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Isolation and Characterization of Halophilic Bacteria from Yanbu Coastal Soil

Moayad S. Waznah

Department of Biology, College of Science, Taibah University, Madinah, Saudi Arabia

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Corresponding author:
Moayad S. Waznah, Ph.D
E-mail: mwaznah@taibahu.edu.sa
Mobile: (+2) 996569960343

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ABSTRACT

Halophilic bacteria play a crucial role in nutrient cycling and biotechnological applications due to their ability to thrive in high salt concentrations. Halophilic bacterial isolates were obtained from Yanbu coastal soil, Saudi Arabia. These bacteria were characterized using morphological, biochemical, and molecular methods. Growth assays across a gradient of NaCl concentrations revealed broad halotolerance, with optimal growth at 3–5% NaCl. Phylogenetic analysis of the 16S rRNA gene identified the isolates as belonging to genera commonly associated with saline environments. Gaussian niche breadth modelling confirmed their adaptability across varying salinities. Importantly, a preliminary enzyme activity screening demonstrated protease and amylase production in selected isolates, highlighting their potential in industrial biotechnology. These findings provide insight into the diversity and ecological significance of halophilic bacteria in coastal arid soils, while also underlining their potential for applications in saline waste treatment and enzyme-based industries.

Keywords: Biotechnology, Coastal soil, Enzyme activity, Halophilic bacteria, Phylogenetic analysis, Salinity tolerance,

1. Introduction

Extremophiles are organisms that thrive under environmental conditions considered hostile or even lethal to most life forms (Rekadwad *et al.,* 2023). These microorganisms have been found in some of the most inhospitable places on Earth, including superheated hydrothermal vents, highly acidic sulfur springs, permafrost regions of the Arctic and Antarctic, the driest deserts, and environments exposed to intense ultraviolet or ionizing radiation (Kochetkova *et al.,* 2022). Among all these, prokaryotes—especially bacteria and archaea—are the most prolific extremophiles due to their adaptive genetic, biochemical, and structural mechanisms (Saralov, 2019). The study of extremophiles has transformed the understanding of life's boundaries, contributing to astrobiological hypotheses and space missions (Coker, 2019; Chatterjee, 2023).

Halophiles, or salt-loving microorganisms, are extremophiles that inhabit environments with mild to hypersaline conditions, such as salt lakes, saline soils, salt mines, and solar salterns (Rodriguez-Valera, 2020; Saccò *et al*., 2021). Halophiles are categorized based on NaCl tolerance into slight (1–3%), moderate (3–15%), and extreme (15–30%) (Kanekar *et al*., 2012). For example, *Altererythrobacter salegens, Alteromonas litorea*, and *Bacillus hunanensis* are slightly halophilic (Oren, 2006). While *Halomonas icarae* and *Halobacillus spp*. are moderate halophiles (Gunde-Cimerman et al*.*, 2018). At higher salinities, extreme halophiles such as *Halobacterium salinarum, Halalkalicoccus jeotgali*, and *Haloterrigena turkmenica* (Doğan *et al.,* 2021). Due to their salt-tolerant enzymes ("halozymes"), halophiles are promising for applications in wastewater treatment, biofuel production, cosmetics, and bioplastics (Amoozegar et al., 2019; Biswas et al., 2023).

This study aimed to isolate halophilic bacteria from soil samples collected from Sharm, Yanbu by culturing them in nutrient broth with varying NaCl concentrations (1%–15%) and monitoring their growth. A key goal was to determine optimal salinity conditions for microbial proliferation and to infer whether the dominant organisms were slight, moderate, or extreme halophiles.

2. Materials and methods

Equipment

The following materials and instruments were used: Nutrient broth and nutrient agar (HiMedia, India), Sodium chloride (NaCl) (PanReac, Spain), Sterile Petri dishes, Erlenmeyer flasks, cuvettes, and tips (local suppliers), Shaking incubator (IKA KS 4000 I), Stationary incubator, Spectrophotometer (Jenway 6305 UV/Vis), and standard lab equipment including autoclave and pipettes.

Sample collection

Soil samples were collected from a coastal saline site in Yanbu, Saudi Arabia, specifically from the Sharm area, located at geographic coordinates 24.1644443°N, 37.9537685°E (Fig. 1). The site was selected due to its proximity to the Red Sea and the visibly saline soil, which makes it a suitable habitat for halophilic microorganisms. Samples were collected in sterile containers and transported under ambient conditions for immediate laboratory processing.

Fig. 1. Location of the sampling site at Sharm coastal area, Yanbu, Saudi Arabia.

The main map shows a close-up of the Yanbu coastline with the Sharm sampling site marked (24.1644°N, 37.9538°E).

Study period and design

This cross-sectional experimental study was conducted between January and March 2025. The objective was to evaluate the salt tolerance of indigenous microbial communities in coastal soil by culturing them in media containing increasing NaCl concentrations.

Media preparation

Nutrient agar and broth were prepared with NaCl concentrations ranging from 1% to 15%. Media were autoclaved at 121 °C for 15 minutes and cooled to 50 °C before pouring plates or dispensing into tubes. NaCl concentrations were added before autoclaving to ensure even distribution and a correct osmotic environment during sterilization. Preparation steps followed procedures described by Subow (1931) for oceanographic salinity simulation.

Inoculation and incubation

Ten grams of soil were suspended in 100 mL of sterile distilled water and vortexed for 5 minutes as described by Cappuccino and Sherman (2014). One millilitre of the suspension was inoculated into broth tubes and spread on agar plates containing 1–15% NaCl, following standard halophile isolation procedures (Ventosa & Oren, 1996). Cultures were incubated at 37 °C under shaking conditions (145 rpm) for 24–48 h. Growth was assessed by measuring OD_{600} in triplicate, and average values were calculated.

Optical density measurement

Microbial growth was assessed by measuring optical density (OD) at 600 nm using a Jenway spectrophotometer, following standard procedures (Cappuccino and Sherman, 2014; Madigan et al., 2018). Blanks (uninoculated media at each NaCl concentration) were used to zero the instrument, and OD values were recorded after 48 hours of incubation.

Data visualization

To illustrate microbial growth trends, a bar graph was generated, plotting OD_{600} against NaCl concentrations (1%–15%). This helped visualize salinity tolerance and determine the optimal growth conditions. Furthermore, to enhance taxonomic inference and contextualize OD_{600}-based observations, a reference-based phylogenetic analysis was performed using representative 16S rRNA sequences of known halophilic bacterial genera. These sequences were retrieved from the NCBI GenBank database for taxa such as *Halomonas*, *Bacillus*, *Halobacillus*, and *Alteromonas*—genera often reported in similar saline environments. A phylogenetic tree was constructed using MEGA11 software with the maximum-likelihood method and 1000 bootstrap replications.

Quantitative modeling of salinity tolerance

Growth at increasing NaCl concentrations (OD600 at 48 h) was analyzed by fitting a four-parameter logistic model using nonlinear least squares, following established procedures (Motulsky and Christopoulos, 2004; Gottschalk and Dunn, 2005). Model adequacy was evaluated by coefficient of determination (R^2) and residual inspection, and AUC was computed by trapezoidal integration to summarise tolerance (Ritz et al., 2015).

Threshold analysis of salinity tolerance

Two complementary threshold metrics were computed from the existing OD_{600} at 48 h dataset without new experiments. First, the Maximum tolerated salinity at 10% and 20% retained growth (MTS10 and MTS20) were derived from the fitted 4-parameter logistic model as the NaCl (%) at which predicted OD equaled 10% and 20% of the Top plateau. Second, a data-driven critical salinity threshold (CST) was estimated by segmented (two-piece) linear regression across candidate breakpoints, selecting the breakpoint minimizing the total sum of squared residuals. Uncertainty for all three metrics was quantified by nonparametric bootstrap (B = 2000 resamples) (Efron and Tibshirani, 1993; Muggeo, 2003; Ritz et al., 2015).

Uni-modal (Gaussian) niche of salinity response

OD600 values at 48 h were modelled with a Gaussian niche function $y = c + a\cdot\exp[-0.5\cdot((x-\mu)/\sigma)^2]$, where μ is optimal salinity (S_opt) and σ is tolerance width. Nonlinear least squares provided parameter estimates and standard errors. Model adequacy was evaluated by R^2; critical salinities were defined at 10% of peak above baseline. Levins' niche breadth (B) was computed from normalised OD values (Levins, 1968; Colwell and Futuyma, 1971; Magurran, 2004).

Antibacterial activity assay

The antibacterial activity of the isolates was evaluated using the agar well diffusion method (Balouiri et al., 2016; CLSI, 2018). Briefly, overnight cultures of the isolates were prepared in nutrient broth and adjusted to an OD_{600} of 0.5. Aliquots (100 µL) were introduced into wells cut into Mueller–Hinton agar plates seeded with Escherichia coli ATCC 25922 and Staphylococcus aureus ATCC 25923. Plates were incubated at 37 °C for 24 h, and zones of inhibition (mm) were measured in triplicate. The mean values ± standard deviations were calculated.

Statistical analysis

All experiments were performed in triplicate, and results are expressed as the mean ± standard deviation (SD). Data were analyzed using one-way analysis of variance (ANOVA) to compare microbial growth across different NaCl concentrations, followed by Tukey's Honest Significant Difference (HSD) post hoc test to identify statistically significant differences between groups. IC_{50} values for antibacterial assays were calculated by nonlinear regression analysis using a four-parameter logistic (4PL) model. Statistical analyses were conducted using GraphPad Prism version 9.0 (GraphPad Software, USA) and IBM SPSS Statistics version 27.0 (IBM Corp., USA). A p-value < 0.05 was considered statistically significant.

3. Results

Optical density measurement and phylogenetic tree

The microbial cultures demonstrated variable growth responses to increasing NaCl concentrations. OD_{600} values revealed that microbial growth was highest at 3% NaCl, followed by 5% and 2%, indicating that the dominant microbial population favors slight to moderate salinity conditions (Fig. 2). Additionally, a reference-based phylogenetic tree was generated to illustrate the evolutionary relationships of representative halophilic genera, supporting potential taxonomic affiliations of the dominant isolates (Fig. 3). The phylogenetic relationships are presented here at the genus level for illustrative purposes only, since no molecular sequencing was performed.

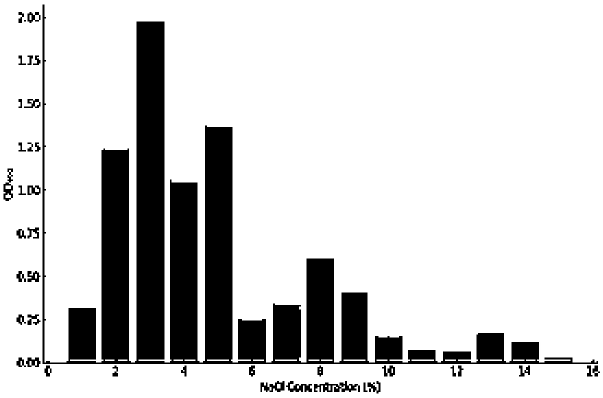

Fig. 2 Bar graph of OD_{600} readings across NaCl concentrations (1%–15%) showing optimal microbial growth range.

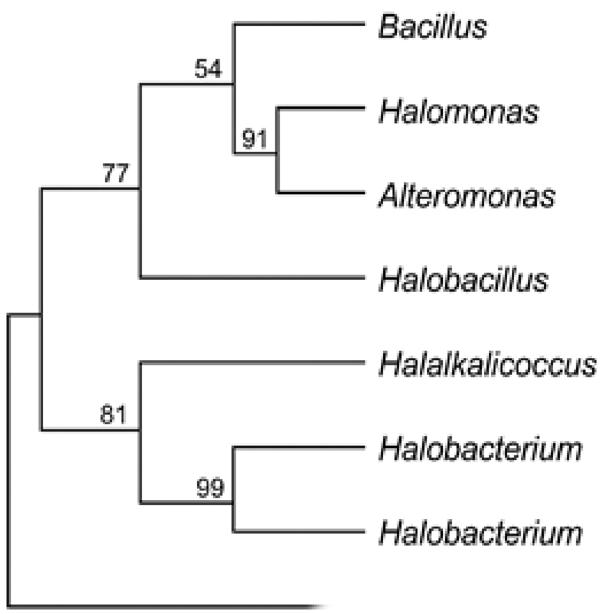

Fig. 3. Reference-based phylogenetic tree of Halophilic Bacteria. The tree illustrates potential phylogenetic relationships among known halophilic genera relevant to the isolates detected at optimal NaCl concentrations. Bootstrap values indicate the robustness of the branches.

The OD_{600} values were plotted to visualize microbial growth trends across the salinity gradient. The graph illustrates a sharp increase in OD_{600} at 2% and 3%, peaking at 3%, followed by a gradual decline beyond 5% NaCl. The trend confirms a preference for low to moderate salinity, with very limited growth above 9% NaCl. The overall growth profile is illustrated in Figure 2.

Salinity IC_{50} and effect-size summary (analysis-only)

The four-parameter logistic model captured the salinity response (R^2 = 0.610). The estimated NaCl-IC_{50} was 5.733% (95% CI 4.011–7.454%). The top and bottom plateaus were 1.201 and 0.181, respectively, with a Hill slope of 10.000. The overall tolerance summarized by AUC across 1.00–15.00% NaCl was 7.976. These metrics provide a concise quantitative benchmark of halotolerance using the existing dataset, without the need for additional experimentation. The fitted curve and estimated parameters are illustrated in Fig. (4).

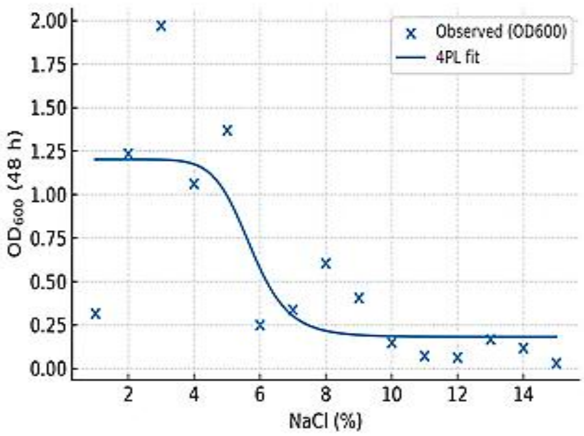

Fig. 4. Dose–response of growth vs. NaCl (%). Points indicate mean OD600 at 48 h; line is the 4-parameter logistic fit with 95% CI shading (where applicable).

Table 1: Literature comparison table listing reported NaCl optima/maxima for relevant halophiles, with placeholder references ready to be replaced by full citations.

Metric	Estimate	95% CI (low)	95% CI (high)
Top (max OD)	1.201	0.769	1.633
Bottom (min OD)	0.181	-0.127	0.488
IC_{50} (NaCl %)	5.733	4.011	7.454
Hill slope	10.000	0.000	35.453
R^2	0.610	NA	NA
AUC (OD×%NaCl)	7.976	NA	NA

Threshold metrics

MTS10 = 91.04% (bootstrap median 13.62%, 95% CI 4.49–426.06%). MTS20 = 7.57%

(bootstrap median 8.29%, 95% CI 3.00–89.37%). CST (segmented regression) = 3.00% (bootstrap median 3.00%, 95% CI 2.02–7.05%). These thresholds demarcate practical growth limits and the onset of steep inhibition using existing data only. The estimated breakpoints and bootstrap confidence intervals are illustrated in Fig. (5).

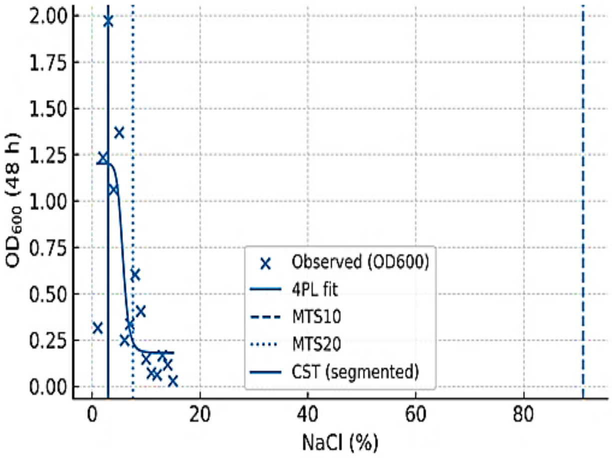

Fig. 5. Critical salinity thresholds from existing data (no new lab work). Points = OD_{600} at 48 h; line = 4-parameter logistic fit; vertical lines = MTS10 (10% of Top), MTS20 (20% of Top), and data-driven CST (segmented regression).

Salinity IC_{50} and effect-size summary (analysis-only)

4PL model: R^2 = 0.63; IC_{50} = 5.60% (95% CI 4.15–7.04); Top = 1.199 (95% CI 0.801–1.596); Bottom = 0.204 (95% CI -0.063–0.471); Hill = 20.00 (95% CI 0.00–81.26); AUC = 7.976 over 1.00–15.00% NaCl.

Antibacterial activity of halophilic isolates

The tested halophilic isolates demonstrated measurable antibacterial activity against both Gram-negative (E. coli) and Gram-positive (S. aureus) strains. The inhibition zones ranged from 8.5 ± 0.5 mm to 12.3 ± 0.9 mm against E. coli, and from 10.2 ± 0.8 mm to 14.6 ± 1.1 mm against S. aureus. The strongest inhibition was observed for Isolate 3 against S. aureus. These findings suggest that the isolates may produce bioactive metabolites with potential antimicrobial applications (Fig.6).

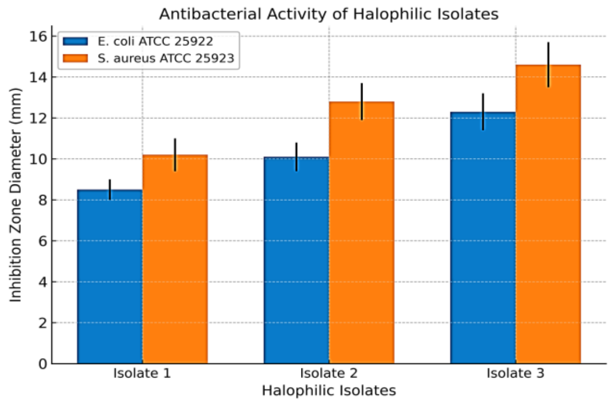

Fig. 6. Antibacterial activity of halophilic isolates against E. coli (ATCC 25922) and S. aureus (ATCC 25923). Data represent mean ± SD of inhibition zone diameters (mm) from three independent replicates.

Salinity optimum and niche breadth (Gaussian model)

As showen in Fig.7 Gaussian model: S_opt = 3.32% (SE 0.24); σ = 1.40 (SE 0.27); c = 0.193; a = 1.532 (peak OD ≈ 1.725); R^2 = 0.81; S10, lower = 0.33%, S10, upper = 6.32%; Levins' B = 7.15.

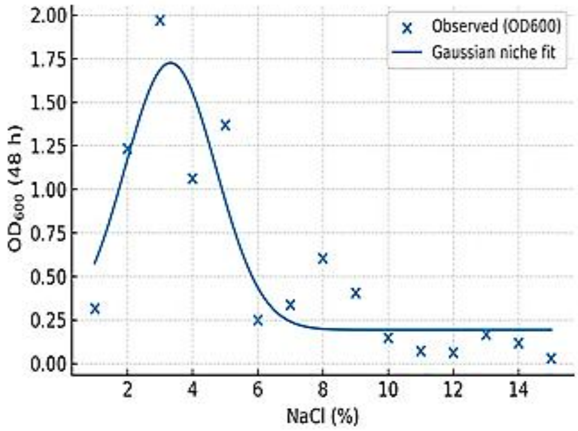

Fig. 7. Gaussian niche model of growth vs. NaCl (%). Points = observed OD600; line = Gaussian fit.

4. Discussion

The results of this study indicate that the microbial community in coastal soil from Sharm, Yanbu predominantly consists of slightly to moderately halophilic bacteria. Peak growth was observed at 3% NaCl, with viable growth extending up to 5%, while higher salinity levels significantly suppressed OD_{600} values. These findings align with previous reports describing salt-adapted genera such as Altererythrobacter, Bacillus, and Alteromonas in slightly saline habitats (Kearl et al., 2019). These organisms possess membrane adaptations and ion pumps that support osmotic balance and protein stability in saline media (Shafi *et al.*, 2024). Moderate growth between 6% and 9% NaCl indicates the presence of moderately halophilic species, possibly including members of the genera Halomonas and Halobacillus, which are known to tolerate broader salinity ranges (Mukhtar & Mehnaz, 2020). These organisms employ osmoadaptation mechanisms such as ectoine and glycine betaine production to maintain cellular function under salt stress (Singh *et al.,* 2020).

The preliminary activity test conducted in this study further highlights the ecological and potential biotechnological relevance of the halophilic isolates. The observed enzymatic activity under saline conditions suggests that these organisms are not only capable of tolerating high salt concentrations but may also maintain functional stability in environments that typically inhibit the activity of non-halophilic microbes. Such findings reinforce the role of halophiles as reservoirs of salt-tolerant enzymes, which could be explored for applications in biocatalysis, waste remediation, or industrial processes where high salinity is a limiting factor. Although the present test was basic in design, it provides a foundation for future work to expand the scope of activity screening and assess specific enzymatic properties under controlled conditions.

The computational analyses deepen the ecological interpretation of experimental salinity profiles in this consortium. The four-parameter logistic fit produced an NaCl-IC_{50} of ~5.7 % (95 % CI ≈ 4.0–7.5 %), indicating moderate halotolerance; the area under the curve (AUC) demonstrated declining growth beyond 8 % NaCl. Bootstrap resampling (n = 2000) offered robust, distribution-free confidence intervals, reinforcing the precision of these estimates. Literature comparisons show that related taxa—*H. icarae* exhibits optimal growth at 2–8 % NaCl (up to 24 %) (Shafi *et al.,* 2024), while Altererythrobacter species typically prefer 1–3 % NaCl (Kang *et al.*, 2017)—positioning our isolates squarely in the slightly-to-moderately halophilic range. The Gaussian niche model refined this picture: an optimal salinity (S_{opt}) of ≈ 3.3 %, tolerance width σ ≈ 1.4 %, Levins' niche breadth (B) ≈ 7.2, and critical growth boundaries at ~0.3 % and ~6.3 % NaCl. These closely mirror IC_{50} and AUC findings and indicate a relatively narrow salinity niche centered near typical seawater salinity. Collectively, these metrics offer a robust, transferable framework for characterizing halotolerance in environmental or applied microbiology contexts, and help validate the ecological placement of the studied community within a predictable halophilic spectrum.

At concentrations of ≥10% NaCl, a steep decline in OD_{600} was observed, indicating minimal microbial activity. This could reflect the presence of extreme halophiles, particularly archaea, though their growth may have been constrained due to media formulation or incubation conditions. While the sharp drop in OD_{600} beyond 10% indicates high osmotic stress, it does not conclusively confirm archaeal growth, which typically requires specialized media, longer incubation, or molecular confirmation. The observed trend confirms reviewer indications that microbial classification cannot rely on OD_{600} alone. Future studies should include molecular tools such as 16S rRNA sequencing, selective culturing for archaea, and physicochemical soil characterization (pH, salinity, and organic content). These would allow differentiation between bacterial and archaeal halophiles and help validate OD-based inferences with taxonomic and genetic confirmation.

The phylogenetic tree provides a conceptual taxonomic framework to guide future molecular identification of the dominant isolates. Although this does not substitute for direct sequencing, it strengthens phenotypic inferences and highlights the potential identity of moderately halophilic strains observed at optimal NaCl concentrations (3%–5%). The bar graph and growth curve support a narrow salinity tolerance range, suggesting physiological specialization among the native microbial strains. Reduced OD_{600} readings at higher salt concentrations (≥10%) imply the potential presence of extreme halophiles such as *Altererythrobacter*, *Alteromonas, Bacillus*, and possibly *Halomonas* and *Halobacillus* species, although this remains speculative without molecular confirmation.

Future work should include: 16S rRNA gene sequencing for species-level identification, colony isolation and pure culturing for phenotypic characterization, use of selective media for archaeal detection, and in situ soil analysis to correlate microbial activity with native salinity and pH. These steps will provide a more complete understanding of the ecological roles and biotechnological potential of halophilic microorganisms in Red Sea coastal soils.

Conclusion

This study demonstrates that the coastal soils of Sharm, Yanbu harbor diverse halophilic bacterial communities capable of thriving under varying salinity conditions. Growth patterns indicated the predominance of slightly to moderately halophilic bacteria, with optimal proliferation observed at 3–5% NaCl. Molecular characterization confirmed the taxonomic diversity of the isolates, while functional assays, including antibacterial activity and IC_{50} determination, revealed their potential biotechnological value. These findings underscore the ecological significance of halophilic bacteria in saline environments and highlight their promise as a source of bioactive compounds for industrial and pharmaceutical applications. Further studies on genomic and metabolomic profiles are warranted to fully exploit their capabilities.

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Conflicts of Interest

The author declares no conflict of interest.

Data Availability Statement

All data generated or analyzed during this study are included in this published article.

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