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Halophilic and Halotolerant Bacteria from Dead Sea–Adjacent Soils as Antibiotic Producers: A Jordan-Centered Bioprospecting and Genome–Metabolome Discovery Framework

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Abstract:
Antimicrobial resistance continues to outpace the discovery of new antibiotics, motivating renewed exploration of under-sampled ecological niches. The Dead Sea region in Jordan is a polyextreme system characterized by high salinity (>34%), an unusual ionic composition enriched in divalent cations, and intense ultraviolet exposure—conditions that select for halophilic and halotolerant microorganisms with distinctive stress-adaptation biology and metabolite repertoires. Here we formulate a Jordan-centered discovery framework that integrates (i) stratified soil sampling from Dead Sea–adjacent gradients (salinity, moisture, vegetation, and anthropogenic influence), (ii) cultivation using high-salt selective media to enrich halophiles and halotolerant taxa, (iii) phenotypic antimicrobial screening against a clinically relevant pathogen panel, (iv) One Strain–Many Compounds (OSMAC) perturbations to activate silent biosynthetic pathways, and (v) paired genome mining and LC-MS/MS dereplication to prioritize novelty. Recent reviews emphasize hypersaline habitats as promising reservoirs of antibiotic chemistry, but also highlight rediscovery risks and the need for modern prioritization pipelines. Accordingly, our workflow couples antiSMASH-based biosynthetic gene cluster (BGC) analysis with molecular networking and dereplication tools to rapidly exclude known scaffolds and focus resources on new chemical space. We propose reporting standards (site metadata, growth conditions, activity metrics, and sequence-verified strain deposition) and a translational roadmap from crude extracts to purified leads, mechanism-of-action hypothesis generation, and early toxicity flags. This article is designed as a practical protocol-style blueprint to accelerate antibiotic discovery from Dead Sea–adjacent soils and to position Jordan as a regional hub for extremophile bio-discovery.

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INTRODUCTION

Hypersaline ecosystems are increasingly recognized as reservoirs of microbial diversity and unusual metabolic solutions to osmotic and ionic stress. Halophilic and halotolerant microorganisms occur across all domains of life and can thrive at salt concentrations exceeding ~100–150 g/L, sometimes approaching saturation (Oren, 2024). Such settings can favor specialized enzymes, membrane chemistries, and protective solutes, which may co-evolve with secondary metabolism and chemical defense. At the same time, the global antibiotic pipeline remains fragile relative to the pace of antimicrobial resistance. Diversifying the search space beyond well-studied soils and actinomycete collections is a strategic necessity. The Dead Sea region offers an especially compelling natural laboratory. Beyond high salinity, the Dead Sea is distinguished by an atypical ion profile dominated by divalent cations and $MgCl_2$ levels near physicochemical limits for life, with surface pH near 6 and high UV exposure (Al-Daghistani *et al.*, 2024). Microbial survival under such polyextreme pressures can select for biosynthetic novelty and may reduce competitive overlap with classical terrestrial microbiomes. Indeed, antimicrobial activity has already been reported for organisms isolated from Dead Sea matrices, such as Bacillus persicus from Dead Sea mud (Al-Karablieh, 2017), supporting the plausibility of further bio-discovery.

However, halophile bio-discovery has historically been constrained by cultivation biases and high rediscovery rates. Recent syntheses emphasize that productive exploration of halophilic habitats requires a combination of enhanced isolation methods, genomic mining, and modern analytical workflows to prioritize novelty (Thompson and Gilmore, 2024). In this article, we formulate a Jordan-centered, implementable framework to discover antibiotic-producing halophilic and halotolerant bacteria from Dead Sea–adjacent soils. Our contribution is not a report of completed experimental results; rather, it is a protocol-style article that integrates best practices from hypersaline microbiology, natural-products analytics, and genome-guided prioritization.

Objectives and Research Questions

The main aim of this research is

1. To isolate and taxonomically authenticate halophilic and halotolerant bacteria from Dead Sea–adjacent soil gradients in Jordan.

2. To screen isolates for antibacterial activity against priority pathogens and to quantify activity using standardized assays (e.g., MIC and time-kill).

3. To expand chemical diversity using OSMAC perturbations (media, salinity, aeration, co-culture, and elicitors).

4. To triage hits via LC-MS/MS dereplication and molecular networking, minimizing rediscovery and focusing on chemically novel candidates (Brittin *et al.*, 2025).

5. To connect antimicrobial phenotypes with biosynthetic potential via BGC detection and comparative genomics using antiSMASH (Blin *et al.*, 2025).

6. To define a translational pathway from crude extract to purified compound(s), with early mechanism-of-action hypotheses and preliminary safety flags.

MATERIALS AND METHODS

Study area and sampling design

Sampling should be stratified across multiple microhabitats bordering the Dead Sea basin (e.g., wadis, saline flats, salt crusts, vegetated margins, sinkhole peripheries, and anthropogenically impacted sites). A gradient design is recommended, with replicate sites spanning

(i) distance from shoreline,

(ii) soil electrical conductivity (EC) and moisture, and

(iii) surface vs. subsurface horizons (e.g., 0–5 cm and 5–20 cm).

For each site, record GPS coordinates, elevation, temperature, date/time, soil texture, visible salt crusting, nearby vegetation, and land use. Collect at least 200–500 g soil per stratum using sterile tools, and transport at 4°C for short-term processing. Given that the Dead Sea is getting saltier and exhibits strong spatial heterogeneity, documenting salinity and ion composition at the sampling point is critical for reproducibility (Al-Daghistani *et al.*, 2024).

Table 1. Recommended site metadata and laboratory measurements.

Category	Field metadata	On-site measurements	Laboratory measurements
Location	GPS, elevation, site code	Ambient temperature	—
Soil	Texture, visible salt crust, color	Moisture (portable)	Gravimetric moisture; EC; pH
Chemistry	Nearby brines/springs (if any)	EC, pH	Major ions (Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, SO_4^{2-})
Ecology	Vegetation, biocrusts, land use	—	Organic matter; nutrients (N, P)

Selective cultivation of halophiles and halotolerant bacteria

To enrich halophilic and halotolerant bacteria, prepare parallel isolation media with graded NaCl (e.g., 3%, 5%, 10%, 15%, 20%) and, where feasible, mimic Dead Sea ionic conditions by supplementing with $MgCl_2$/$CaCl_2$ in controlled ranges. Common base media include marine agar, modified ISP media, and low-nutrient R2A variants. Incubate plates at multiple temperatures (e.g., 25°C, 30°C, 37°C) for up to 2–4 weeks to recover slow growers. Pick morphologically distinct colonies, re-streak to purity, and preserve as glycerol stocks at −80°C. Halophile definitions should be documented (e.g., growth optima across salt gradients) in line with operational thresholds for hypersaline life (Oren, 2024).

Molecular identification and strain deposition

Perform 16S rRNA gene sequencing for bacterial isolates (and ITS for fungi if targeted), supported by whole-genome sequencing (WGS) for prioritized bioactive strains. Use phylogenetic placement and average nucleotide identity (ANI) to assess novelty. Deposit lead strains in an accessible culture collection and deposit sequences in public repositories with rich metadata to enhance reproducibility and downstream collaboration.

Primary antimicrobial screening

Use a two-stage screen: (i) rapid agar-based inhibition assays using cell-free supernatants and crude extracts (Khalid *et al.*, 2016), followed by (ii) quantitative broth microdilution to determine minimum inhibitory concentrations (MICs) for confirmed hits. Prioritize pathogens aligned with WHO and clinical priorities (e.g., methicillin-resistant *Staphylococcus aureus*, *Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*). Because high-salt matrices can interfere with diffusion and growth, include appropriate salt-matched controls and, when needed, desalting steps prior to testing.

Table 2. Example pathogen panel and readouts for a staged antimicrobial screen.

Stage	Assay	Primary readout(s)
Stage 1	Agar overlay / well diffusion / cross-streak	Zone of inhibition; qualitative rank
Stage 2	Broth microdilution (CLSI/EUCAST-aligned)	MIC (µg/mL); MBC where relevant
Stage 3	Time-kill / biofilm inhibition (optional)	Log reduction; MBIC/MBEC; synergy screens

OSMAC and elicitation to expand chemical diversity

The OSMAC strategy systematically varies cultivation parameters to activate otherwise silent biosynthetic pathways. Classic levers include carbon/nitrogen sources, salinity, aeration, vessel geometry, incubation time, and co-culture; modern implementations also use small-molecule elicitors and enzyme inhibitors (Wei *et al.*, 2010). Recent OSMAC studies continue to demonstrate that changing media can substantially alter metabolite profiles and antimicrobial potency (de Andrade *et al.*, 2025). For Dead Sea–derived isolates, salinity and divalent cation levels are particularly meaningful experimental factors.

Table 3. Illustrative OSMAC matrix for a single lead strain (example).

Condition group	Variable	Levels (example)	Rationale
Medium	Carbon source	Glucose; glycerol; starch	Shifts precursor supply
Salinity	NaCl	5%; 10%; 15%	Stress-responsive pathways
Ions	$MgCl_2$	0; 0.5 M; 1.0 M	Dead Sea–like pressure
Ecology	Co-culture	Lead strain + competitor	Induces defensive metabolites
Chemistry	Elicitors	Sub-inhibitory antibiotics; HDAC inhibitors	De-repress silent BGCs

Extraction, LC-MS/MS dereplication, and metabolomics triage

Extract culture supernatants and biomass using solvent systems suitable for halophilic matrices (e.g., ethyl acetate for supernatants, methanol for biomass), followed by salt removal/desalting as needed. Apply high-resolution LC-MS/MS to generate spectral fingerprints. To avoid spending resources on known scaffolds, integrate dereplication early by matching MS/MS spectra to reference libraries and by applying orthogonal prioritization. For example, coupling LC-MS/MS with functional genomics profiles has been shown to minimize rediscovery and rapidly flag undesirable compound classes (Brittin *et al.*, 2025). Molecular networking (e.g., GNPS) and in silico annotation tools (e.g., SIRIUS) can further assist in identifying families of related metabolites.

Genome sequencing and biosynthetic gene cluster mining

Whole-genome sequencing of prioritized strains enables biosynthetic potential mapping and hypothesis generation about chemical classes and tailoring enzymes. antiSMASH is a leading platform for detection and analysis of secondary metabolite biosynthetic gene clusters; the current antiSMASH 8.0 release expands detectable cluster types and improves analyses of modular enzymes and tailoring chemistry (Blin *et al.*, 2025). Comparative genomics against reference halophile genomes can identify unique BGCs and guide targeted activation (e.g., via OSMAC or genetic approaches) when clusters appear silent.

Hit prioritization and progression criteria

Advance candidates based on a transparent scoring rubric combining (i) antimicrobial potency (MIC), (ii) spectrum and selectivity, (iii) novelty signals (dereplication ‘no-hit’, unique molecular network family), (iv) genomic novelty (unique BGC architecture), and (v) preliminary safety flags (hemolysis, cytotoxicity in mammalian cell lines). This aligns with calls to integrate enhanced isolation, metagenomics/genomics, and prioritization tools to overcome rediscovery barriers in halophile biodiscovery (Thompson and Gilmore, 2024).

EXPECTED RESULTS AND DISCUSSION

Using the proposed workflow, the project is expected to deliver:

(1) a curated culture collection of Dead Sea–adjacent halophilic/halotolerant bacterial isolates with sequence-verified taxonomy;

(2) a shortlist of bioactive strains with quantified MICs;

(3) LC-MS/MS datasets and molecular networks enabling dereplication and family-level novelty assessment;

(4) genomes and BGC annotations for prioritized strains, including candidate clusters linked to activity; and

(5) at least one optimized production condition (OSMAC) that increases yield or unveils new bioactivity.

Why the Dead Sea–adjacent soil niche is strategically valuable

The Dead Sea region's polyextreme conditions may enrich for microorganisms with distinctive stress-response systems, which can be coupled to specialized secondary metabolism. Beyond salinity, the dominance of divalent cations and $MgCl_2$ near limits of life may constrain community composition and favor unique adaptations (Al-Daghistani *et al.*, 2024). From a discovery standpoint, these constraints can be advantageous: unusual selective pressures may reduce overlap with well-mined terrestrial collections and, therefore, potentially lower rediscovery of classical scaffolds.

Nevertheless, novelty is not guaranteed. Hypersaline environments can still yield known antibiotic classes, and halophilic actinomycetes and Bacillus-like taxa may reproduce familiar chemistry (Thompson and Gilmore, 2024). Accordingly, the strongest contribution of this framework is the deliberate coupling of cultivation-based recovery with modern dereplication and genome-guided triage. In particular, early LC-MS/MS dereplication can rapidly identify known compounds and prevent costly downstream work (Brittin *et al.*, 2025), while antiSMASH-based BGC mining can reveal whether strains carry rare or uncharacterized biosynthetic architectures (Blin *et al.*, 2025). This combined 'metabolome-genome' logic is well-suited to resource-constrained discovery programs and can be implemented within Jordanian research infrastructures through targeted instrumentation partnerships.

Limitations and Risk Mitigation

Key limitations include cultivation bias (many halophiles remain uncultured), assay interference from salts, and the possibility that active compounds are produced at low titers. Mitigation strategies include using multiple media and incubation conditions, incorporating 'culturomics' thinking for halophiles (Oren, 2024), applying OSMAC perturbations (de Andrade *et al.*, 2025; Wei *et al.*, 2010), and investing early in analytical sensitivity (high-resolution LC-MS/MS). Another practical risk is that some bioactivities arise from general toxicity rather than specific antibacterial action; this underscores the importance of counter-screens (hemolysis, cytotoxicity) and basic mechanism-of-action hypothesis generation.

CONCLUSION

This protocol-style article outlines a Jordan-centered, implementable framework to discover antibiotic-producing halophilic and halotolerant bacteria from Dead Sea–adjacent soils. By combining stratified sampling, high-salt cultivation, staged antimicrobial screening, OSMAC-driven chemical diversification, and integrated genome-metabolome prioritization, the approach aims to maximize novelty while minimizing rediscovery. The Dead Sea region is uniquely positioned to contribute to extremophile biodiscovery, and systematic implementation of this workflow can generate publishable datasets, transferable strains, and prioritized leads for downstream drug-discovery collaboration.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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