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# ISOLATION AND CHARACTERIZATION OF HALOPHILIC BACTERIA FROM COASTAL REGIONS OF GUJARAT, INDIA AND EVALUATION OF THEIR ANTIBACTERIAL ACTIVITY AGAINST *ESCHERICHIA COLI*

Microbiology

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## ABSTRACT

**Background:** The emergence of multidrug-resistant pathogens has intensified the search for novel antimicrobial compounds. Marine and coastal environments are considered promising sources of microorganisms capable of producing bioactive secondary metabolites. The present study aimed to isolate and characterize halophilic bacteria from coastal regions of Gujarat, India and evaluate their antagonistic activity against Escherichia coli. **Methodology:** Coastal saline soil samples were collected from different coastal regions of Gujarat, India. Samples were collected aseptically from a depth of 5-10 cm. Samples were serially diluted and cultured on nutrient agar supplemented with 5% NaCl for selective isolation of halophilic bacteria. The antibacterial activity of isolates against Escherichia coli (ATCC 8739) was evaluated using agar well diffusion method. The isolates were characterized by colony morphology, Gram staining and VITEK-2 identification. **Results:** Five bacterial strains were isolated from four different coastal regions of Gujarat (Porbandar, Anand, Amreli and Mandvi). Among the isolates, one strain identified as Halobacillus sp, demonstrated antimicrobial activity against E. coli. The observed inhibition zone was comparatively small (1.36 ± 0.1 mm) indicating limited antibacterial activity under the tested condition. **Conclusion:** This research provides preliminary evidence on the antibacterial activity exhibited by Halobacillus sp. isolated from coastal saline soils. The findings suggest that coastal saline environments of Gujarat may harbor bacteria capable of producing bioactive compounds. Further studies involving purification and characterization of active compounds are required to evaluate their potential pharmaceutical applications.

## KEYWORDS

Halophilic bacteria; Coastal saline soil; Antibacterial Activity; Escherichia coli; Halobacillus sp.; Marine environments; Gujarat.

## INTRODUCTION

The use of bioactive compounds for prevention and treatment of diseases has been practiced since ancient times. In modern medicine, the discovery of antibiotics and antimicrobial compounds is considered as one of the most significant advancements of the 20th century, contributing substantially to reduction of infectious disease [1]. Despite the rise of synthetic chemistry, natural products particularly those derived from microorganisms, remain a dominant source of therapeutic agents. Between 1981 and 2014, approximately 65% of small-molecule drugs approved by the United States Food and Drug Administration (USFDA) were natural products or their derivative, underscoring their pivotal role in pharmaceutical innovation [2]. Among these, microbial metabolites both primary and secondary are recognized for their structural diversity and diverse biological activities, ranging from antibacterial to anticancer properties [2,3]. Recent progress in the discovery of microbial natural products has continued to support pharmaceutical innovation, particularly secondary metabolites produced by microorganisms serve as an important foundation for modern drug development.

Microbial ecosystems continue to represent important sources for the discovery of new antimicrobial compounds, particularly in the face of growing antimicrobial resistance (AMR) [4,5,6]. Antimicrobial resistance (AMR) has emerged as one of the most pressing global public health challenges, with drug-resistant bacterial infections contributing to millions of deaths each year and highlighting the urgent need for new antimicrobial therapies [7,8].

Soil remains one of the most prolific sources of bioactive microorganisms, harbouring vast microbial diversity in complex ecological niches. Yet, it is estimated that nearly 99% of soil microbial species remain uncultured or unexplored. These unexplored microbial populations may serve as potential sources of antimicrobial compounds. Isolation and screening of bacteria from diverse soil environments such as agricultural fields, forest soils, compost, and rhizospheres are therefore critical for discovering new natural products with clinical potential [9,10]. Marine ecosystems have emerged as a valuable reservoir of structurally diverse and biologically active secondary metabolites produced by microorganisms, with numerous studies highlighting their potential as novel antimicrobial agents [11,12]. Among these microorganisms, halophilic bacteria from hypersaline coastal and marine habitats have attracted particular interest due to their ability to synthesize a wide range of unique bioactive metabolites, including compounds with antimicrobial and antitumor activities. These characteristics make them promising resources for future drug discovery efforts [13,14,15].

Recent studies highlight the antimicrobial potential of halophilic bacteria. Halomonas spp. have shown inhibitory activity against Staphylococcus aureus, Pseudomonas aeruginosa, and Candida albicans, while Pseudoalteromonas spp. isolated from saltern environments have demonstrated particularly strong antibacterial effects against S. aureus, indicating their promise as a source of novel antimicrobial compounds [16,17]. In view of the increasing antimicrobial resistance crisis and the limited exploration of microorganisms inhabiting saline coastal environments, the present study focuses on isolating and screening antimicrobial metabolite-producing bacteria from soil samples collected from different coastal regions of Gujarat, India, to evaluate the antibacterial activity of halophilic bacterial isolates obtained from coastal soil samples.

## MATERIALS AND METHODS

### Sample collection

Soil samples (50 g each) were collected from the coastal regions of Porbandar, Anand, Amreli, and Mandvi, Gujarat, India, from a depth of approximately 5-10 cm using sterile containers. One representative soil sample was collected aseptically from each sampling location. Samples were transported to the laboratory in an ice box under controlled temperature conditions and processed within 24-48 h of collection. Enrichment culture and isolation were initiated within 24 h after transportation to laboratory.

**Table 1: Sample Collection Details**

| Sr. No. | Soil Type | Sampling Depth | Ecological Significance | Sampling Region | Area/Location | Sampling Date |
|---|---|---|---|---|---|---|
| 1 | Coastal soil | 5-10 cm | High saline and marine environment | Coastal site of ocean | Mandvi-Kutchh | 12/01/2026 |
| 2 | Coastal soil | 5-10 cm | High saline and marine environment | Coastal site of ocean | Porbandar | 03/03/2026 |
| 3 | Coastal soil | 5-10 cm | High saline and marine environment | Coastal site of ocean | Amreli | 05/01/2026 |
| 4 | Coastal soil | 5-10 cm | High saline and marine environment | Coastal site of ocean | Anand | 08/01/2026 |

### Sample Testing

Each soil sample was sieved through a sterile 2 mm sieve to remove debris and other unwanted materials. Sieved sample was dried at 90 C for 2 hrs. Samples were analysed for electrical conductivity, Total

Dissolved Solids (TDS) and pH and compared with NaCl solution of 1%, 2%, 5% and 10%. The physicochemical parameters were analysed using calibrated instruments following calibrated in-house validated procedures. All measurements were performed in triplicate, and the average values were recorded.

**Table 2: Physicochemical Analysis of Standard NaCl Solution**

| Samples | NaCl [Sodium Chloride] | | | |
|---|---|---|---|---|
| | 1% | 2% | 5% | 10% |
| Electrical Conductivity [mS/cm] | 10.80 | 17.75 | 3.3 | 64 |
| TDS [ppm] | 6.8 | 11.35 | 24.3 | 39.2 |
| pH | 6.08 | 6.0 | 5.8 | 5.65 |

**Table 3: Physiochemical Analysis of Soil Samples**

| Soil Samples | Electrical conductivity [mS/cm] | TDS [ppm] | pH |
|---|---|---|---|
| **Mandvi** | **2.9** | **10.2** | **6.2** |
| **Porbandar** | 3.0 | 11 | 6.3 |
| **Amreli** | 3.1 | 10.8 | 6.0 |
| **Anand** | 3.0 | 10.5 | 6.7 |

**Isolation of Bacteria**

One gram of soil sample was suspended in 10 mL sterile distilled water and mixed thoroughly under aseptic conditions. The suspension was allowed to settle for 1 h, and the supernatant was collected. Serial ten-fold dilutions were prepared by transferring 1 mL of sample into 9 mL sterile diluent up to dilution factor of $10^{-4}$. Appropriate dilutions were spread onto nutrient agar supplemented with 5% NaCl for selective isolation of halophilic bacteria. The plates were incubated at 32.5°C for 48 h.

Morphologically distinct colonies were observed and recorded based on colony colour, texture and morphology. Well-isolated colonies were selected and purified by repeated streaking on fresh nutrient agar plates supplemented with 5% NaCl until pure cultures were obtained. Pure isolates were maintained in nutrient broth supplemented with 5% NaCl at 32.5°C for further analysis.


**Mandvi**


**Porbandar**


**Amreli**


**Anand**

**Figure 1: Sampling Locations**

**Antimicrobial assay**

The agar well diffusion method was employed to assess the antimicrobial properties of the isolates against the *Escherichia coli (ATCC 8739)*, procured from Microbiologics. One ml of broth was taken and centrifuged at 7000 rpm for 15 min and supernatant was collected. The collected extracts were stored under refrigerated conditions (2-8°C) until further analysis. The test organism Escherichia coli (ATCC 8739) was inoculated onto SCDA plates using a sterile swab under aseptic conditions.

A single well with a diameter of approximately 6 mm was prepared on the agar surface using a sterile cup borer. The well was filled with 100 µL of the bacterial extract, 5% NaCl solution, distilled water and Streptomycin respectively. The plates were incubated at 37°C for 24 hours and the inhibition zone was measured. All experiments were performed in triplicate. Streptomycin was used as the positive control, while 5% NaCl solution and distilled water were used as negative controls.

**Table 4: Information on Test Sample**

| Plate No. | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| **Purpose** | Test Sample | Negative Control | Negative Control | Positive Control |
| **Test Item** | Bacterial extracts | 5% NaCl solution | Distilled water | Streptomycin [Antibiotic] |
| **No. of Well** | 01 | 01 | 01 | 01 |

**Molecular Identification and characterization of isolated species**

The isolated bacteria species was initially characterized by morphological and gram staining characteristics. Further identification of the specie was done using Vitek-2 technique.

**Statistical analysis**

Statistical analysis was done by using validated Microsoft excel sheet and results of zones of inhibition on tested microorganism were expressed as mean ± standard deviation.

## RESULTS

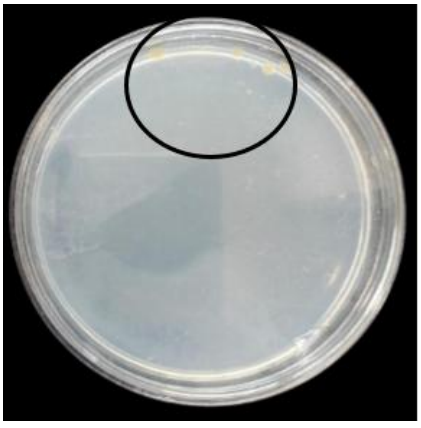
### Biochemical characterization of halophilic bacteria

In the present study, five halophilic bacterial isolates were obtained Coastal soil samples collected from different regions of Gujarat, India. The isolates were preliminary characterized based on colony morphology, pigmentation. The morphological characteristics of isolates are summarized in **Table 5**.

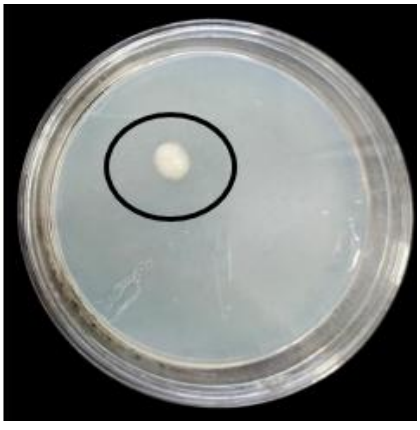
**Table 5: Sample Location and Observed Colonies**

| Sample | No. of Colony | Colony Color | Colony size | Colony texture | Cell Morphology |
|---|---|---|---|---|---|
| **Mandvi** | Yellow | large | Smooth | Rod | Yellow |
| **Porbandar** | White | medium | Smooth | Short rod | White |
| **Amreli** | Yellow | medium | smooth | cocci | Yellow |
| **Anand** | Yellow | Large | Rough | Cocci | Yellow |
| | White | medium | Smooth | Short rod | White |
| | White | Small | Smooth | cocci | White |

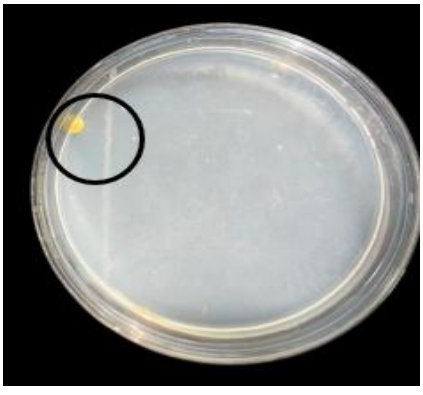
The antibacterial activity of halophilic bacterial isolates against Escherichia coli (ATCC 8739) was evaluated using agar well diffusion method. The bacterial extract demonstrated a small inhibition zone against E. coli (1.36±0.1 mm), indicating limited antibacterial activity under tested conditions.



**Mandvi**



**Porbandar**



**Amreli**


**Anand**

**Figure 2: Agar plate showing growth of halophilic bacterial isolates from coastal soil samples Antimicrobial activity of Halophilic bacteria**

**Table 6: Antimicrobial Assay of Isolates**

| Plate No. | Zone of Inhibition [mm] | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | Mean ± SD |
| Bacterial extracts | 1.5 | 1.2 | 1.4 | 1.36 ± 0.1 mm |
| 5% NaCl solution | 0 | 0 | 0 | 0 |
| Distilled Water | 0 | 0 | 0 | 0 |
| Streptomycin (1 mg/mL) [Antibiotic] | 5 | 5.5 | 5.7 | 5.4 |

## DISCUSSION

In the recent time, use of antibiotics has been increased exponentially both ethical and unethical way due to lack of awareness and sincerity towards negative impact on the human health. This practice causing increase in resistance to many first line antibiotics drugs being used in treatment of infection as well critical situation posed by the severe infections [18,19]. The burden of AMR continues to grow worldwide, with resistant pathogens projected to cause millions of deaths each year by 2050. This alarming trend highlights the urgent need for the development of new antimicrobial strategies [20].

One of the source which brings hope and have potential to provide new molecules is those bacteria who usher in harsh conditions such as salty plains of ocean coastal or deserted areas. The isolates obtained from these bacteria may serves as potential antibiotics which may use against the resisted bacteria and open treatment options for human kind [21]. Environmental bacteria, as a type of microorganism, display various advantageous characteristics within the medical and pharmaceutical fields. These advantages include the ability to absorb heavy metals, detoxify harmful substances, and produce antimicrobial peptides and antibiotics. Halophilic bacteria have great potential as a new source of bioactive substances, including antimicrobial and antitumor agents [22]. Several studies have highlighted the antimicrobial potential of halophilic bacteria isolated from hypersaline environments. In particular, halophilic actinobacteria and members of Bacillus-related genera have been reported to produce secondary metabolites that exhibit activity against a range of clinically relevant pathogens [13,14]. Similarly, metabolites derived from Halobacillus species isolated from salt pans have shown both antibacterial and antioxidant activities. Further chemical characterization by GC–MS identified diketopiperazines and cyclic peptide-like compounds among the major bioactive constituents responsible for these effects [23].

In the present study, the results of Vitek-2 technique determined that the isolated bacteria belonged to halobacillus sp. The genus halobacillus consists of rod-shaped, endospore-forming, and Gram +Ve variable bacteria included in the family Bacillaceae. Similar findings have been reported from other hypersaline environments. For example, an Alkalihalobacillus species isolated from saline soils in Shushtar, Iran exhibited antimicrobial activity against Enterococcus faecalis, with a minimum inhibitory concentration (MIC) of 25 µg/mL, highlighting the therapeutic potential of bacteria from saline habitats [24]. Likewise, halophilic bacteria recovered from the Khewra Salt Mines in Pakistan, including Halobacillus karajiensis, were found to

produce biosurfactants with antibacterial activity against both Gram-positive and Gram-negative bacteria, including Escherichia coli [25]. The similar research from the Haj Aligholi salt desert and Dagh Biarjmand of Shahrood in Iran indicated that the D6A, Dar, and D8B strains have antimicrobial properties against various pathogens such as Candida albicans, Fusarium oxysporum, Aspergillus flavus, Neurospora crassa, Botrytis cinerea, and Pseudomonas syringae pv. Syringae. Additionally, a phylogenetic analysis revealed that the D6A and Dar strains belong to Bacillus subtilis species, while the D8B strain is classified under Virgibacillus olivae [26].

The literatures reviewed and the present study findings suggest that exploring new environments could lead to the discovery of novel antimicrobial substances that target specific pathogens. Although the antibacterial activity observed was limited, the results suggest that Gujarat's coastal saline soils, being similarly unexplored, represent a valuable ecological niche for future bioprospecting [27]. A limitation of the present study is the relatively small number of isolates recovered and the evaluation of activity against only a single test organism. Further studies involving molecular identification, characterization of bioactive metabolites, determination of MIC values, and evaluation against a wider range of clinically relevant pathogens are needed to better understand their therapeutic potential.

## CONCLUSIONS

According to recent studies, further investigation is necessary to understand the composition of antimicrobial agents using appropriate techniques to determine their chemical properties, structure and mode of action. The aim of this study was to isolate formidable antibiotic-producing bacteria from Coastal region of Gujarat, India. The research reveals that isolated bacteria from coastal Mandvi region has the ability to produce antimicrobial agents as well bacteriocins that inhibit the growth of closely related strains of bacteria. The results from the antibacterial assessments on these bacteria suggest that the coastal soil in this region may serve as a valuable source of new antimicrobial substances.

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