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STATISTICAL, MOLECULAR, AND ULTRASTRUCTURAL INSIGHTS INTO THE STRESS-ALLEVIATING POTENTIAL OF AUTOCHTHONOUS STEPPE BACTERIAL LINEAGES

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ABSTRACT

The identification and characterization of multi-stress tolerant Plant Growth-Promoting Bacteria (PGPB) are essential for developing sustainable agricultural strategies to mitigate the effects of abiotic stress factors and intensive agrochemical usage. This study aimed to evaluate the stress tolerance of eleven autochthonous strains isolated from steppe zone previously exposed to glyphosate application. Taxonomic identity was confirmed by 16S rRNA gene sequencing, resulting in a phylogenetically diverse collection of isolates belonging to the genera *Pseudomonas* spp., *Paenibacillus* spp., and *Paenarthrobacter* spp. The strains were subjected to a comparative tolerance screening under abiotic and xenobiotic stress, including osmotic, saline, and glyphosate exposure. Quantitative growth histograms revealed distribution of strains depending on stress factors, while principal component analysis (PCA) identified distinct clustering patterns, indicating genus-specific tolerance thresholds. Molecular screening detected the *groL* and *ectA* genes in *Paenibacillus* isolates, indicating potential roles in protein folding and osmoadaptation. Furthermore, ultrastructural scanning and transmission microscopic imaging revealed exopolysaccharide (EPS) production, suggesting its role in cellular protection. Overall, the eleven indigenous strains showed synergistic physiological, genetic, and structural adaptations, highlighting their potential as multi-functional bio-inoculants for stress-resilient agriculture.

Keywords: PGPB, *Pseudomonas*, *Paenibacillus*, *Paenarthrobacter*, glyphosate tolerance, EPS, SEM/TEM, abiotic stress factors.

INTRODUCTION

Modern agriculture is increasingly exposed to various abiotic stress factors, including pesticide pollution, soil salinization, and drought conditions, all of which substantially affect crop productivity and soil health [1, 2]. The extensive application of pesticides has resulted in significant soil contamination worldwide [3]. Consequently, the soil microbiome, which plays a crucial role in maintaining soil fertility and ecosystem stability, becomes severely affected. Recent studies indicated pesticide pollution as one of the major factors negatively influencing soil microbial diversity [4]. In parallel, saline soils are widely distributed across the globe, while continuous salinization has become a serious threat to sustainable agricultural production [2]. Furthermore, climate change imposes additional pressure on agricultural systems, as increasing global temperatures intensify drought stress and reduce crop resilience [5].

Soil functionality and plant productivity are strongly dependent on balanced soil microbiome [6]. Disturbance of beneficial microbial communities may lead to long-term negative effects on soil fertility, nutrient cycling, and agricultural productivity [7]. Therefore, maintaining stable and beneficial microbial populations represents one of the key foundations of sustainable agriculture. Among the promising approaches for alleviating abiotic stress in crops is the application of plant growth-promoting bacteria (PGPB) [1]. Numerous studies have demonstrated the positive effects of PGPB on different agricultural crops under diverse stress conditions, highlighting their considerable potential for sustainable agricultural applications [8-10].

A common strategy for the development of effective PGPB-based preparations involves the isolation of native bacte-

rial strains followed by their comprehensive evaluation under laboratory, greenhouse, and field conditions [11]. In this context, autochthonous bacterial strains represent particularly valuable candidates because they are naturally adapted to local environmental conditions and may integrate into indigenous microbial communities with minimal disturbance to native microbiome structure and diversity. Therefore, comprehensive characterization of indigenous soil bacterial communities is required, including phenotypic examination, molecular insights, statistical evaluation, and stress-response mechanisms [12]. Such holistic investigations provide important insights into the microbial populations and the physiological and molecular mechanisms underlying their stress tolerance. Therefore, the present study aimed to isolate glyphosate-tolerant bacterial species from the local steppe zone, with subsequent phenotypic, molecular, ultrastructural, and statistical analyses to evaluate the stress-alleviating potential of indigenous steppe bacterial lineages.

MATERIALS AND METHODS

Isolation and identification of indigenous steppe bacterial lineages

The bacterial strains were isolated from the rhizosphere of soft spring wheat (*Triticum aestivum* L. cv. "Aqmola 2") collected in Northern Kazakhstan. Soil suspensions (5 g in 40 mL of 0.9% NaCl) were serially diluted, and 50 µL aliquots were plated onto *Pseudomonas* Minimal Medium (PMM) [13]. To select glyphosate-utilizing strains, glyphosate ("Uragan Forte", 1 g/L) was used as the sole carbon source. The medium was supplemented with nystatin (0.05 g/L) and fluconazole (0.05 g/L) to prevent fungal contamination. In addition, enrichment culture approach using liquid culture grown in PMM was applied at two-step passage. After incubation

at 25 °C for 3-5 days, glyphosate-utilizing colonies were obtained.

Taxonomic identification of purified isolates was performed using 16S rRNA gene sequencing. Genomic DNA was extracted, and the 16S rRNA gene was amplified using universal bacterial primers (Eub 341F and Eub 1060R) [14]. Sequences were analyzed using BLAST, and the 2, 3, and 6 isolates were assigned to the genera *Pseudomonas*, *Paenarthrobacter*, and *Paenibacillus*, respectively.

Evaluation of tolerance to the presence of glyphosate, saline and osmotic stress factors

The tolerance of bacterial isolates to glyphosate, salinity, and osmotic stress was evaluated using growth-based assays under controlled conditions.

Glyphosate tolerance was assessed using a microplate growth assay. YEG medium (g/L: yeast extract 5; glucose 10; agar 20) was supplemented with glyphosate at final concentrations of 0, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, and 5.0 g/L. Overnight cultures were used to prepare cell suspensions, and 180 µL of amended medium with 20 µL inoculum was added per well of a 96-well plate. All treatments were performed in triplicate. Plates were incubated at 25 °C with shaking at 200 rpm using CAPP Rondo 4-Place Incubating Shaker, CRPI-412X (AHN Biotechnologie, Germany). Bacterial growth was monitored by measuring optical density at OD₆₂₀ after 24 and 48 h using a Multiskan SkyHigh microplate spectrophotometer (Thermo Fisher Scientific, USA). The longer incubation time point was selected for all analyses since certain strains needed prolonged time to adapt to stress exposure.

Salinity tolerance was evaluated by culturing bacterial isolates in liquid YEG medium supplemented with different NaCl concentrations (0, 2, 5, 10, 15, 20, 25, and 50 g/L). Osmotic stress tolerance was determined using YEG supplemented with polyethylene glycol (PEG 6000 at 0, 25, 50, 100, 150, 200, 250, and 300 g/L) to simulate drought conditions. Inoculation, incubation, and measurements were performed as described above.

Principal component analysis (PCA) of bacterial tolerance across the genera

To evaluate the metabolic response patterns of the investigated bacterial lineages (*Paenarthrobacter*, *Paenibacillus*, and *Pseudomonas*) under various abiotic stress conditions, Principal Component Analysis (PCA) was performed. Growth data, measured as optical density (OD₆₂₀), were first transformed from a long to a wide format for each stress factor: glyphosate (0-5 g/L), NaCl (0-50 g/L), and PEG 6000 (0-300 g/L). All multivariate analyses were conducted in R (v. R-4.3.1, <https://www.r-project.org/>) and RStudio (v. 2023.06.1, <https://posit.co/>) The PCA was executed using the *prcomp* function with data centering and scaling to ensure equal weighting of the concentration gradients. Visualization of the individual factor maps was performed using the *factoextra* [15] and *ggplot2* [16] packages. Confidence ellipses were calculated at a 95% confidence level to define the multivariate space occupied by each genus. Final multi-panel figures were assembled using the *patchwork* library [17], employing a unified theme to ensure publication quality and consistency.

PCR-based detection of genes involved in stress tolerance

PCR screening was performed to detect genes associated

with abiotic stress tolerance in the bacterial isolates. Two target genes were analyzed: *ectA*, associated with ectoine biosynthesis and osmoprotection, and *groL*, general stress response and protein folding.

Amplification of the *ectA* gene was carried out using the primers FW: 5'-CTGTTAAAGTAACACTCGGAC-CGAAAG-3' and RV: 5'-CAGCGTTGAATGTACCACG-CAGTTTG-3'. PCR conditions included an initial denaturation at 98 °C for 3 min, followed by 31 cycles of 98 °C for 15 s, 69 °C for 20 s, and 72 °C for 18 s, with a final extension at 72 °C for 2 min.

The *groL* gene was amplified using primers FW: 5'-CGAACGATGTAGCTGGTGTATGGTAC-3' and RV: 5'-CAGCGTTGAATGTACCACGCAGTTTG-3' under the same cycling conditions.

Scanning and transmission ultrastructural characterization of EPS as stress-alleviating factor

EPS production was evaluated using liquid EPS-inducing medium composed of (g/L): yeast extract 20; MgSO₄ 20; K₂HPO₄ 20; CaCl₂ 0.02; NaCl 0.01; FeSO₄*7 H₂O 0.01; and MnSO₄*H₂O 0.01; supplemented with 50 g/L sucrose or glucose [18, 19] according to the method described by [20]. Cell suspensions prepared at 10⁸ CFU/mL in 0.9% NaCl were inoculated (5 µL) in sterile paper disk placed on EPS agar. The appearance of mucous colonies around the disk after 24-h incubation at 25 °C was attributed for EPS production.

Subsequently, *Paenibacillus* sp. ZHS-9 and *Pseudomonas* sp. ADL-13 were selected as representative strains for SEM and TEM analyses based on their high stress tolerance, positive EPS production, and taxonomic representation. Cultures were incubated in liquid EPS-inducing medium under static conditions at 28 °C for 24 h, with an initial cell density of approximately 1 x 10⁵ CFU/mL. Following incubation, samples of 1 mL were gently mixed with 1 mL of 2.5% glutaraldehyde (GA) and fixed at 4 °C for overnight. Scanning electron microscopy (SEM) and Transmission electron microscopy (TEM) were performed using an external service (Electron Microscopy Laboratory, Core Facilities and HPC, Nazarbayev University). The obtained images were used to evaluate cell morphology, ultrastructural features, and EPS production.

RESULTS

Isolation and identification of indigenous steppe bacterial lineages

A total of 11 bacterial strains were successfully isolated from soft spring wheat (Aqmola-2) fields in Kazakhstan. Primary screening via serial dilution on selective medium supplemented with 1 g/L glyphosate revealed diverse colony morphologies. To clarify their taxonomic status and functional capabilities, these isolates were further characterized for their molecular identification and environmental resilience. The isolated strains, taxonomic identification, and tolerance profiles of these autochthonous steppe bacterial lineages to various abiotic stress factors and EPS production are summarized in Table 1.

Molecular identification based on 16S rRNA gene sequencing confirmed the taxonomic affiliation of the isolated bacterial strains. The obtained sequences showed high similarity to members of the genera *Paenarthrobacter* (ZHS-4, ZHS-

Table 1. Isolation and identification of autochronous steppe bacterial lineages and their tolerance to abiotic stress factors and EPS synthesis.

Bacterial strain	16S rRNA-based identification and NCBI accession number	Tolerance to abiotic stress factors			EPS synthesis
		Glyphosate, g/L	NaCl, g/L	PEG 6000, g/L	
ZHS-4	<i>Paenarthrobacter</i> sp. [PZ467928]				-
ZHS-5	<i>Paenarthrobacter</i> sp. [PZ467929]				-
ZHS-6	<i>Paenarthrobacter</i> sp. [PZ467930]				-
ZHS-8	<i>Paenibacillus</i> sp. [PZ467931]				+
ZHS-9	<i>Paenibacillus</i> sp. [PZ467932]				+
JSM-10	<i>Paenibacillus</i> sp. [PZ059921]				+
AZ-1	<i>Paenibacillus</i> sp. [PZ467923]				+
AZ-2	<i>Paenibacillus</i> sp. [PZ467924]				+
AZ-3	<i>Paenibacillus</i> sp. [PZ467925]				+
ADL-13	<i>Pseudomonas</i> sp. [PZ467926]				+
ADL-20	<i>Pseudomonas</i> sp. [PZ467927]				+

Green and red colours in the abiotic stress tolerance section represent the significant ($p < 0.05$) and insignificant ($p > 0.05$) tolerance of the strains, respectively comparing to control without stress factor. The + and – signs in the EPS production indicate the presence and the absence of EPS synthesis, accordingly.

5, and ZHS-6), *Paenibacillus* (strains ZHS-8, ZHS-9, JSM-10, AZ-1, AZ-2, and AZ-3), as well as *Pseudomonas* (strains ADL-13 and ADL-20). The 16S rRNA analysis revealed phylogenetic diversity among the steppe-derived isolates suggesting their phylogenetic diversity.

Stress tolerance for glyphosate, saline, and osmotic stress factors was set as 0.1 g/L, 15 g/L [21], and 40 g/L [22], respectively. Statistical analysis indicated no significant differences in bacterial growth of the majority of the strains between the control and several high-stress treatments ($p > 0.05$), demonstrating the ability of tolerant isolates to maintain growth under elevated concentrations of glyphosate, NaCl, and PEG 6000. These results confirm the sufficient abiotic stress tolerance of the selected bacterial strains.

PCA of bacterial lineages for tested abiotic stress factors

Principal component analysis (PCA) revealed clear genus-dependent trends in tolerance profiles across all tested stress conditions (Figure 1).

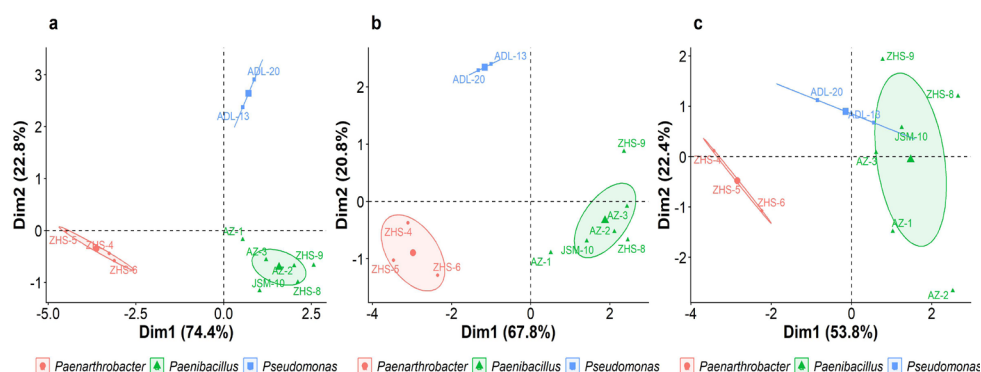
For glyphosate, the first principal component (PC1, 74.4% variance explained) distinctly separated *Paenarthrobacter*

strains from *Paenibacillus* and *Pseudomonas*, indicating fundamentally different response patterns (Figure 1a). *Paenibacillus* strains formed a relatively broad cluster, suggesting intra-genus variability, whereas *Pseudomonas* strains were separated along PC2, reflecting a distinct response trajectory.

A similar pattern was observed under NaCl stress, where PC1 (67.8%) again discriminated *Paenarthrobacter* from the other genera (Figure 1b). In contrast, *Paenibacillus* strains formed a more compact cluster, indicating more uniform salt tolerance. *Pseudomonas* strains remained separated along the secondary axis, suggesting consistent differences in stress response mechanisms.

Under osmotic stress (PEG), the overall variance explained by PC1 decreased (53.8%), reflecting increased phenotypic complexity (Figure 1c). While *Paenarthrobacter* strains remained clearly separated, *Paenibacillus* exhibited pronounced dispersion, indicating substantial intra-genus variability. *Pseudomonas* strains again occupied a distinct position along PC2.

Overall, PCA consistently indicated genus-dependent pat-

**Figure 1.** PCA across different genera towards abiotic stress factors: (a) glyphosate; (b) NaCl; (c) PEG 6000

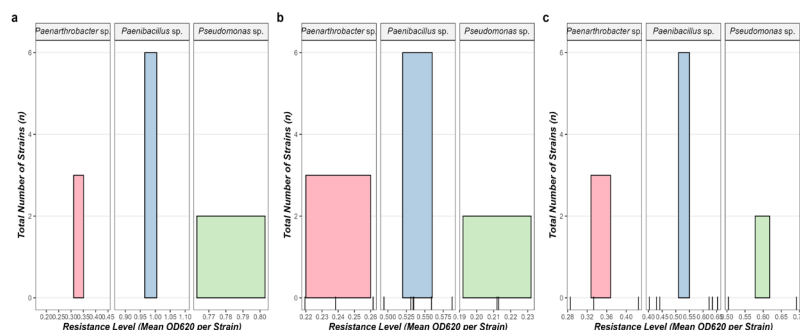


Figure 2. Histogram showing distribution of bacterial lineages across different abiotic stress factors: (a) glyphosate; (b) NaCl; (c) PEG 6000

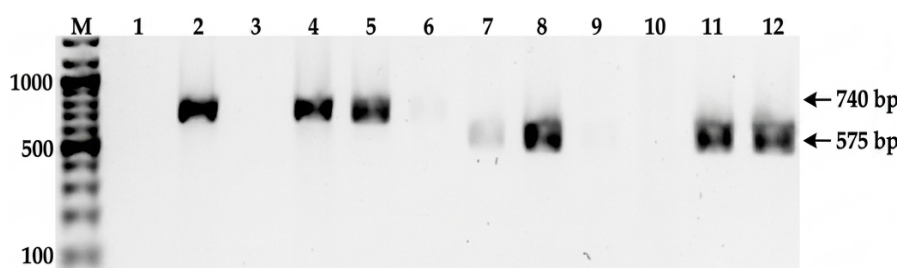


Figure 3. PCR-based detection of genes putatively involved in stress alleviation. Lines 1-6 are *ectA* PCR results of AZ-1, AZ-2, AZ-3, ZHS-8, ZHS-9, JSM-10, respectively; lines 7-12 are *groL* PCR results of AZ-1, AZ-2, AZ-3, ZHS-8, ZHS-9, JSM-10, accordingly.

terns in stress responses among the investigated genera of *Paenarthrobacter*, *Paenibacillus*, and *Pseudomonas*, exhibiting different tolerance profiles across stress conditions.

Bacterial tolerance to glyphosate, NaCl and PEG 6000 distributed by their genera

The distribution of bacterial tolerance across different genera was evaluated under three abiotic stress conditions, including glyphosate, NaCl, and PEG 6000 (Figure 2). Overall, clear genus-dependent differences were observed in response to all tested stress factors.

Genus-dependent distribution of bacterial tolerance based on mean optical density (OD_{620}). Under glyphosate (Figure 2a) and salinity (Figure 2b) stress, *Paenibacillus* sp. showed the highest resilience, forming a high-cell density cluster (0.525-0.550), while *Paenarthrobacter* sp. and *Pseudomonas* sp. exhibited lower growth levels. Under PEG 6000-induced osmotic stress (Figure 2c), *Paenibacillus* sp. remained dominant (0.95-1.00), *Pseudomonas* sp. showed intermediate tolerance, and *Paenarthrobacter* sp. demonstrated the lowest biomass accumulation (0.28-0.33).

PCR-based detection of stress-response genes

To evaluate the genetic potential of the isolated *Paenibacillus* strains to mitigate environmental stress, the presence of functional genes *ectA* (saline and osmotic stress response) and *groL* (saline conditions) was analyzed via PCR (Figure 3).

PCR analysis confirmed the presence of the stress-associated genes *ectA* and *groL* in several *Paenibacillus* isolates (Figure 3). Amplicons of the expected sizes, 740 bp for *ectA* and 575 bp for *groL*, were detected by agarose gel electrophoresis. The *ectA* gene was identified in strains corresponding to lanes AZ-2, ZHS-8, ZHS-9, and JSM-10, whereas *groL* amplification was observed in lanes AZ-1, AZ-2, ZHS-9, and JSM-

10. No amplification products were detected in the remaining isolates. These findings indicate the presence of the stress-associated genes, suggesting a potential role in osmotic and saline stress adaptation.

SEM and TEM images of EPS produced by *Paenibacillus* and *Pseudomonas* strains

All strains of *Paenibacillus* and *Pseudomonas* showed mucous and translucent colonies on EPS-inducing medium, suggesting their potential of EPS synthesis. Subsequently, visual ultrastructural obtained confirmation of extracellular polymeric substance (EPS) production by the representative isolates was obtained using SEM and TEM electron microscopy (Figure 4).

Electron microscopy demonstrated EPS production in *Paenibacillus* sp. ZHS-9 and *Pseudomonas* sp. ADL-13 cultivated in EPS-induced medium. SEM images showed dense extracellular networks surrounding bacterial cells and promoting attachment to the crystalline substrate (Figure 4a, c). After dehydration, the EPS matrix exhibited a porous film-like structure. TEM analysis further revealed an electron-dense capsule-like layer around the cell envelope, confirming extracellular polymer accumulation in both strains (Figure 4b, d). Differences in matrix density and organization suggested strain-specific EPS architecture under stress conditions.

DISCUSSION

Agricultural production is increasingly challenged by pesticide exposure and abiotic stresses such as drought and salinity. It leads to reduce crop health, disrupt plant metabolism, and limit yield. Under these conditions, autochthonous steppe bacterial lineages represent a promising resource for sustainable crop protection because native plant-associated microbes

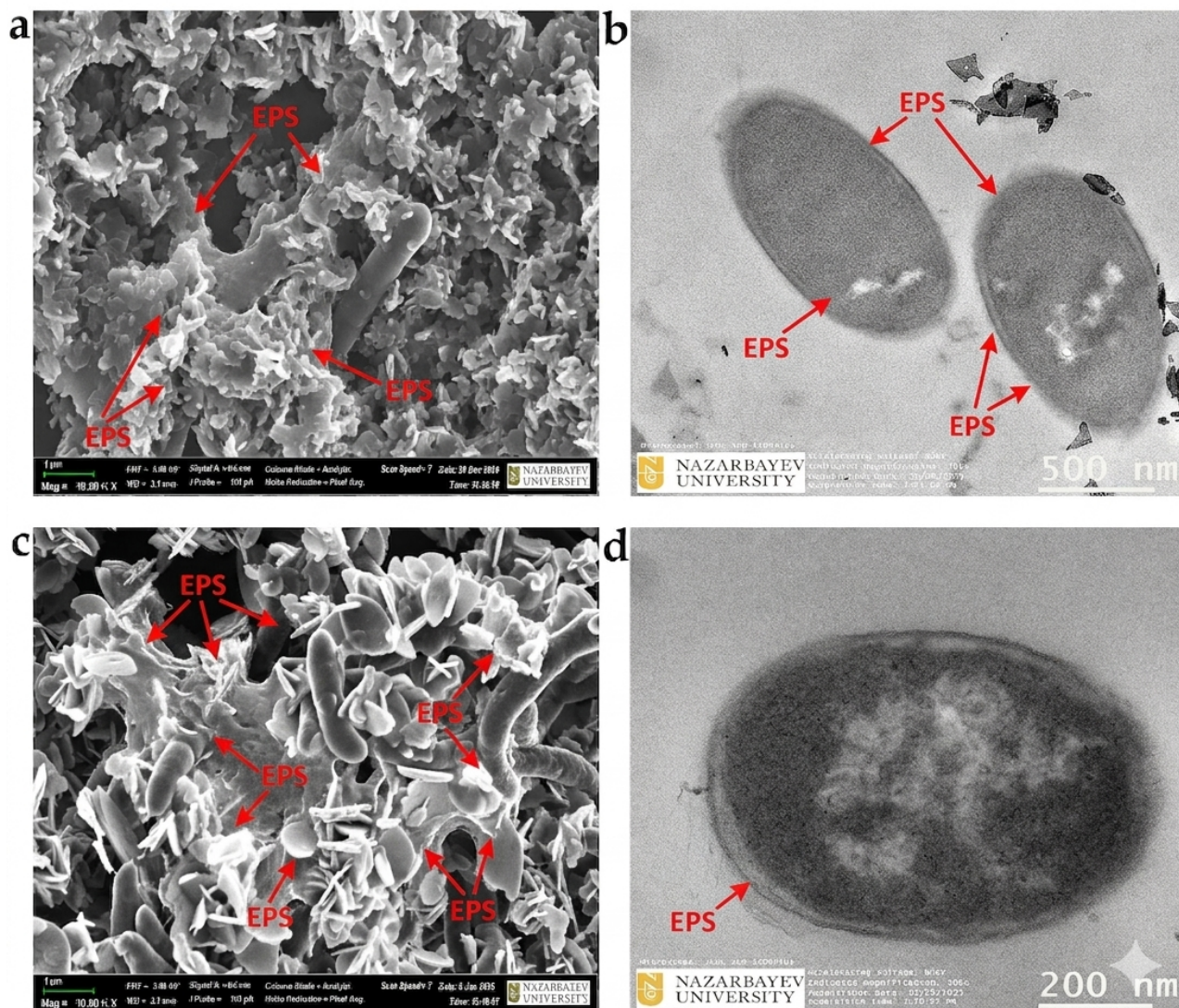


Figure 4. Electron micrographs of EPS-producing bacterial strains grown in EPS-induced medium. (a) SEM and (b) TEM image of *Paenibacillus* sp. ZHS-9; (c) SEM and (d) TEM image of *Pseudomonas* sp. ADL-13. Arrows indicate extracellular polymeric substances (EPS).

are often better adapted to harsh local environments.

PCA analysis indicated genus-dependent patterns of the bacterial lineages to the tested abiotic stress factors, indicating functional diversity among the isolates. Several strains exhibited high stress tolerance associated with effective osmotic regulation, antioxidant activity, and rhizosphere adaptation. PCA analysis showed clear differences between the bacterial lineages in their responses to the tested abiotic stress factors, indicating functional diversity among the isolates. *Paenarthrobacter* strains formed a separate cluster, suggesting distinct stress-response characteristics. *Pseudomonas* isolates were closely grouped and showed stable distribution patterns associated with strong stress tolerance. In contrast, *Paenibacillus* strains were more widely distributed across the PCA plots, reflecting greater physiological variability and different adaptation strategies under stress conditions. The high percentage of variance explained by the first principal component supported the reliability of the clustering results.

The PCA pattern observed for our isolates is consistent with previous reports on stress-adapted bacterial strains. For example, *Arthrobacter* sp. Helios are known for strong des-

iccation-related adaptation [23], while *Pseudomonas putida* 12D6 has been reported as a drought-mitigating PGPB [24]. In the case of *Paenibacillus*, strains such as *P. polymyxa* SC2 and HY96-2 show salt tolerance and biofilm-associated stress adaptation, which may explain the broader dispersion of *Paenibacillus* isolates in our PCA plots [25, 26]

Functional gene profiling identified *ectA* and *groL*, indicating multiple stress-adaptive traits in the studied strain. The *ectA* gene is involved in ectoine biosynthesis, suggesting osmotic and abiotic stress tolerance. The *groL* gene encodes the GroEL chaperonin, which supports protein folding and cellular stability under stress conditions. Together, these genes indicate the strain's potential for detoxification, osmoprotection, and survival in environmentally stressed soils [27].

In our study, these genes were specifically identified in the *Paenibacillus* strain, and all functional interpretations are therefore restricted to this genus. The *ectA* gene indicates that the *Paenibacillus* strain can produce ectoine, a compatible solute known to enhance osmotic and salt stress tolerance in halotolerant and plant-growth-promoting bacteria. Our isolate remained viable at salinity levels up to 15 g/L NaCl, in line

with the reported role of ectoine-related pathways in osmoprotection. Likewise, *groL* points to efficient GroEL-mediated protein folding and cellular homeostasis under heat and other abiotic stresses, consistent with the stress-protective functions of GroEL in diverse bacteria. The strains maintained higher viability under both salinity stress (up to 15 g/L NaCl) and thermal shock conditions, emphasizing the importance of chaperone systems in stress adaptation. Taken together, the co-occurrence of *ectA* and *groL* in this *Paenibacillus* strain suggests a multifunctional stress-adaptation strategy that combines detoxification, osmoprotection, and chaperone-dependent protein stability, potentially enhancing its fitness in contaminated or saline soils. Future studies incorporating a broader panel of stress-associated genes and gene expression analyses will further improve the understanding of the molecular mechanisms underlying bacterial stress tolerance.

Beyond specific genetic mechanisms, osmotic and saline stress tolerance are mediated through alternative molecular and microbial systems. In addition to specific genetic determinants, bacterial adaptation may also involve antioxidant defense systems that protect cells from stress-induced reactive oxygen species, highlighting that different bacterial species can employ distinct adaptive strategies under adverse environmental conditions [28]. Furthermore, membrane lipid remodeling has been recognized as an important adaptive response that helps maintain membrane integrity under oxidative stress in microorganisms [29]. Stress adaptation may also involve regulatory networks that preserve cell envelope homeostasis, such as the *Cpx* envelope stress response system characterized in *Escherichia coli* [30].

Representative strains of *Paenibacillus* and *Pseudomonas* ZHS-9 and ADL-13 produced exopolysaccharides (EPS), indicating that EPS formation may contribute to their stress adaptation. EPS production is known to support biofilm formation, tolerance to drought and heavy metals, and improvement of soil structure, thereby helping bacteria survive under stressful environmental conditions [31].

In *Paenibacillus*, several strains have been reported to synthesize high-yield EPS under optimized fermentation conditions. For example, *Paenibacillus polymyxa* and related species were shown to produce levan-type EPS or curd-like polymers, with yields reaching up to ~35-40 g/L depending on carbon source and culture parameters. Such EPS-producing *Paenibacillus* strains are often used as bio-fertilizers partly because their EPS improves root colonization, soil structure, and stress tolerance [32].

Among *Pseudomonas* species, EPS-producing strains have been isolated from soil and rhizosphere, with EPS contributing to acid tolerance, biofilm formation, and protection against desiccation and heavy metals. A study on EPS-producing soil bacteria classified many isolates as *Pseudomonas*. EPS from these strains promoted acid tolerance and enhanced soil aggregation, highlighting its role in adaptation to chemically stressful soils [33].

Arthrobacter species are also well-known EPS producers, particularly in soil and polluted environments. Their EPS is associated with metal binding and biofilm formation, helping the bacteria survive in contaminated or nutrient-limited habitats [34]. In the present study, EPS production was detected in selected strains of genera *Paenibacillus* and *Pseudomonas*.

This suggests that EPS-mediated protection may contribute to stress-adaptation strategy in the investigated representative strains of stepped-derived soil bacteria.

Although EPS is considered important for stress alleviation in certain bacteria, different microorganisms adapt to abiotic stress using a variety of strategies. For example, bacterial populations may differ substantially in the physiological and regulatory mechanisms underlying stress resistance, even among closely related strains [35].

The present study focused on the holistic physiological, molecular, and ultrastructural characterization of indigenous stress-tolerant bacterial isolates. The limitations of the present study primarily relate to the scope of the molecular and functional validation. Future studies incorporating gene expression analyses, additional stress-associated genes, and plant-based validation will further extend the understanding of their stress-alleviating potential. Together, these investigations will provide a stronger scientific foundation for the future application of such bacteria in sustainable agriculture. Furthermore, microbial inoculants can be integrated into alternative agricultural strategies to improve crop resistance to abiotic stress factors. These strategies include crop breeding for stress-tolerant cultivars, crop improvement programs, and sustainable soil management practices, which have all been shown to enhance resilience under drought and salinity [36]. Collectively, these complementary approaches have the potential to contribute to more resilient and sustainable agricultural production under changing environmental conditions.

CONCLUSION

This study demonstrated that indigenous steppe bacterial lineages belonging to *Pseudomonas*, *Paenibacillus*, and *Paenarthrobacter* exhibit distinct genus-dependent tolerance to multiple abiotic stresses, including glyphosate, salinity, and osmotic stress. Among the tested isolates, *Pseudomonas* and *Paenibacillus* sp. showed the highest overall stress resilience, while *Paenarthrobacter* sp. demonstrated comparatively lower tolerance, particularly under osmotic stress conditions.

Integration of physiological (OD_{620}), statistical (PCA), molecular (PCR-based detection of *ectA* and *groL*), and ultrastructural (SEM/TEM) analyses confirmed multiple complementary adaptation mechanisms. These include osmoprotection, protein stability maintenance, and exopolysaccharide (EPS)-mediated protection.

Overall, the results highlight the multifunctional stress-adaptive potential of the studied indigenous bacteria, identifying them as promising candidates for future evaluation as bioinoculants for stress-affected agricultural soils.

AUTHOR CONTRIBUTIONS

All authors contributed to the article and approved the submitted version. **Zh.Zh, A.S.** - methodology, formal analysis, investigation, discussion, writing - original draft preparation; writing - review and editing **S.A, A.Z** - conceptualization, funding acquisition, project administration, data curation, supervision, writing - review and editing.

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CONFLICTS OF INTEREST

The authors declare the absence of any conflict of interest.

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ДАЛАЛЫҚ ЭКОЖҮЙЕЛЕРДІҢ АВТОХТОНДЫ БАКТЕРИЯЛЫҚ ЛИНИЯЛАРЫНЫҢ СТРЕСС ӘСЕРІН ТӨМЕНДЕТУ ӘЛЕУЕТІНІҢ СТАТИСТИКАЛЫҚ, МОЛЕКУЛАЛЫҚ ЖӘНЕ УЛЬТРАҚҰРЫЛЫМДЫҚ АСПЕКТІЛЕРІ

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АНДАТПА

Көптеген стресс факторларына төзімді өсімдік өсуін ынталандыратын бактерияларды (PGPB) анықтау және сипаттау абиотикалық стресс факторлары мен агрохимикаттарды қарқынды қолданудың теріс әсерін төмендетуге бағытталған тұрақты ауылшаруашылық стратегияларын әзірлеу үшін маңызды болып табылады. Бұл зерттеудің мақсаты - бұрын глифосат әсеріне ұшыраған далалық аймақтан бөлініп алынған он бір автохтонды штаммның стресс-төзімділігін бағалау. Таксономиялық сәйкестендіру 16S рРНҚ генін секвенирлеу арқылы жүргізіліп, нәтижесінде *Pseudomonas* spp., *Paenibacillus* spp. және *Paenarthrobacter* spp. туыстарына жататын филогенетикалық тұрғыдан әртүрлі изоляттар коллекциясы анықталды. Штаммдар осмотық, тұздық және глифосат әсерін қамтитын абиотикалық және ксенобиотикалық стресс жағдайларында салыстырмалы төзімділікке зерттелді. Сандық өсу гистограммалары штаммдардың стресс факторларына байланысты таралуын көрсетті, ал негізгі компоненттер анализі (PCA) туысқа тән төзімділік шектерін сипаттайтын айқын кластерлену үлгілерін анықтады. Молекулалық скрининг нәтижесінде *Paenibacillus* изоляттарында *groL* және *ectA* гендері анықталып, олардың ақуыздардың дұрыс жиналуы мен осмоадаптациядағы ықтимал рөлін көрсетті. Сонымен қатар, сканирлеуші және трансмиссиялық электрондық микроскопия арқылы жүргізілген ультрақұрылымдық талдау экзополисахаридтердің (EPS) түзілуін анықтап, олардың жасушалық қорғаныстағы рөлін болжауға мүмкіндік берді. Жалпы алғанда, он бір жергілікті штамм физиологиялық, генетикалық және құрылымдық бейімделулердің синергиялық үйлесімін көрсетті, бұл олардың стресс жағдайларына төзімді ауыл шаруашылығына арналған көпфункционалды биоинокулянттар ретіндегі әлеуетін айқындайды.

Түйін сөздер: PGPB, *Pseudomonas*, *Paenibacillus*, *Paenarthrobacter*, глифосатқа төзімділік, EPS, SEM/TEM, абиотикалық стресс факторлары.

СТАТИСТИЧЕСКИЕ, МОЛЕКУЛЯРНЫЕ И УЛЬТРАСТРУКТУРНЫЕ АСПЕКТЫ СТРЕСС-ПРОТЕКТОРНОГО ПОТЕНЦИАЛА АВТОХТОННЫХ БАКТЕРИАЛЬНЫХ ЛИНИЙ СТЕПНЫХ ЭКОСИСТЕМ

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АННОТАЦИЯ

Идентификация и характеристика устойчивых к множественным стрессовым факторам ризобактерий, стимулирующих рост растений (PGPB), имеют важное значение для разработки устойчивых агротехнологий, направленных на снижение негативного воздействия абиотических стрессов и интенсивного применения агрохимикатов. Целью данного исследования была оценка стрессоустойчивости одиннадцати автохтонных штаммов, выделенных из степных почв, ранее подвергавшейся воздействию глифосата. Таксономическая принадлежность была подтверждена секвенированием гена 16S рРНҚ, в результате чего была сформирована филогенетически разнообразная коллекция изолятов, относящихся к родам *Pseudomonas* spp., *Paenibacillus* spp. и *Paenarthrobacter* spp.. Штаммы были подвергнуты сравнительному анализу устойчивости в условиях абиотического и ксенобиотического стресса, включая осмотическое, солевое и глифосатное воздействие. Количественный анализ роста в виде гистограмм показал распределение штаммов в зависимости от стрессовых факторов, тогда как анализ главных компонент (PCA) выявил отчетливые паттерны кластеризации, свидетельствующие о родоспецифических порогах устойчивости. Молекулярный скрининг выявил наличие генов *groL* и *ectA* у изолятов *Paenibacillus*, что указывает на их возможную роль в сворачивании белков и осмоадаптации. Кроме того, ультраструктурный анализ с использованием сканирующей и просвечивающей электронной микроскопии выявил продукцию экзополисахаридов (EPS), предполагая их участие в клеточной защите. В целом, одиннадцать автохтонных штаммов продемонстрировали синергизм физиологических, генетических и структурных адаптаций, что подчеркивает их потенциал в качестве многофункциональных биоинокулянтов для стрессоустойчивого сельского хозяйства.

Ключевые слова: PGPB, *Pseudomonas*, *Paenibacillus*, *Paenarthrobacter*, устойчивость к глифосату, EPS, SEM/TEM, абиотические стрессовые факторы.