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ISOLATION AND SCREENING OF HALOPHILIC ANTIMICROBIAL-PRODUCING BACTERIA FROM AGRICULTURAL SOIL OF KUTCHH, GUJARAT COASTAL REGION AGAINST *STAPHYLOCOCCUS AUREUS*

Microbiology

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ABSTRACT

Background: In response to the growing global shortage of novel antibiotics, the present study sought to identify new bacterial strains capable of producing effective antimicrobial compounds. To mitigate the side effects of widespread resistance, there is an urgent need for "targeted inhibition"-the discovery of antimicrobial agents that specifically target that specifically target single pathogen without disrupting the surrounding microbial ecology. Marine and coastal ecosystems, characterized by high salinity, harbor unique microbial diversity with the potential to produce specialized secondary metabolites. Therefore, this study aimed to isolate and characterize halophilic bacteria from agricultural soils of Mandvi (Kutchh), Gujarat, India, and to evaluate their antagonistic activity against *Staphylococcus aureus.* **Methodology:** Agricultural coastal saline soil sample was collected from farmland located in Mandvi, Kutch, Gujarat, India. Sample was collected aseptically from a depth of 5-10 cm. Serial dilutions of the samples were prepared and plated on nutrient agar supplemented with 10% (w/v) NaCl to facilitate the selective isolation of halophilic bacteria. The antibacterial activity of isolates against *Staphylococcus aureus (ATCC 6538)* was evaluated using agar well diffusion method. The isolates were characterized by colony morphology, Gram staining and VITEK-2 identification. **Results:** Two different bacterial strains were isolated from coastal region agricultural soil of Mandvi (kutchh). Among the isolates, one strain identified as *Geobacillus thermoleovorans,* demonstrated antimicrobial activity against *Staphylococcus aureus.* The observed inhibition zone was comparatively small (1.33 ± 0.1 cm) indicating limited antibacterial activity under the tested condition. second strain identified as *Bacillus firmus,* demonstrated antimicrobial activity against *Staphylococcus aureus (ATCC 6538)*. The observed inhibition zone was comparatively small (1.53 ± 0.1 cm) indicating limited antibacterial activity under the tested condition. **Conclusion:** This study provides preliminary evidence of the antibacterial potential of *Halobacillus sp*. isolated from agricultural coastal saline soils. The results indicate that saline agricultural environments in Gujarat represent a promising source of microorganisms capable of producing bioactive antimicrobial compounds. Further investigations involving the isolation, purification, and characterization of the active metabolites are necessary to determine their chemical nature and assess their potential pharmaceutical and biotechnological applications.

KEYWORDS

Halophilic Bacteria; Coastal Saline Soil; Agricultural Saline Soil; Antibacterial Activity; Staphylococcus Aureus; Halobacillus Sp.; Marine Environments; Gujarat; Kutchh

INTRODUCTION

The emergence and rapid spread of antimicrobial resistance (AMR) has become one of the most serious global public health threats, significantly reducing the effectiveness of existing antibiotics and increasing morbidity and mortality worldwide (1). The continued rise of multidrug-resistant bacterial pathogens has created an urgent need for the discovery of novel antimicrobial compounds with new mechanisms of action (2).

Microorganisms remain one of the most important sources of antibiotics, with a large proportion of clinically used drugs originally derived from bacterial secondary metabolites (3). These secondary metabolites include chemically diverse compounds such as polyketides, non-ribosomal peptides, and alkaloids, which play important roles in microbial competition and survival (4).

In recent years, extreme environments have gained considerable attention as potential reservoirs of novel microorganisms capable of producing unique bioactive compounds (5). Among these, saline and hypersaline ecosystems are particularly important due to the presence of halophilic and halotolerant microorganisms adapted to high salt concentrations (6).

Halophilic bacteria have evolved specialized physiological mechanisms to survive in high-salt environments, often resulting in the production of structurally unique secondary metabolites with antimicrobial and pharmaceutical potential (7). Several studies have reported that halophilic genera such as *Bacillus, Halobacillus,* and *Halomonas* exhibit antimicrobial activity against clinically important pathogens (8).

Coastal saline soils represent ecologically distinct environments influenced by seawater intrusion, evaporation, and fluctuating salinity levels, supporting diverse microbial communities (9). Such environments are still relatively underexplored, particularly in regions such as Gujarat, India, despite their potential for harboring novel bioactive microorganisms (10).

The Kutch region of Gujarat is one of the largest saline ecosystems in India, characterized by extreme environmental conditions that support unique microbial diversity (11). Previous studies have indicated that this region contains a variety of halophilic and halotolerant bacteria with biotechnological potential (10).

Staphylococcus aureus is a clinically significant pathogen responsible for a wide range of infections, including skin infections, pneumonia, and bloodstream infections (12). The increasing prevalence of *methicillin-resistant Staphylococcus aureus (MRSA)* has further complicated treatment strategies and intensified the search for alternative antimicrobial agents (12).

Therefore, the present study aims to isolate and screen halophilic bacteria from agricultural coastal saline soils of the Kutch region, Gujarat, India, and evaluate their antagonistic activity against *Staphylococcus aureus (ATCC 6538)*, with the objective of identifying potential sources of novel antimicrobial compounds.

MATERIALS AND METHODS

Sample collection

50 g of Soil sample (50 g each) was collected from the coastal agricultural regions of Mandvi, Kutchh, Gujarat, India, from a depth of approximately 5-10 cm using sterile containers. Sample was 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
1	Coastal soil	5-10 cm	High saline and marine environment	Agricultural coastal soil	Mandvi-Kutchh

Sample Testing

The collected soil sample was passed through a sterile 2 mm sieve to remove debris, stones, and other extraneous materials. The sieved samples were subsequently dried at 90°C for 2 hours prior to analysis. Sample was analysed for electrical conductivity, Total Dissolved Solids (TDS) and pH. 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: Physiochemical Analysis of Soil Sample

Soil Samples	Electrical conductivity [mS/cm]	TDS [ppm]	pH
Mandvi	0.140	0.088	6.88

Isolation of Bacteria

For the isolation of halophilic bacteria, 1 g of soil sample was suspended in 10 mL of sterile distilled water and homogenized thoroughly under aseptic conditions. The suspension was allowed to stand undisturbed for 1 h to facilitate sedimentation of particulate matter, after which the supernatant was collected. Serial ten-fold dilutions were prepared by transferring 1 mL of the supernatant into 9 mL of sterile diluent, up to a dilution factor of 10^{-4}. Aliquots from appropriate dilutions were spread onto nutrient agar supplemented with 10% (w/v) NaCl for the selective isolation of halophilic bacteria. The inoculated plates were incubated at 32.5°C for 48 h, and distinct colonies were subsequently selected for further characterization. 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 10% NaCl until pure cultures were obtained. Pure isolates were maintained in nutrient broth supplemented with 10% NaCl at 32.5°C for further analysis.

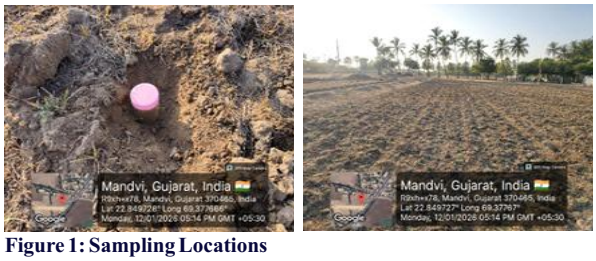

Figure 1: Sampling Locations

Antimicrobial assay

The agar well diffusion method was employed to assess the antimicrobial properties of the isolates against the *Staphylococcus aureus (ATCC 6538)*, procured from standard source. 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 *Staphylococcus aureus (ATCC 6538)* 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, 10% 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 10% NaCl solution and distilled water were used as negative controls.

Table 3: Information on Test Sample

Plate No.	1	2	3	4
Purpose	Test Sample	Negative Control	Negative Control	Positive Control
Test Item	Bacterial extracts	10% 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, two halophilic bacterial isolates were obtained Coastal agricultural soil sample collected from Mandvi, Kutchh, Gujarat, India. The isolates were preliminary characterized based on colony morphology, pigmentation. The morphological characteristics of isolates are summarized in **Table 4**.

Table 4: Sample Location and Observed Colonies

No of colonies	Colony color	Colony size	Colony texture	Cell Shape
2	White	Medium,	Smooth	Rod
	Creamish	Medium	Smooth	Rod

The antibacterial activity of halophilic bacterial isolates against *Staphylococcus aureus (ATCC 6538)* was evaluated using agar well diffusion method. The bacterial extract demonstrated a small inhibition zone against *S. aureus* (1.53±0.1 cm & 1.33 ±0.1 cm) indicating limited antibacterial activity under tested conditions.

Figure 2: Agar plate showing growth of halophilic bacterial isolates from agricultural coastal soil samples

Table 5: Antimicrobial Assay of Isolates

No. of Plates	Zone of Inhibition [cm]			
	1	2	3	Mean ± SD
Bacterial extracts (colony 01)	1.2	1.5	1.7	1.53
Bacterial extracts (colony 02)	1.5	1.2	1.3	1.33
10% NaCl solution	0	0	0	0
Distilled Water	0	0	0	0
Streptomycin (5 mg/ml) [Antibiotic]	6.2	6.5	6.3	6.33

Figure 3: Agar plate showing zone of inhibition from the extract of colony no. 1 against *Staphylococcus aureus (ATCC 6538)*

Figure 4: Agar plate showing zone of inhibition from the extract of colony no. 2 against Staphylococcus aureus (ATCC 6538)

DISCUSSION

The emergence of antimicrobial resistance among pathogenic microorganisms has intensified the search for novel antimicrobial agents from diverse environmental sources (14,15). Saline and coastal agricultural soils represent unique ecological niches that harbor microorganisms adapted to high salinity and fluctuating environmental conditions. Such microorganisms often possess distinctive metabolic capabilities and may produce secondary metabolites with potential antimicrobial properties (13,16). In the present study, two bacterial isolates obtained from coastal agricultural saline soils of Mandvi, Kutch, Gujarat, were identified as *Geobacillus thermoleovorans* and *Bacillus firmus*, and their antibacterial activity

against *Staphylococcus aureus (ATCC 6538)* was evaluated.

Both isolates demonstrated inhibitory activity against *S. aureus*, indicating their ability to produce compounds with antibacterial properties. The inhibition zones observed for *G. thermoleovorans* (1.33 ± 0.1 cm) and *B. firmus* (1.53 ± 0.1 cm) were relatively small, suggesting limited antibacterial activity under the experimental conditions employed. Nevertheless, the detection of measurable inhibition supports the presence of bioactive metabolites capable of affecting the growth of Gram-positive bacteria. The modest activity observed may be attributed to several factors, including suboptimal culture conditions, low concentrations of antimicrobial metabolites, restricted diffusion of active compounds in the agar medium, or the inherent sensitivity of the test organism (18).

Members of the genus *Bacillus* are well recognized for their ability to synthesize a wide range of antimicrobial compounds, including bacteriocins, lipopeptides, and peptide antibiotics (14,15). Previous studies have reported that *Bacillus firmus* produces extracellular metabolites exhibiting antibacterial activity against various pathogenic bacteria (14). The antibacterial activity observed in the present study is consistent with these reports and further supports the biotechnological significance of this species as a potential source of antimicrobial agents (14,15).

Similarly, species belonging to the genus *Geobacillus* have attracted increasing attention due to their thermophilic nature and capacity to produce thermostable enzymes and bioactive compounds (13,16). Although *Geobacillus thermoleovorans* has been investigated primarily for industrial enzyme production and biodegradation applications (13). Several studies have indicated that thermophilic bacteria can synthesize antimicrobial substances with potential pharmaceutical value. The antibacterial activity demonstrated by the isolate in the current study suggests that coastal saline soils may serve as reservoirs for such metabolically versatile microorganisms.

The slightly greater inhibitory effect exhibited by *B. firmus* compared with *G. thermoleovorans* may reflect differences in the nature, concentration, or spectrum of antimicrobial metabolites produced by the two species. However, the small difference in inhibition zone diameters indicates that both isolates possess comparable levels of antibacterial activity under the tested conditions. Additional studies involving optimization of growth parameters, nutrient composition, incubation conditions, and extraction methodologies may enhance metabolite production and provide a more accurate assessment of their antimicrobial potential (14,16).

The findings of this study also highlight the importance of exploring under-investigated environments such as coastal saline agricultural soils for microbial diversity and novel bioactive compounds (13,16). Gujarat possesses extensive coastal regions characterized by unique physicochemical conditions that may support microorganisms capable of producing structurally diverse secondary metabolites. Such habitats remain relatively unexplored compared with terrestrial and marine ecosystems and therefore represent promising sources for bioprospecting efforts (13,16).

Despite the encouraging findings, the study has certain limitations. Antibacterial activity was evaluated against only a single test organism, and the active compounds responsible for the observed inhibition were not isolated or characterized. Therefore, it is not possible to determine the specific mechanisms of action or the broader antimicrobial spectrum of the isolates. Future investigations should include screening against a wider range of Gram-positive and Gram-negative pathogens, determination of minimum inhibitory concentrations (MICs), purification and structural characterization of active metabolites, and assessment of their safety and therapeutic potential (19). Overall, the present study demonstrates that *Geobacillus thermoleovorans* and *Bacillus firmus* isolated from coastal agricultural saline soils possess detectable antibacterial activity against *Staphylococcus aureus*. Although the observed activity was limited, these findings provide a basis for further exploration of saline soil microorganisms as potential sources of novel antimicrobial compounds and contribute to the growing body of knowledge on the biotechnological significance of extremotolerant bacterial species (13,16).

CONCLUSIONS

Under this study, *Geobacillus thermoleovorans* and *Bacillus firmus* were successfully isolated and identified from coastal agricultural saline soils of Mandvi, Kutch, Gujarat. Both isolates exhibited inhibitory activity against *Staphylococcus aureus (ATCC 6538)*, indicating their ability to produce antibacterial metabolites. While the inhibition zones observed were relatively small, represents limited antibacterial efficacy under the experimental conditions employed. These findings contribute to the growing evidence that saline soil ecosystems are reservoirs of microorganisms with potential antimicrobial metabolites. Future research should focus on optimizing fermentation conditions and identifying the bioactive compounds responsible for the observed activity to assess their suitability for development as antimicrobial agents.

REFERENCES:

1. Murray, C.J., Ikuta, K.S., Sharara, F., Swetschinski, L., Aguilar, G.R., Gray, A., Han, C., Bisignano, C., Rao, P., Wool, E. and Johnson, S.C., 2022. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. The lancet, 399(10325), pp.629-655.
2. World Health Organization. Antimicrobial resistance fact sheet. WHO; 2023. Available from:http:// www.tbcindia.org
3. Newman, D.J. and Cragg, G.M., 2020. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. Journal of natural products, 83(3), pp.770-803.
4. Bérdy, J., 2012. Thoughts and facts about antibiotics: where we are now and where we are heading. The Journal of antibiotics, 65(8), pp.385-395.
5. Hentschel U, Piel J, Degnan SM. Marine microbial natural product research. *Nat Rev Microbiol*. 2021;19:1–14.
6. Ventosa A, Nieto JJ, Oren A. Biology of moderately halophilic bacteria. *Extremophiles*. 2021;25:1–15.
7. Hamedi J, Mohammadipanah F. Halophilic bacteria as sources of bioactive compounds. *Front Microbiol*. 2022;13:968053.
8. Kushwaha M, et al. Antimicrobial metabolites from halophilic bacteria. *Front Microbiol*. 2022;13:968053.
9. Patel S, et al. Microbial diversity in saline soils and biotechnological potential. *Sci Rep*. 2022;12:8151.
10. Gupta A, et al. Halophilic bacterial diversity of Kutch saline desert. *Front Mar Sci*. 2022;9:769043.
11. Joshi J, et al. Microbial diversity in Kutch desert ecosystem. *J Arid Environ*. 2021;190:104506.
12. Tong SYC, Davis JS, Eichenberger E, Holland TL, Fowler VG Jr. *Staphylococcus aureus*: epidemiology and pathogenesis. *Clin Microbiol Rev*. 2021;34(4):e00018-19.
13. Najar, I. N., & Thakur, N. (2020). A systematic review of the genera Geobacillus and Parageobacillus: their evolution, current taxonomic status and major applications. Microbiology, 166(9), 800-816.
14. Tran, C., Cock, I. E., Chen, X., & Feng, Y. (2022). Antimicrobial Bacillus: Metabolites and Their Mode of Action. Antibiotics (Basel, Switzerland), 11(1), 88.
15. Caulier, S., Nannan, C., Gillis, A., Licciardi, F., Bragard, C., & Mahillon, J. (2019). Overview of the antimicrobial compounds produced by members of the Bacillus subtilis group. Frontiers in microbiology, 10, 302.
16. Zebrowska, J., Witkowska, M., Struck, A., Laszuk, P. E., Raczuk, E., Ponikowska, M., ... & Zylicz-Stachula, A. (2022). Antimicrobial potential of the genera Geobacillus and Parageobacillus, as well as endolysins biosynthesized by their bacteriophages. Antibiotics, 11(2), 242.
17. Mahdi, M. S., Ibrahim, H. K., & Al-ugaili, D. N. (2024). Antimicrobial activity of Geobacillus thermoleovorans Ir1 active compounds against pathogenic bacteria. Iraqi Journal of Science, 1286-1296.
18. Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. Journal of pharmaceutical analysis, 6(2), 71-79.
19. Wiegand, I., Hilpert, K., & Hancock, R. E. (2008). Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nature protocols, 3(2), 163-175.