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SALT TOLERANCE, PHENOTYPIC CHARACTERISTICS AND FUNCTIONAL SCREENING OF *BACILLUS* ISOLATES FROM SALINE SOIL

D. Azizova [1], M. Isokulov [2], S. Kamolova [1], S. Zakiryaeva [2], Kh. Khamidova [2] and H. Karimov [2, *]

[1] Department of Microbiology and Biotechnology, Faculty of Chemistry and Biology, Karshi State University, Karshi, Uzbekistan.
[2] Institute of Microbiology, Academy of Sciences of the Republic of Uzbekistan, Tashkent, Uzbekistan.
* Corresponding Author

ORCID Details
M. Isokulov: https://orcid.org/0009-0008-1638-0075
S. Zakiryaeva: https://orcid.org/0000-0002-3309-1258
Kh. Khamidova: https://orcid.org/0000-0001-7923-2813
H. Karimov: https://orcid.org/0000-0001-7262-1672

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ABSTRACT

This study assessed the taxonomic affiliation, sodium chloride tolerance, and preliminary phytohormone-associated colorimetric responses of culturable bacteria isolated from saline soil in Qamashi District, Uzbekistan. Thirteen isolates were characterized by Gram staining and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). The identifications obtained were *Bacillus halotolerans*, *B. rugosus*, *B. safensis*, and *B. pumilus*. Growth was screened on nutrient agar supplemented with 5%, 10%, and 15% (w/v) NaCl. The proportions of isolates showing visible growth were 84.6% (11/13), 76.9% (10/13), and 53.8% (7/13), respectively. Cell-free culture filtrates were evaluated using colorimetric assays associated with indole-3-acetic acid (IAA) and gibberellin-like compounds. The highest gibberellin-associated absorbance was observed for *B. rugosus* isolate 13 (OD750 = 0.805), whereas *B. pumilus* isolate 10 showed the highest IAA-associated response (OD450 = 0.776). *B. halotolerans* isolate 7 combined growth at 15% NaCl with a relatively high gibberellin-associated response (OD750 = 0.734). These findings identify promising locally adapted isolates for further study; however, the colorimetric responses do not establish hormone identity or concentration, and plant growth promotion remains to be experimentally demonstrated.

Keywords: Saline Soil; Bacillus; Salt Tolerance; MALDI-TOF MS; Indole-3-Acetic Acid; Gibberellin-Like Compounds.

1 INTRODUCTION

Soil salinization is a major abiotic constraint on agricultural productivity and agroecosystem sustainability. Climate change, drought, high evapotranspiration, saline irrigation water, inadequate drainage and rising groundwater levels exacerbate this problem. The Food and Agriculture Organization reported that approximately 1.381 billion hectares, or 10.7% of global land area, are affected by salts, with substantial additional areas at risk [1]. The challenge is particularly acute in arid and semi-arid regions dependent on irrigation [2].

Excess soluble salts lower soil-solution water potential and limit root water uptake, resulting in osmotic stress. Prolonged exposure may lead to sodium and chloride toxicity, disturbed potassium and calcium homeostasis, impaired photosynthesis, altered antioxidant metabolism, reduced biomass accumulation and yield losses [3]. Salinity also affects soil microbial diversity, community structure and metabolic activity, potentially disrupting organic-matter decomposition and nutrient cycling [4].

Not all microorganisms respond equally to saline conditions. Halotolerant bacteria can grow without requiring NaCl but withstand elevated salt concentrations, whereas obligate halophiles depend on salt for growth. Mechanisms of bacterial adaptation include ion transport and Na+/H+ antiporters, regulation of intracellular K+/Na+ ratios, accumulation of compatible solutes such as proline, glycine betaine and trehalose, and changes in exopolysaccharide production and membrane properties [5]. Screening growth across NaCl concentrations is therefore a useful first step in identifying stress-tolerant isolates.

Salt-tolerant plant growth-promoting bacteria (ST-PGPB) have attracted interest as potential biological resources for saline agriculture [6]. Reported mechanisms include nutrient mobilization, siderophore production, 1-aminocyclopropane-1-carboxylate deaminase activity, exopolysaccharide synthesis and modulation of plant hormone signaling [6,7]. Bacterial indole-3-acetic acid (IAA) may affect root architecture, lateral roots and root hairs [8]. Gibberellin-producing bacteria have also been associated with improved germination and early growth under saline conditions in certain experimental systems [9]. Nevertheless, salt tolerance alone does not demonstrate plant growth-promoting activity, and these functional traits vary among strains [10,11].

Phenotypic characteristics provide an initial description of isolates but generally do not support definitive species-level identification. MALDI-TOF MS compares microbial protein mass spectra with reference databases and can provide rapid identification, although confidence depends on reference coverage and identification scores [12]. The combination of taxonomic characterization, salinity screening and preliminary functional assays is therefore useful for selecting isolates for subsequent confirmation.

Salinization is an important issue in Uzbekistan, where irrigated agriculture is widespread [1,13]. Locally isolated bacteria from Qashqadaryo Province may represent a useful source of candidates adapted to regional environmental conditions. The objective of this study was to characterize culturable bacterial isolates from saline soil in Qamashi District using phenotypic observations and MALDI-TOF MS, assess their visible growth at 5%, 10% and 15% NaCl, and compare preliminary IAA- and gibberellin-associated colorimetric responses.

2 MATERIALS AND METHODS

2.1 Study site, soil sampling and bacterial isolation

Soil was collected on 18 April 2026 from a saline site in Qamashi District, Qashqadaryo Province, Uzbekistan (38.874484° N, 66.505119° E). Fifteen bulk-soil samples, not directly associated with plant roots, were taken from a depth of 0–30 cm and transported to the laboratory. The samples were combined to prepare one composite sample. The reported pH was 7.8 and electrical conductivity (EC) was 4–8 dS m−1;

A 10-g portion of composite soil was suspended in 90 mL of sterile distilled water (initial 10−1 dilution). Tenfold serial dilutions were prepared, and aliquots from 10−3 to 10−6 were plated on soil agar and nutrient agar (NA). Plates were incubated at 28–32 °C for up to four days. Colonies with distinct morphologies were selected and repeatedly subcultured to obtain pure cultures. Isolates were evaluated according to colony color, shape, size, margin, elevation, surface, transparency and consistency. Cultures were stored in 10% (v/v) glycerol at −80 °C; working cultures were maintained at 4–8 °C. Thirteen isolates were included in the identification and NaCl screening datasets; their original isolate identifiers were retained (1–10, 12, 13 and 15).

2.2 MALDI-TOF MS identification and microscopic characterization

Fresh colonies of pure bacterial cultures were analyzed using a MALDI-TOF mass spectrometric system at the Institute of Microbiology, Academy of Sciences of Uzbekistan. Protein mass spectra were compared with reference spectra to obtain tentative taxonomic identifications. The instrument model, sample preparation protocol, reference database and identification scores were not reported in the source records and should be added for reproducibility. Colony morphology was examined, and smears from pure cultures were Gram stained. Cells were observed using an N-300M light microscope with a 100× oil-immersion objective and an 18× eyepiece. Cell shape, arrangement and possible spore-like structures were recorded. Gram staining alone was not considered definitive evidence of endospore formation.

2.3 Salt-tolerance screening

The thirteen isolates were inoculated separately onto nutrient agar supplemented with 5%, 10% or 15% (w/v) NaCl, corresponding approximately to 856, 1711 and 2567 mM, respectively. Nutrient agar without additional NaCl served as the control. Plates were incubated at 30–32 °C for 48 h. Visible growth was recorded as positive (+), and absence of visible growth as negative (−). The highest tested concentration permitting visible growth was used as an operational screening endpoint rather than a universal tolerance threshold. The percentage of isolates exhibiting growth was calculated as P (%) = (n/N) × 100, where n is the number of isolates growing at a given concentration and N = 13. Descriptive comparisons were made; no quantitative growth-rate or inferential statistical analysis was reported.

2.4 Preliminary colorimetric screening for phytohormone-associated compounds

Cell-free culture filtrates were used for two colorimetric tests. For the gibberellin-associated assay, 1.0 mL of filtrate was mixed with 0.5 mL of diluted Folin reagent (1:1, v/v) and 2.5 mL of concentrated HCl. After incubation for 40 min in the dark, absorbance was measured at 750 nm. For the IAA-associated Salkowski assay, 0.5 mL of filtrate was combined with 4.0 mL of Salkowski reagent containing FeCl3 and concentrated H2SO4. The mixture was kept in the dark for 20 min and absorbance was recorded at 450 nm. Sterile medium treated with the corresponding reagents served as the blank. Absorbance values were used only as comparative screening indicators. In the absence of calibration curves and confirmatory chemical analyses, they were not interpreted as absolute hormone concentrations or proof of compound identity.

3 RESULTS

3.1 Phenotypic and microscopic characteristics

All thirteen isolates included in the identification dataset exhibited Gram-positive, rod-shaped cells. Some cells were predominantly solitary, whereas others occurred in chains. Spore-like structures were noted in selected isolates, but their identity was not confirmed by a specific endospore stain. The observed variation in cell arrangement and sporulation-like appearance provides a preliminary phenotypic description, not a basis for definitive species identification (Table 1; Figure 1).

Table 1: Gram reaction and microscopic morphology of the bacterial isolates

Isolate	Gram reaction	Cell morphology	Arrangement	Spore-like structures
B. halotolerans 1	Positive	Rods	Single	Not observed
B. rugosus 2	Positive	Rods	Single	Not observed
B. rugosus 3	Positive	Rods	Single	Not observed
B. halotolerans 4	Positive	Rods	Chains	Observed
B. halotolerans 5	Positive	Rods	Chains	Observed
B. rugosus 6	Positive	Rods	Chains	Observed
B. halotolerans 7	Positive	Rods	Chains	Observed
B. safensis 8	Positive	Rods	Single	Not observed
B. rugosus 9	Positive	Rods	Single	Not observed
B. pumilus 10	Positive	Rods	Single	Not observed
B. halotolerans 12	Positive	Rods	Chains	Not observed
B. rugosus 13	Positive	Rods	Chains	Not observed
B. safensis 15	Positive	Rods	Single	Not observed

Note: “Not observed” means that spore-like structures were not detected in the examined Gram-stained preparation; it does not establish an inability to form endospores.

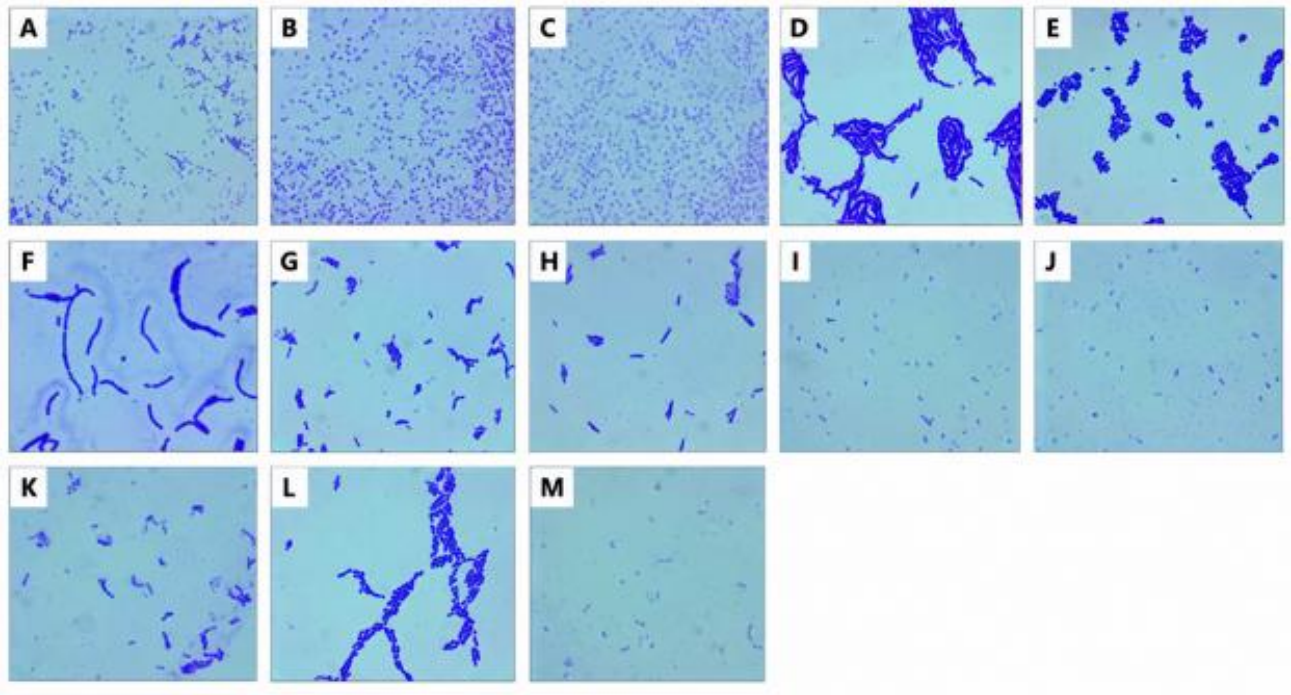


Figure 1: Gram-stained cells of bacterial isolates obtained from saline soil. Panels A–M represent 13 bacterial isolates examined using an N-300M light microscope equipped with a 100× oil-immersion objective.

3.2 MALDI-TOF MS identification

MALDI-TOF MS assigned the thirteen isolates to four Bacillus species: *B. halotolerans* (isolates 1, 4, 5, 7 and 12), *B. rugosus* (2, 3, 6, 9 and 13), *B. safensis* (8 and 15), and *B. pumilus* (10). These assignments were broadly consistent with the observed Gram-positive rod morphology (Table 2). Species-level confidence cannot be independently evaluated without the MALDI-TOF MS scores and reference database details.

Table 2: Taxonomic assignments obtained by MALDI-TOF MS

Isolate identifier	MALDI-TOF MS assignment
1	*B. halotolerans*
2	*B. rugosus*
3	*B. rugosus*
4	*B. halotolerans*
5	*B. halotolerans*
6	*B. rugosus*
7	*B. halotolerans*
8	*B. safensis*
9	*B. rugosus*
10	*B. pumilus*
12	*B. halotolerans*
13	*B. rugosus*
15	*B. safensis*

3.3 Growth under increasing NaCl concentrations

Visible growth was recorded for 11 of 13 isolates (84.6%) at 5% NaCl, 10 isolates (76.9%) at 10% NaCl, and seven isolates (53.8%) at 15% NaCl. *B. rugosus* isolates 2 and 3 did not grow at any of the tested NaCl concentrations. *B. safensis*

isolate 15 grew at 5% but not at 10% or 15%. Seven isolates—*B. halotolerans* 1, 4, 5, 7 and 12, and *B. rugosus* 6 and 9—retained visible growth at 15% NaCl (Tables 3 and 4; Figure 2).

Table 3: Visible growth of isolates on nutrient agar containing different NaCl concentrations

Isolate	5% NaCl	10% NaCl	15% NaCl	Highest tested level with growth
B. halotolerans 1	+	+	+	15%
B. rugosus 2	-	-	-	<5%
B. rugosus 3	-	-	-	<5%
B. halotolerans 4	+	+	+	15%
B. halotolerans 5	+	+	+	15%
B. rugosus 6	+	+	+	15%
B. halotolerans 7	+	+	+	15%
B. safensis 8	+	+	-	10%
B. rugosus 9	+	+	+	15%
B. pumilus 10	+	+	-	10%
B. halotolerans 12	+	+	+	15%
B. rugosus 13	+	+	-	10%
B. safensis 15	+	-	-	5%

Note: +, visible growth after 48 h; –, no visible growth under the tested conditions. The last column indicates the highest concentration tested at which growth was observed, not a quantitatively determined maximum.

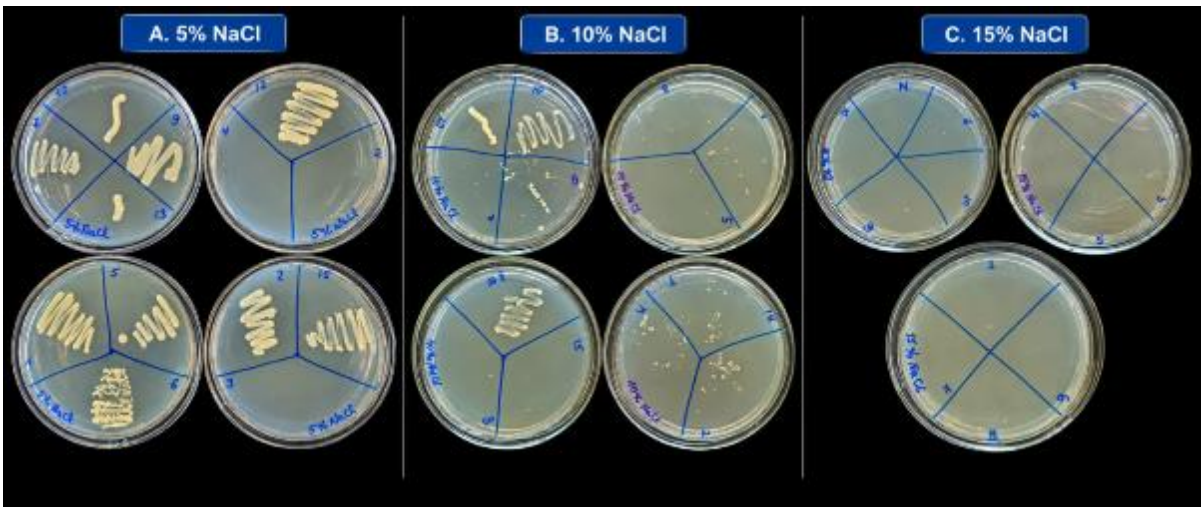


Figure 2: Representative growth of bacterial isolates on nutrient agar containing (A) 5%, (B) 10% and (C) 15% NaCl. The images illustrate the original screening experiment.

Table 4: Proportion of isolates exhibiting visible growth at each NaCl concentration

NaCl (% w/v)	NaCl (mM)	Isolates tested (n)	Growth positive (n)	Growth negative (n)	Growth positive (%)
5	856	13	11	2	84.6
10	1711	13	10	3	76.9
15	2567	13	7	6	53.8

The proportion of isolates retaining growth declined with increasing salinity. Growth intensity was not measured quantitatively; therefore, these results characterize the presence or absence of visible growth rather than growth kinetics or comparative growth rates.

3.4 IAA- and gibberellin-associated colorimetric responses

The colorimetric responses differed across isolates (Table 5). The highest absorbance in the gibberellin-associated assay was observed for *B. rugosus* isolate 13 (OD750 = 0.805), followed by *B. halotolerans* isolate 7 (0.734) and *B. safensis* isolate 8 (0.541). The highest IAA-associated response was recorded for *B. pumilus* isolate 10 (OD450 = 0.776), followed by *B. safensis* isolate 8 (0.608) and *B. rugosus* isolate 13 (0.525). No gibberellin-associated absorbance value was available for *B. halotolerans* isolate 12.

Table 5: Absorbance values in preliminary IAA- and gibberellin-associated colorimetric assays

Isolate	Gibberellin-associated OD750	IAA-associated OD450
B. halotolerans 4	0.239	0.207
B. halotolerans 5	0.279	0.272
B. rugosus 6	0.106	0.103
B. halotolerans 7	0.734	0.057
B. safensis 8	0.541	0.608
B. rugosus 9	0.200	0.071
B. pumilus 10	0.277	0.776
B. halotolerans 12	Not determined	0.086
B. rugosus 13	0.805	0.525
B. safensis 15	0.145	0.340

The response patterns were not parallel across the two assays. *B. halotolerans* isolate 7 showed a relatively high gibberellin-associated signal but a low IAA-associated signal (0.057), whereas *B. pumilus* isolate 10 showed the highest IAA-associated response and a lower gibberellin-associated response (0.277). *B. safensis* isolate 8 and *B. rugosus* isolate 13 showed relatively strong signals in both assays. These results identify differences in colorimetric reactivity among culture filtrates; they do not confirm hormone biosynthesis without compound-specific analysis.

4 DISCUSSION

The thirteen culturable Bacillus isolates differed markedly in their ability to grow on media containing elevated NaCl. Although the proportion exhibiting visible growth decreased from 84.6% at 5% NaCl to 53.8% at 15%, more than half retained growth at the highest concentration tested. The observed tolerance is consistent with reports of highly salt-tolerant Bacillus strains. For example, the literature describes *B. halotolerans* strain 105 growing at high salinity, with genomic features associated with osmotic protection and ion homeostasis [14]. However, neither osmoprotectants nor ion-transport mechanisms were measured in the present study, and these mechanisms should not be inferred for the isolates analyzed here.

Salt tolerance also varied among isolates assigned to the same species. *B. rugosus* isolates 6 and 9 grew at 15% NaCl, isolate 13 grew at 10% but not 15%, and isolates 2 and 3 did not exhibit visible growth at 5%. This heterogeneity emphasizes that taxonomic assignment alone is insufficient to predict a strain's response to salt stress. Studies of plant-associated, stress-tolerant bacteria have likewise described strain-dependent functional diversity [15].

The preliminary colorimetric data further demonstrated that salt tolerance and phytohormone-associated responses were not consistently aligned. *B. rugosus* isolate 13 produced the highest gibberellin-associated absorbance and a relatively high IAA-associated response, but failed to grow at 15% NaCl. *B. safensis* isolate 8 also showed strong colorimetric responses but lacked growth at 15%. Conversely, *B. halotolerans* isolate 7 combined visible growth at 15% NaCl with a relatively high gibberellin-associated absorbance. Thus, isolate 7 is a candidate for targeted follow-up, rather than a confirmed plant growth-promoting strain.

Previous studies have reported growth-promoting traits in *B. safensis* PM22 and several *B. pumilus* strains, including IAA-related activity, ACC deaminase, siderophore production, phosphate solubilization and improved crop performance

under salinity [16–19]. These published findings provide a rationale for further screening, but cannot be extrapolated as experimentally demonstrated properties of the present isolates. Chemical confirmation of IAA and gibberellins using chromatographic or mass-spectrometric methods, together with calibration and independent biological replicates, is needed before claiming phytohormone production.

The MALDI-TOF MS results suggest that the isolates belong to *B. halotolerans*, *B. rugosus*, *B. safensis* and *B. pumilus*. The absence of reported instrument settings, reference-library coverage and identification scores limits the strength of species-level assignments. Where high taxonomic certainty is required, sequence-based confirmation should be considered [12]. In addition, the soil sampling relied on one composite sample, and the screening used qualitative growth endpoints. Consequently, the results describe the investigated isolate collection and should not be generalized to the full bacterial community or interpreted as replicated field-level prevalence estimates.

Overall, the study provides a descriptive screening dataset from saline soil in Qashqadaryo Province. Follow-up experiments should prioritize standardized quantitative salt-growth assays, validated phytohormone measurements, and seedling or greenhouse trials under defined salinity to determine whether the selected isolates confer measurable plant benefits. Potential biosafety considerations should also be assessed before agricultural application.

5 CONCLUSION

Thirteen culturable isolates from saline soil were assigned by MALDI-TOF MS to four Bacillus species and displayed heterogeneous salt tolerance. Seven isolates (53.8%) maintained visible growth at 15% NaCl. Preliminary colorimetric screening revealed isolate-dependent IAA- and gibberellin-associated responses; *B. halotolerans* isolate 7 combined tolerance to 15% NaCl with a relatively high gibberellin-associated signal. These findings support the selection of local candidates for further characterization but do not establish phytohormone identity or plant growth-promoting efficacy. Confirmatory analytical and plant-based experiments are necessary before biotechnological application.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

The authors declare no conflicts of interest regarding this manuscript.

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