physicochemical parameters of the five different soil samples.

Samples	pH	EC (ds/m)	Phosphorus (kg/ha)	Potassium (kg/ha)	Carbon (%)
	mean $\pm$ SD				
Sample 1	8.23 $\pm$ 0.08	0.73 $\pm$ 0.05	513 $\pm$ 22	1059 $\pm$ 50	0.66 $\pm$ 0.03
Sample 2	8.91 $\pm$ 0.09	0.27 $\pm$ 0.02	30 $\pm$ 2	164 $\pm$ 9	0.89 $\pm$ 0.04
Sample 3	8.62 $\pm$ 0.07	1.74 $\pm$ 0.12	444 $\pm$ 21	618 $\pm$ 33	0.69 $\pm$ 0.03
Sample 4	7.43 $\pm$ 0.06	1.79 $\pm$ 0.10	70 $\pm$ 4	660 $\pm$ 30	0.81 $\pm$ 0.03
Sample 5	7.99 $\pm$ 0.08	2.13 $\pm$ 0.13	97 $\pm$ 5	563 $\pm$ 28	0.57 $\pm$ 0.02



Figure 1: Bacterial isolates obtained through spreading plate method from soil samples.

lates such as S_4WZ_3 and S_6O_2C showed limited or no growth under higher metal concentrations, suggesting lower tolerance (Figure 10).



Figure 2: Different bacterial isolates.

Antibiotic sensitivity test for the organism

The presence of antibiotic-sensitive bacteria reduces selective pressure for resistance genes in environments (Table 5 and figure 11).

Table 2: Colony characteristics of halotolerant bacteria.

Isolates	Size	Shape	Margin	Elevation	Consistency	Pigmentation
S ₂ T _c	Small	Round	Eros	Flat	Moist	White
S ₂ T ₃	Medium	Irregular	Eros	Flat	Dry	Orange
S ₃ T ₇	Medium	Irregular	Eros	Raised	Dry	Yellow
S ₁ Z ₁	Small	Round	Eros	Convex	Moist	White
S ₁ Z ₇	Small	Round	Even	Raised	Dry	Yellow
S ₃ Z ₅	Medium	Round	Entire	Flat	Moist	Off white
S ₄ WZ ₃	Small	Pinhead	Even	Convex	Dry	Orange
S ₄ WZ ₅	Small	Round	Even	Raised	Moist	white
S ₄ PZ ₅	Medium	Round	Entire	Convex	Moist	Off white
S ₅ WZ ₁	Large	Irregular	Eros	Raised	Dry	white
S ₅ Z ₇	Small	Round	Entire	Flat	Moist	white
S ₆ O ₂ C	Small	Round	Eros	Raised	Dry	Yellow
S ₆ WZ ₁	Small	Pinhead	Entire	Flat	Moist	Orange
S ₇ LZ ₃	Large	Irregular	Eros	Flat	Moist	Off white

Antagonistic activity

It helps to control harmful pathogens. Out of 14 isolates eight isolates show antagonistic activity against both bacteria and fungi, while five showed activities against bacteria only (Figure 12). Higher zone of inhibition observed against bacteria in S₂T₃ and S₅Z₇ (Table 6). The highest antifungal activity observed is S₃Z₅ and S₁LZ₃ (Figure 13).

Co-existence

The coexistence assay is used to assess whether different probiotic bacterial strains can grow together without inhibiting each other. A stable coexistence indicates potential for multi-strain probiotic use (Table 7). Isolate no. 8 coexists to isolate no. 14 but do not coexist to isolate no. 12 and 13. Isolates 4 coexists to isolates 5, 6, 7, 9 but do not coexist to no. 8 (Figure 14).

Physiological characterization

Optimization of carbon source

The findings suggest that simple sugars, particularly glucose, are more easily metabolized by isolates. The decreased activity with lactose indicates these bacteria may lack efficient metabolic pathways or transport systems for complex carbohydrates. This behavior aligns with halotolerant organisms' adaptations, where energy efficiency in high-salt environments may favor rapidly metabolizable substrates like glucose (Figure 15).

Optimization of nitrogen source

Yeast extract appears to be the most effective nitrogen source overall, followed by peptone. Ammonium sulphate is the least effective for most isolates (Figure 16).

Optimization of temperatures

The optimal temperature for the bacterial isolates appears to be around 37 °C, with decreased growth observed at temperatures above and below this point. The sharp decline in activity at elevated temperatures suggests that these isolations are adapted to moderate temperatures and may be sensitive to extreme heat (Figure 17).

Optimization of pH

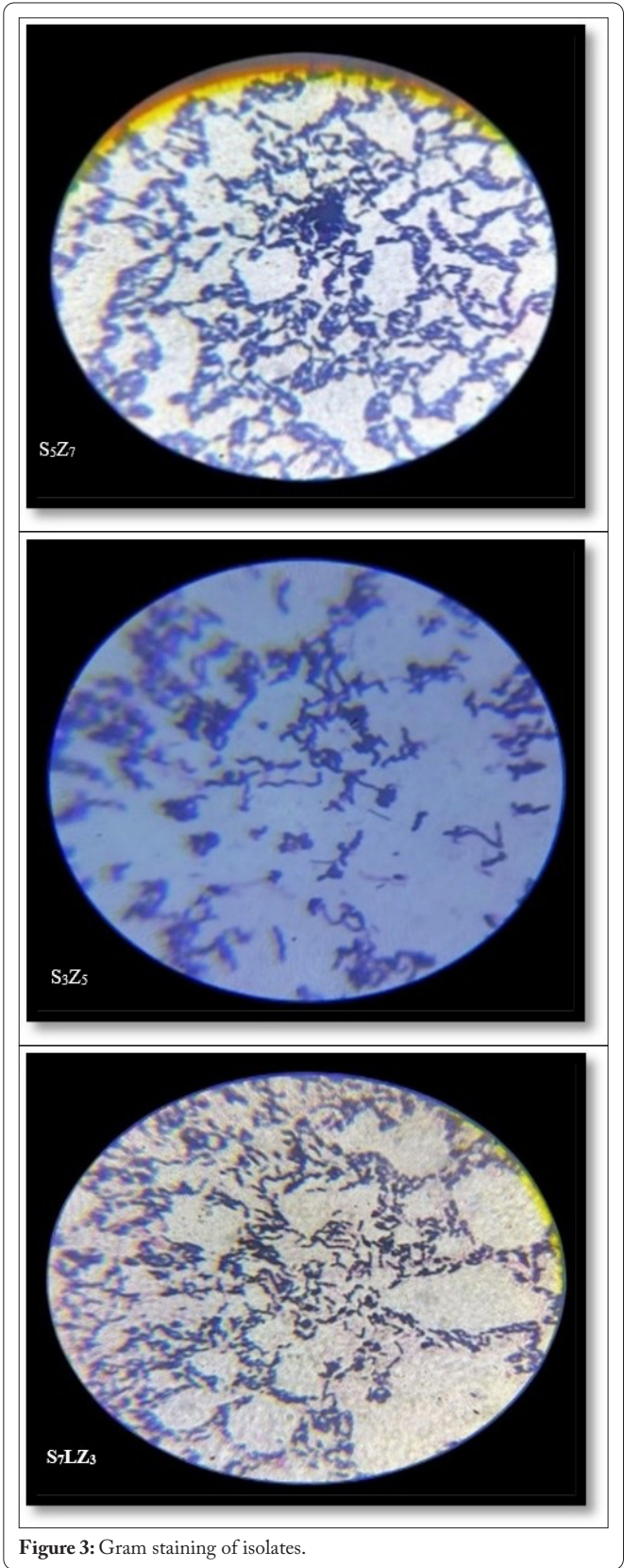
The bacterial isolates exhibit optimal growth around pH 9, indicating they are alkaliphilic or alkali tolerant in nature. The significant decline in growth at lower pH values suggests sensitivity to acidic conditions, while the decrease at higher pH values indicates an optimal range for growth rather than tolerance for extreme alkalinity (Figure 18).

Optimization of NaCl concentration

The bacterial isolates exhibit optimal growth at 5% NaCl, indicating adaptation to moderately saline environments. Reduced growth at lower salt concentrations suggests a requirement for some level of salinity, while the significant decline beyond 7% NaCl highlights the limits of their salt tolerance and the impact of salt stress on their growth (Figure 19).

Discussion

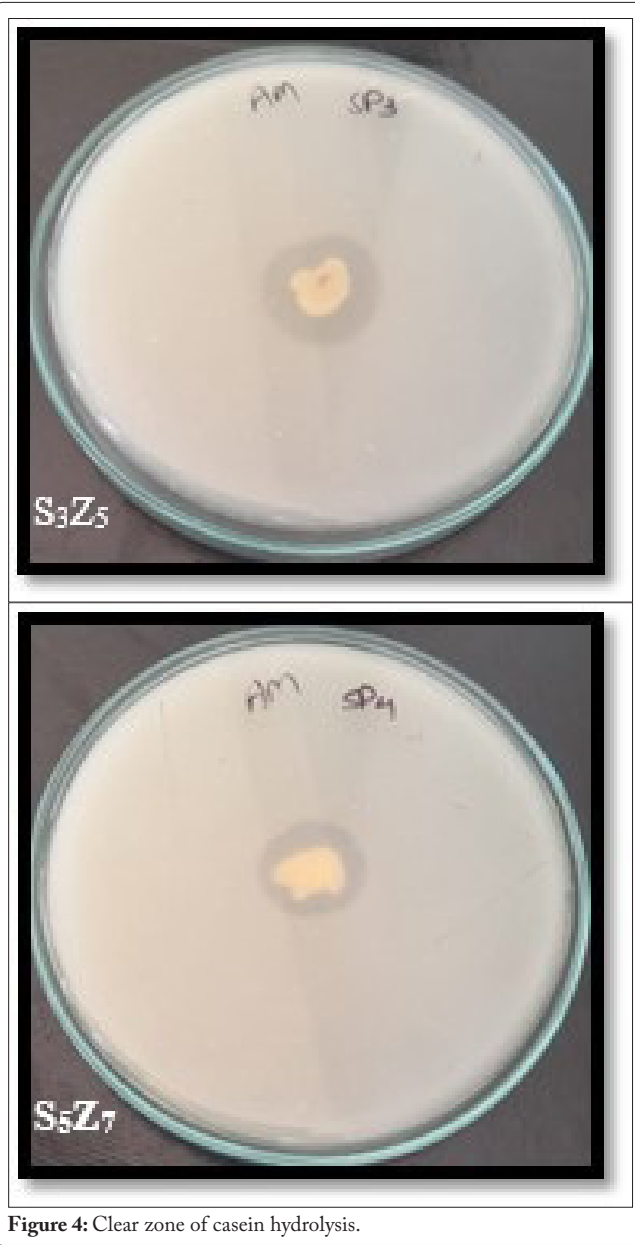
This research focused on the isolation and characterization of halotolerant bacteria from coastal soil samples of Veraval and Kodinar, Gujarat, India. The soil samples from five locations showed mild to moderate alkaline pH ranging from 7.4 - 8.9, which is in accordance with general saline coastal soils [32]. The diverse values of EC reflected the diverse salinity levels, which had a varying effect on the microbial diversity of these soils [33]. 36 morphologically different bacterial colonies were obtained on nutrient agar with 2% NaCl. Most isolates grew well at 6% NaCl, which proves to be halotolerant, while a few had growths at 10%, showing salt resistance at an extremely elevated level. These results validate that the isolates are compatible with the coastal soil's saline environments as described in existing research [34]. Gram staining confirmed that all were Gram-positive, indicative of robust peptidoglycan-rich cell walls, as reported earlier for halotolerant bacteria [35]. Biochemical analysis confirmed the presence of catalase and dehydrogenase activities in most of the isolates, and varied metabolic capabilities towards the metabolism of sugars, further indicating their environmental proficiency and utility for industrial purposes [36]. Functional assays reflect

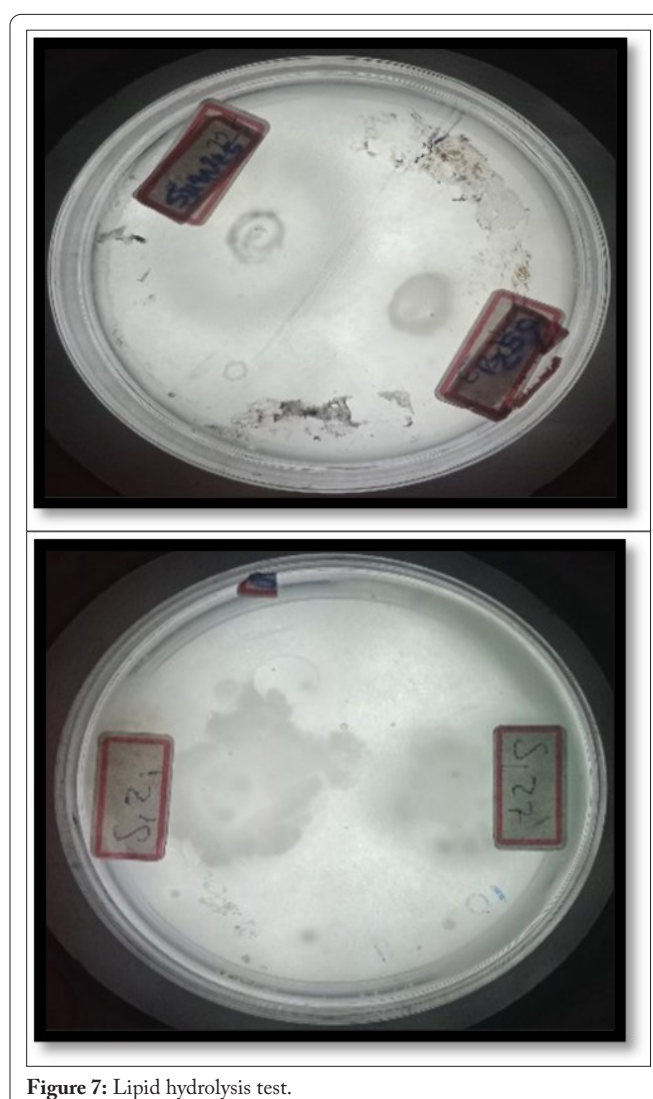
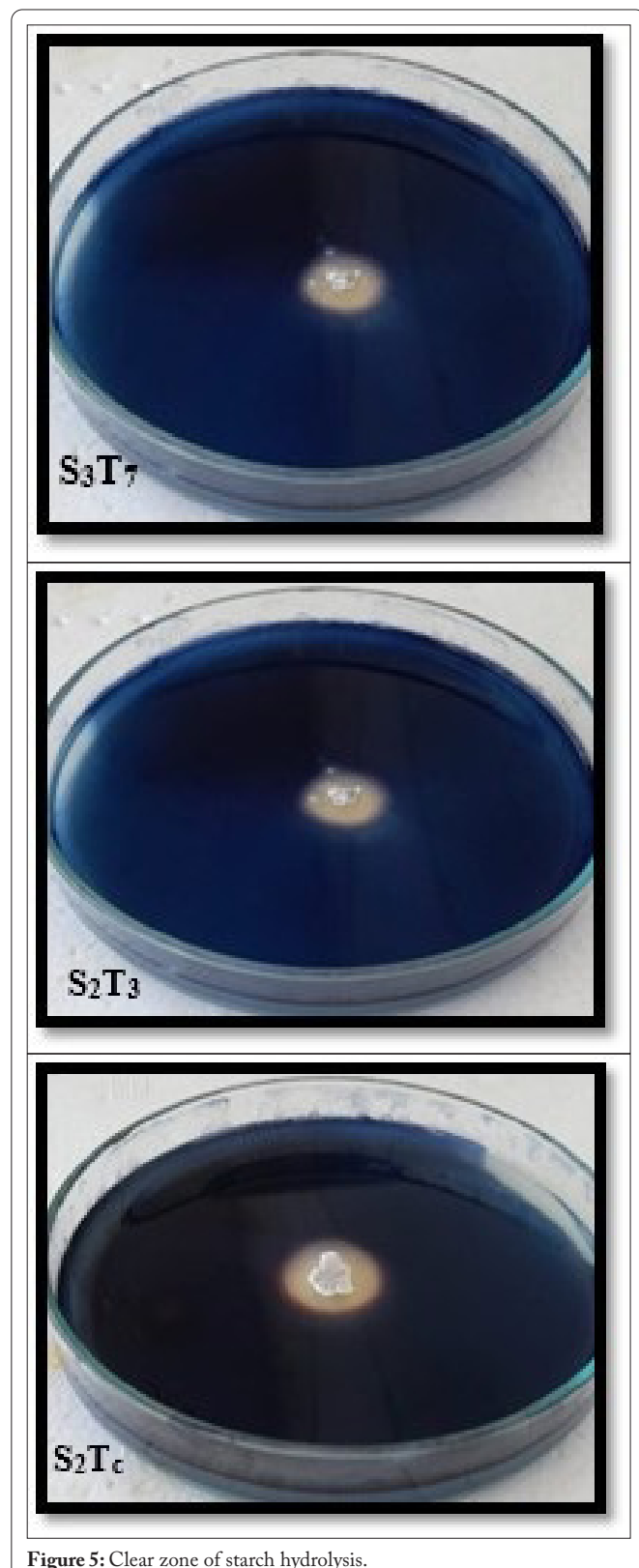


the ecological versatility of these bacteria. The isolates ranged from moderate to elevated levels of enzyme production, such as casein hydrolysis, starch hydrolysis, gelatin hydrolysis, and lipid hydrolysis. These enzymes can be utilized in several industries like detergent, food, and pharmaceutical [37]. The isolates exhibited differential biofilm production, EPS yield, and tolerance to heavy metals. Such traits are beneficial for

Table 3: Enzyme production by halotolerant bacterial isolates.

Isolates	Casein hydrolysis test	Starch hydrolysis test	Gelatin hydrolysis test	Lipid hydrolysis test
S ₂ T _c	+	-	+	-
S ₂ T ₃	+	+	+	+
S ₃ T ₇	+	+	+	+
S ₁ Z ₁	+	+	+	+
S ₁ Z ₇	+	+	-	-
S ₃ Z ₅	+	+	+	+
S ₄ WZ ₃	+	+	+	-
S ₄ WZ ₅	-	+	-	+
S ₄ PZ ₅	+	+	+	+
S ₅ WZ ₁	+	-	-	+
S ₅ Z ₇	+	+	+	+
S ₆ OZ _C	-	-	+	+
S ₆ WZ ₁	-	+	+	-
S ₇ LZ ₃	+	+	+	+





the survival and colonization of bacteria in varied niches [38]. The antibiotic sensitivity profile showed some isolates being sensitive to specific antibiotics, lowering selective pressure for resistance genes in niches [39].

The isolates were found to have antagonistic activity both against bacteria and fungi and suggested use in biocontrol and probiotics [40]. In the coexistence assay, it was found

that some isolates would grow together without preventing each other from doing so, showing promise for use as multi-strain biofertilizers [41]. The isolates had optimal growth at temperatures, pH, and NaCl levels. The best temperature for growth was approximately 37 °C and lower growth rates at higher and lower temperatures, as reported in earlier work on mesophilic bacteria [42]. The isolates were alkaliphilic or al-

Table 4: Heavy metal tolerance tests result of halotolerant isolates.

Isolates	50 mM				100 mM			
	Nickel	Lead	Copper	Cobalt	Nickel	Lead	Copper	Cobalt
S ₂ T _c	+	+	+	+	+	+	+	+
S ₂ T ₃	+	+	-	+	+	+	-	D+
S ₃ T ₇	+	+	+	D+	D+	+	D+	D+
S ₁ Z ₁	+	+	D+	+	+	+	D+	D+
S ₁ Z ₇	+	+	+	+	+	+	+	+
S ₃ Z ₅	+	+	+	+	+	+	+	D+
S ₄ WZ ₃	D+	+	D+	-	-	+	-	-
S ₄ WZ ₅	+	+	+	+	+	+	+	D+
S ₄ PZ ₅	+	+	+	D+	-	+	D+	-
S ₅ WZ ₁	+	+	+	+	+	+	+	D+
S ₅ Z ₇	+	+	+	+	+	+	D+	D+
S ₆ O ₂ C	-	+	-	-	-	+	-	-
S ₆ WZ ₁	+	+	+	D+	+	+	D+	-
S ₇ LZ ₃	+	+	+	+	+	+	+	D+



Figure 8: Detection of biofilm production.

kali tolerant in nature, exhibiting optimal growth around pH 9, like other halophilic microorganisms that thrive in alkaline environments [43]. The bacterial isolates exhibited optimal growth at 5% NaCl, indicating adaptation to moderately saline environments, which is in line with the findings on halotolerant bacteria from coastal ecosystems [44].

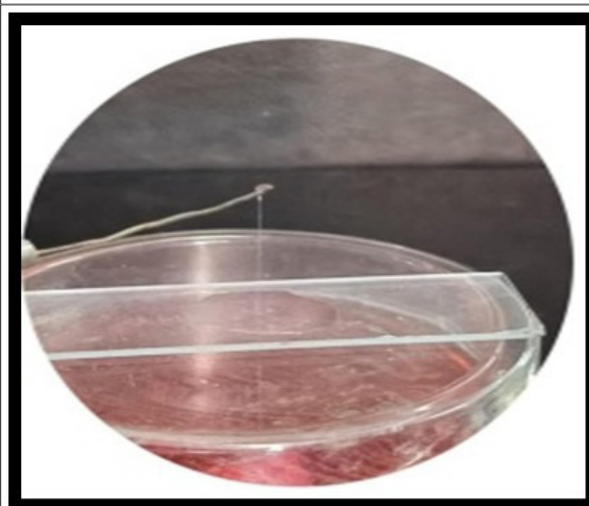
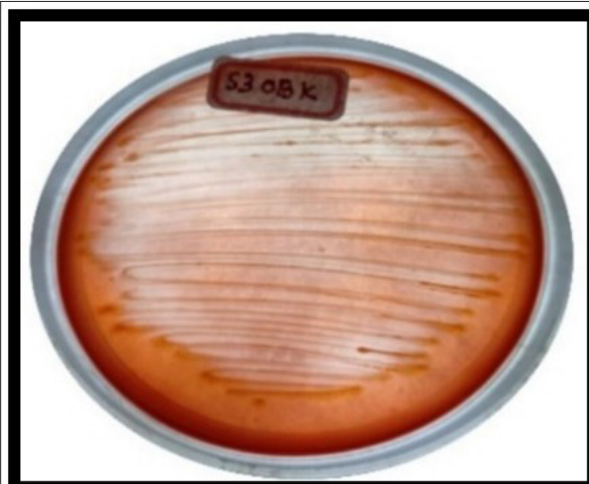


Figure 9: EPS production.

Conclusion

This study isolated and characterized 36 morphologically distinct halotolerant bacterial isolates from coastal soils in

Table 5: Standard deviation for antibiotic sensitivity (in mm).

Isolates	Antibiotics											
	AS	BA	PR	TE	CF	RC	QB	LZ	CX	AT	LM	GM
S ₂ T _c	0.360555	0.251661	0.404145	0.43589	0.208167	0.1	0.251661	0.351188	0.4	0.929157	0.556776	0.503322
S ₂ T ₃	0.945163	0.655744	0.351188	0.862168	0.458258	1.234234	0.85049	0.754983	1.081665	1.301281	0.251661	0.458258
S ₃ T ₇	0.655744	0.264575	0.602771	0.873689	0.360555	2.042058	0.916515	0.737111	0.404145	1.601041	1.113553	0.737111
S ₁ Z ₁	0.602771	0.665833	0.984886	0.750555	0.251661	0.929157	0.754983	0.556776	0.7	0.85049	0.3	1.113553
S ₁ Z ₇	0.152753	0.802081	0.550757	1.106044	0.3	0.916515	1.137248	0.702377	0.550757	0.6245	0.650641	0.4
S ₃ Z ₅	0.665833	0.602771	0.702377	0.650641	0.305505	0.602771	0.960902	0.907377	1.352775	0.702377	1.656301	0.929157
S ₄ WZ ₃	NaN	NaN	NaN	NaN	1.357694	0.862168	0.984886	0.907377	0.351188	0.208167	0.251661	0.305505
S ₄ WZ ₅	0.85049	0.8	1.159023	1.159023	0.9	1.305118	0.754983	0.971253	0.960902	0.70946	1.123981	1.410674
S ₁ PZ ₅	0.960902	0.916515	0.6	0.832666	0.793725	0.916515	0.8	0.85049	1.069268	1.721434	0.85049	1.250333
S ₅ WZ ₁	0.70946	1.320353	0.945163	0.208167	0.953939	0.7	0.802081	0.655744	0.556776	0.9	0.3	0.776745
S ₅ Z ₇	0.665833	0.873689	0.472582	0.7	0.550757	0.70946	0.602771	0.602771	0.585947	0.793725	0.360555	0.655744
S ₆ O ₂ C	0.650641	1.069268	0.650641	0.585947	0.61101	0.70946	1.167619	1.106044	0.61101	1.404754	1.053565	0.754983
S ₆ WZ ₁	0.702377	0.7	1.410674	0.929157	0.916515	0.550757	0.916515	0.907377	0.61101	0.85049	0.665833	0.61101
S ₇ LZ ₃	0.737111	0.862168	0.945163	1.301281	0.862168	1.68226	0.85049	0.793725	0.655744	0.85049	0.585947	0.556776

Note: Ampicillin/Sulbactam - AS, Co-trimoxazole - BA, Cephalexin - PR, Tetracycline - TE, Cefotaxime - CF, Ciprofloxacin - RC, Levofloxacin - QB, Linezolid - LZ, Cloxacillin - CX, Roxythromycin - AT, Lincomycin - LN, and Gentamicin - Gm.



Figure 10: Bacteria growth.

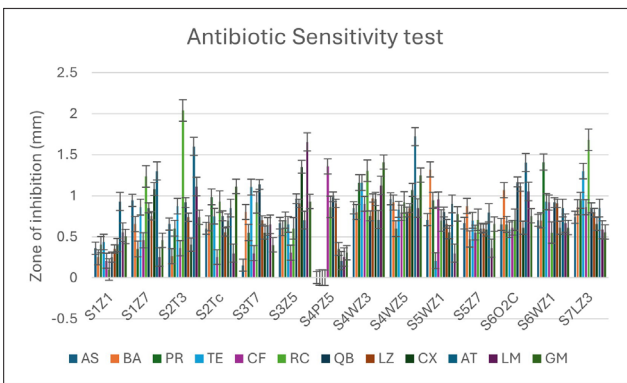


Figure 11: Antibiotic sensitivity assay.

pases, and displaying functional traits such as biofilm formation and antimicrobial activity. Physiological optimization revealed optimal conditions: glucose as the carbon source, yeast extract as the nitrogen source, 37 °C temperature, pH 9, and 5% NaCl concentration. The halotolerant bacteria demonstrate promising biotechnological potential due to their adaptability, enzyme production, and functional traits. Their optimal growth conditions provide essential parameters for scaling up applications in sustainable agriculture, bioremediation, enzyme production, and other industries. Future studies on genomic characterization, metabolite profiling, and scale-up fermentations can further explore their commercial potential in pharmaceuticals, environmental biotechnology, and marine bio industries.

Acknowledgements

None.

Conflict of Interest

None.

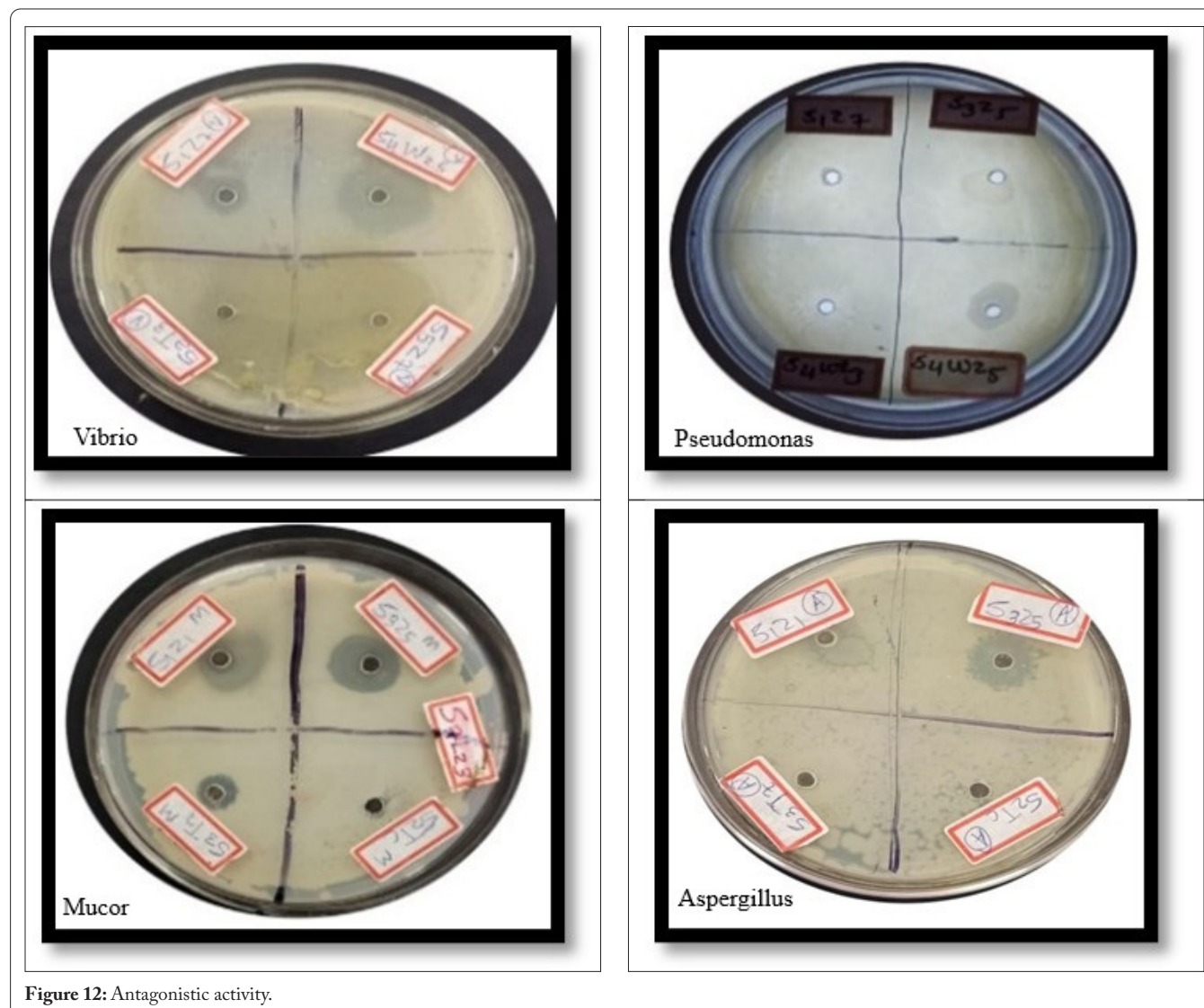


Figure 12: Antagonistic activity.

Table 6: Standard deviation for antagonistic activity (in mm).

Isolates	<i>Vibrio</i> spp.	<i>Pseudomonas</i> spp.	<i>Mucor</i>	<i>Aspergillus</i>
S ₂ T _c	0.450925	0.416333	0.2	0.251661
S ₂ T ₃	0.305505	0	0	0.251661
S ₃ T ₇	0.818535	0.458258	0	0
S ₁ Z ₁	0.1	0.1	0	0
S ₁ Z ₇	0.378594	0	0.251661	0.152753
S ₃ Z ₅	0.776745	0.351188	0.916515	0.793725
S ₄ WZ ₃	0.1	0.208167	0	0
S ₄ WZ ₅	0	0	0	0
S ₄ PZ ₅	0	0	0	0
S ₃ WZ ₁	0	0	0	0
S ₃ Z ₇	0.665833	0.550757	0.152753	0.305505
S ₆ O ₂ C	0	0.2	0.208167	0
S ₆ WZ ₁	0.568624	0.251661	0.6	0.52915
S ₇ LZ ₃	0.472582	0.472582	0.70946	0.472582

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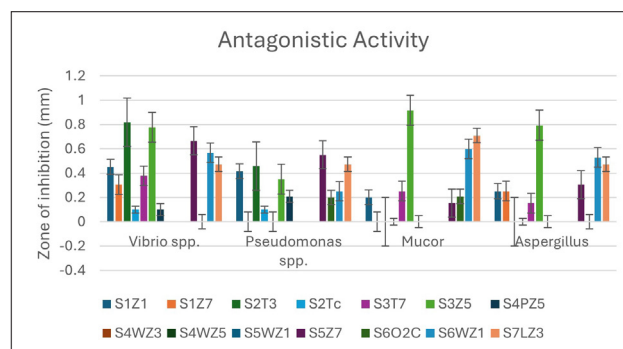


Figure 13: Antagonistic activity against pathogens.

Table 7: Coexistence assay of isolates.

Isolates	S ₂ T _c	S ₂ T ₃	S ₃ T ₇	S ₁ Z ₁	S ₁ Z ₇	S ₃ Z ₅	S ₄ WZ ₃	S ₄ WZ ₅	S ₄ PZ ₅	S ₅ WZ ₁	S ₅ Z ₇	S ₆ O ₂ C	S ₆ WZ ₁	S ₇ LZ ₃
S ₂ T _c	0	+	-	+	+	+	+	-	+	+	-	-	+	+
S ₂ T ₃	+	0	-	+	+	+	+	-	+	+	-	-	+	+
S ₃ T ₇	-	-	0	+	+	+	+	+	+	+	+	-	+	-
S ₁ Z ₁	+	+	+	0	+	+	+	-	+	+	+	+	+	+
S ₁ Z ₇	+	+	+	+	0	+	+	+	+	+	+	-	+	+
S ₃ Z ₅	+	+	+	+	+	0	+	+	+	+	+	-	+	+
S ₄ WZ ₃	+	+	+	+	+	+	0	+	+	+	+	+	+	-
S ₄ WZ ₅	-	-	+	-	+	+	+	0	+	+	+	+	-	+
S ₄ PZ ₅	+	+	+	+	+	+	+	+	0	+	+	-	+	+
S ₅ WZ ₁	+	+	+	+	+	+	+	+	+	0	+	-	+	+
S ₅ Z ₇	-	-	+	+	+	+	+	+	+	+	0	+	-	-
S ₆ O ₂ C	-	-	-	+	-	-	+	+	-	-	+	0	+	-
S ₆ WZ ₁	+	+	+	+	+	+	+	-	+	+	-	+	0	-
S ₇ LZ ₃	+	+	-	+	+	+	-	+	+	+	-	-	-	0

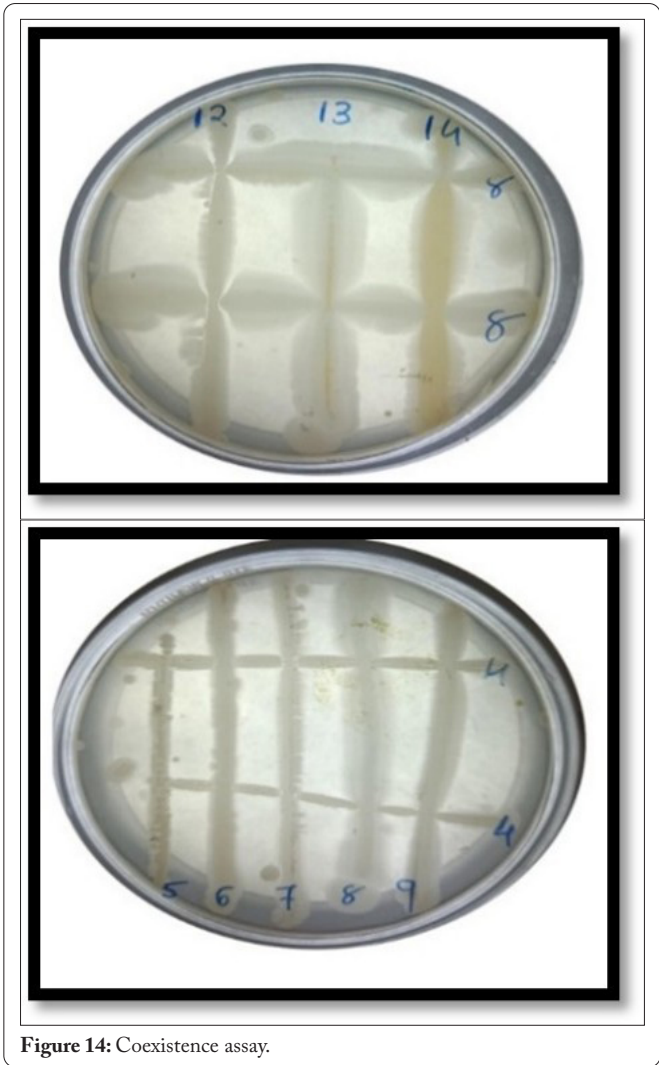


Figure 14: Coexistence assay.

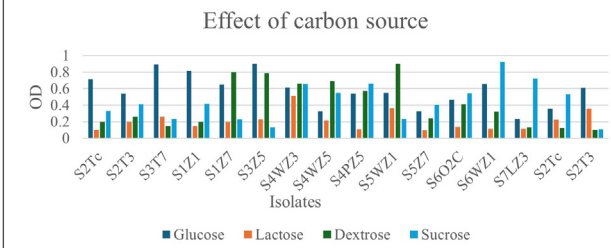


Figure 15: Optimization of carbon source.

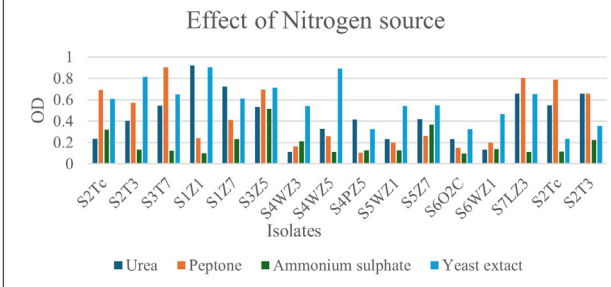


Figure 16: Optimization of nitrogen source.

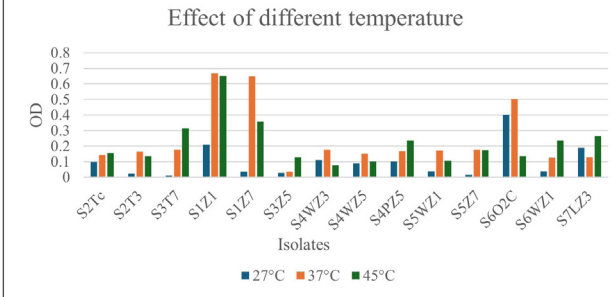


Figure 17: Optimization of temperatures.

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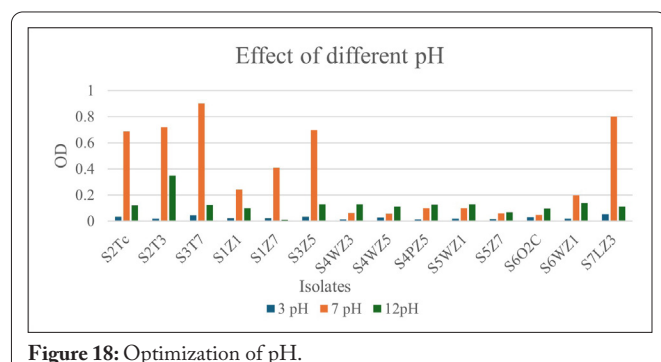


Figure 18: Optimization of pH.

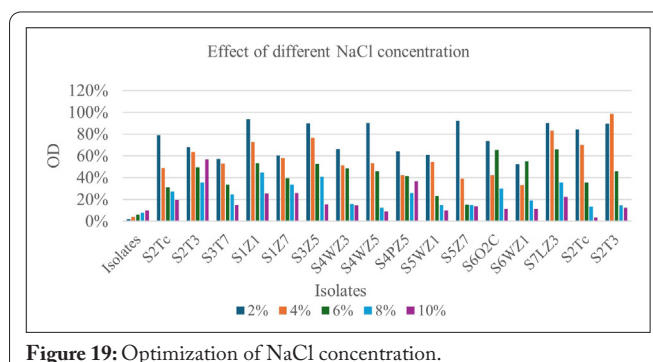


Figure 19: Optimization of NaCl concentration.

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