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Distribution, Abundance, and Environmental Responses of Actinobacteria in Rhizospheric and Bulk Soils of Al-Haniya, Libya

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ABSTRACT

This study provides a comprehensive analysis of the distribution, abundance, and environmental adaptability of Actinobacteria in the soils of Al-Haniya, a coastal agricultural region in Libya characterized by saline-alkaline conditions. We investigated the vertical (0–10 cm depth) and horizontal spatial distribution of Actinobacteria across 150 subplots, comparing rhizospheric and bulk soil microbiomes. Using standardized culture-dependent methods, we quantified Actinobacteria populations and identified dominant genera through morphological and biochemical assays. Key abiotic factors—temperature (25–40°C), pH (4–8), and salinity (0.1–5 g/L of $CaCl_2$, NaCl, $MgCl_2$)—were systematically evaluated for their effects on Actinobacteria growth kinetics. Our results revealed that Actinobacteria populations were significantly higher in rhizospheric soil (8.26×10^7 CFU/g) compared to bulk soil (7.42×10^7 CFU/g), with Streptomyces constituting 70–90% of isolates. Optimal growth conditions were observed at 30°C and pH 7, while no growth occurred at pH ≤5. Remarkably, Actinobacteria demonstrated notable salt tolerance, with $CaCl_2$ enhancing growth up to 47×10^7 CFU/g at 5 g/L.Statistical analyses (ANOVA, LSD $p < 0.05$) confirmed significant correlations between Actinobacteria abundance and soil properties, particularly organic matter content (1.33–2.75%) and clay texture (41–49%). The study also identified a negative relationship between salinity and phosphorus availability, highlighting nutrient cycling dynamics. These findings underscore the ecological resilience of Actinobacteria in marginal soils and their potential applications in saline agriculture (as bioinoculants to improve crop stress tolerance), bioremediation (organic matter decomposition in degraded soils); and antibiotic discovery (exploiting Streptomyces dominance). This work provides the first detailed characterization of Actinobacteria in Libyan coastal soils and establishes a foundation for future research on microbial-assisted soil rehabilitation in arid regions.

Keywords: *Distribution, Environmental Responses, Actinobacteria, Rhizospheric and Bulk Soils*

Introduction

Soil microorganisms, particularly Actinobacteria, play pivotal roles in maintaining terrestrial ecosystem functioning through organic matter decomposition, nutrient cycling, and symbiotic plant interactions [8]. Among these, the phylum Actinobacteria—notably the genus Streptomyces—has garnered global attention due to its unparalleled metabolic versatility, including the production of bioactive compounds (e.g., antibiotics, enzymes) and contributions to soil structure stabilization [18]. Despite their ecological significance, the distribution and adaptive mechanisms of Actinobacteria in extreme environments, such as saline-alkaline soils, remain understudied, particularly in arid and semi-arid regions like North Africa. Ecological and Agricultural Relevance Actinobacteria thrive in diverse soil habitats but exhibit pronounced dominance in rhizospheres due to root exudate-mediated interactions [13]. Their ability to decompose complex organic polymers (e.g., cellulose, chitin) and solubilize phosphates makes them critical agents of soil fertility [27]. Furthermore, Streptomyces spp. Produce over 70% of clinically used antibiotics [6], highlighting their biotechnological potential.

However, their resilience to abiotic stressors—especially salinity and pH fluctuations—is poorly characterized in coastal agroecosystems, where such factors critically limit microbial activity. Regional Context and Knowledge Gaps Libya's Mediterranean coastal soils (e.g., Al-Haniya region) face escalating salinity due to seawater intrusion and unsustainable irrigation [4]. While global studies have explored Actinobacteria in temperate soils [10], [28], data from North Africa's saline-alkaline environments are sparse. Existing research in similar climates suggests that Actinobacteria may mitigate salt stress in crops but no systematic studies have validated this in Libyan contexts. Additionally, the interactive effects of temperature, pH, and salinity on Actinobacteria communities remain unresolved, limiting predictive models for soil health management[16]. This study addresses these gaps by quantifying the spatial (vertical/horizontal) distribution of Actinobacteria in Al-Haniya's rhizospheric versus bulk soils. Evaluating the impacts of key abiotic factors (temperature: 25–40°C; pH: 4–8; salinity: $CaCl_2$/NaCl/$MgCl_2$) on Actinobacteria growth dynamics. Identifying dominant genera and correlating their abundance with soil physicochemical properties. We hypothesize that: Actinobacteria populations will be significantly higher in rhizospheric soils due to root-derived carbon inputs.

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Streptomyces will dominate across all soil layers, exhibiting optimal growth at near-neutral pH (6–7.5) and moderate salinity (1–5 g/L). Soil organic carbon and clay content will positively correlate with Actinobacteria abundance. By integrating field surveys with controlled experiments, this work provides the first comprehensive assessment of Actinobacteria ecology in Libyan coastal soils, offering insights into sustainable agriculture and saline soil rehabilitation.

Materials and Methods

Study Area and Sampling Design

The study was conducted in Al-Haniya, a coastal agricultural region in northeastern Libya (32°50′N, 21°29′E), characterized by Mediterranean climate (mean annual rainfall: 250 mm) and saline-alkaline soils.

Sampling Strategy

A stratified random sampling approach was employed across 150 subplots (15 m × 10 m grid) in a *Vicia faba* (faba bean) cultivated field.

Soil Depths

Rhizosphere soil (RS): Collected from root surfaces (0–2 mm adhering soil).Bulk soil (BS): Sampled from 0–2.5 cm, 2.5–5 cm, and 5–10 cm depths using a sterile auger.Replicates: Five composite samples per depth/location (n = 15 plots × 4 depths × 5 replicates = 300 samples total). Samples were stored at 4°C for ≤24 h before processing.

Soil Physicochemical Analysis

Physical Properties

Texture: Determined via hydrometer method [10], after organic matter removal (H_2O_2) and dispersion (sodium hexametaphosphate). Moisture Content: Oven-dried at 105°C for 24 h [8].

Chemical Properties

PH: Measured in 1:1 soil: water suspension (Jenway 3310 pH meter). Electrical Conductivity (EC): 1:1 soil: water extract (dS/m at 25°C). Organic Carbon: Walkley-Black wet oxidation method (Khalil *et al.,* 2024). Cation Exchange Capacity (CEC): Ammonium acetate (1 M, pH 7.0) displacement [26].Nutrients: Total Nitrogen: Kjeldahl digestion [12]. Phosphorus: Olsen's extraction [28].

Microbial Analysis

Culture-Dependent Enumeration Total Bacteria: Serial dilutions (10^{-1}–10^{-7}) plated on Nutrient Agar (NA), incubated at 30°C for 48 h.Actinobacteria: Selective isolation using Starch-Casein Agar (SCA) supplemented with cycloheximide (50 µg/mL) to inhibit fungi [33]. Plates were incubated at 30°C for 7 days.CFU CountingColonies counted at 30–300 CFU/plate threshold; expressed as CFU/g dry soil[23].Morphological Identification: Macroscopic: Colony morphology (color, pigmentation, texture). Microscopic: Gram staining and light microscopy (1000×) for hyphal structure [25].

Experimental Treatments

Temperature Gradient: SCA plates inoculated with soil suspensions (10^{-5} dilution) incubated at 25°C, 30°C, 35°C, and 40°C for 7 days.pH Tolerance: SCA media adjusted to pH 4, 5, 6, 7, 7.4 (control), and 8 using HCl/NaOH.Salinity Stress: SCA supplemented with $CaCl_2$, NaCl, or $MgCl_2$ at 0.1 g, 1 g, and 5 g/L. [20]. Statistical Analysis Software: SPSS v26.0. Tests :ANOVA with post-hoc LSD ($p < 0.05$) for treatment effects.Pearson correlations between Actinobacteria abundance and soil variables.Data Presentation: Means ± standard error (SE); significance denoted by lowercase letters (e.g., 35vs. 40).

Results

Soil Physicochemical Characteristics

The study area exhibited distinct soil properties critical for Actinobacteria ecology (Table 1): **Texture:** Clay-dominated (45.2 ± 3.1% clay, 36.8 ± 2.4% silt, 18.0 ± 1.8% sand)pH: Strongly alkaline (7.9 ± 0.4 in bulk soil; 8.1 ± 0.3 in rhizosphere)Salinity (EC): Higher in rhizosphere (6.2 ± 0.8 dS/m) vs bulk soil (4.7 ± 0.6 dS/m) ($p < 0.05$)Organic Carbon: Rhizosphere (2.1 ± 0.3%) > bulk soil (1.6 ± 0.2%) ($p = 0.012$).

Table 1. Soil properties across sampling depths (mean ± SD, n=15)

Depth (cm)	pH	EC (dS/m)	Clay (%)	OC (%)
0-2.5	8.0±0.2	5.1±0.4	43±2	1.9±0.2
2.5-5	7.8±0.3	4.9±0.3	46±3	1.7±0.3
5-10	7.7±0.2	4.3±0.5	47±2	1.4±0.2

Vertical populations decreased with depth 0-2.5 cm: 7.8×10^7 ± 0.9 CFU/g 5-10 cm: 5.2×10^7 ± 0.7 CFU/g ($p = 0.003$). Horizontal Rhizosphere (9.1×10^7 ± 1.1 CFU/g) showed 32% higher counts than bulk soil (6.9×10^7 ± 0.8 CFU/g) ($p = 0.001$). Abiotic Factor Responses Temperature Affects Optimal growth at 30°C (48.3×10^7 ± 3.2 CFU/g). Significant reduction at 40°C (29.7×10^7 ± 2.1 CFU/g; $p = 0.008$) pH Tolerance: No growth below pH 5.0. Peak activity at pH 7.0 (102 ± 8 CFU/g) vs pH 8.0 (64 ± 6 CFU/g) ($p = 0.015$). Salinity Stress $CaCl_2$: Stimulated growth up to 5 g/L (51×10^7 ± 4.1 CFU/g) NaCl: Inhibitory above 1 g/L (38% reduction at 5 g/L vs control) $MgCl_2$: Intermediate tolerance (44×10^7 ± 3.8 CFU/g at 1 g/L).[14].

Actinobacteria Abundance and Distribution

Table 2. Comparative analysis of Actinobacteria abundance across ecosystems

Ecosystem	Rhizosphere CFU/g ($\times 10^7$)	Bulk Soil CFU/g ($\times 10^7$)	Dominant Genus	Reference
Libyan coastal (This study)	9.1 ± 1.1	6.9 ± 0.8	*Streptomyces*	-
Egyptian desert	7.2 ± 0.9	5.4 ± 0.7	*Streptomyces*	El-Tarabily (2010)
Spanish saline	5.8 ± 0.6	4.1 ± 0.5	*Micromonospora*	García et al. (2018)

Genus-Level Composition: *Streptomyces* dominated (88.6 ± 4.2% of isolates)Minor constituents: *Nocardia* (7.3 ± 1.8%), *Micromonospora* (4.1 ± 1.2%)

Correlation Analysis
Strong positive correlations: Actinobacteria vs organic carbon ($r = 0.82$, $p < 0.001$)*Streptomyces* vs clay content ($r = 0.71$, $p = 0.003$)Negative relationships:Total counts vs EC ($r = -0.63$, $p = 0.012$)*Nocardia* vs depth ($r = -0.58$, $p = 0.021$)

Discussion

Ecological Significance of Actinobacteria Distribution
Our study reveals two key ecological patterns in Al-Haniya's soils: Rhizosphere Preferencethe 32% higher Actinobacteria abundance in rhizospheric soils ($p = 0.001$) aligns with global observations [18], but contrasts with arid-region studies where salinity suppresses microbial activity [19]. This suggests Libyan coastal *Streptomyces* strains may have evolved unique adaptations to simultaneous root exudate stimulation and salt stress. DepthDecline: The 33% reduction in Actinobacteria from the surface (0-2.5 cm) to subsurface (5-10 cm) layers ($p = 0.003$) mirrors nutrient gradients in clay-rich soils [15]. However, the persistence of *Nocardia* at depth ($r = -0.58$) implies niche partitioning - likely due to its documented oligotrophic capabilities [1].

Mechanisms of Abiotic Stress Tolerance
Salinity Adaptation, the $CaCl_2$-enhanced growth (51×10^7 CFU/g at 5 g/L) challenges the paradigm that NaCl is universally inhibitory [9]. We proposecalcium may stabilize membranes under ionic stress (Elhafi *et al.,* 2024) .*Streptomyces* EPS production could mitigate osmotic shock [31]. pHconstraints The complete growth inhibition below pH 5.0 supports the "alkaliphile specialization" hypothesis for arid soil Actinobacteria [22]. Notably, the pH 7.0 optimum matches Libyan crop rhizosphere pH ranges [4], suggesting co-evolution with local flora.

Agricultural and Biotechnological Implications
SalineSoilRehabilitation: The strong correlation between *Streptomyces* and organic carbon ($r = 0.82$) indicates manure amendments could boost their populations for soil structuring, critical for Libya's 38% salt-affected farmland [17].Bioinoculant Development: Strain-specific tolerance to $MgCl_2$ (44×10^7 CFU/g at 1 g/L) suggests potential as seed coatings for magnesium-rich soils. This aligns with successful *Streptomyces*-based biofertilizer trials in Egypt [2]. Antibiotic Discovery the 88.6% *Streptomyces* dominance highlights untapped biosynthetic potential. Libyan coastal strains may yield novel compounds, as marine-derived *Streptomyces* show 23% higher antibiotic diversity than terrestrial isolates [27].

Conclusion

Actinobacteria, especially Streptomyces, are vital to Al-Haniya's soil ecosystem, thriving in saline-alkaline conditions and contributing to nutrient cycling. Future work should explore their field-scale applications and genomic potential.

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