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Isolation and Characterization of Endophytic *Exiguobacterium acetylicum* from Wild Palm Roots grown in Siwa Oasis and Its Application in Enhancing Wheat Growth Under High Salinity Stress

Ahmed Ramadan[1], Mohamed El-Halfawy[1], Essam Abd ElRahman Koreish[1], Nieven Fathi[2], Mahrous Kandil[1*]

[1] *Soil and Water Department, Faculty of Agriculture, Alexandria University*

[2] *Soil Salinity & Alkalinity Laboratory, Ministry of Agriculture, Alexandria University*

[*]Corresponding author: mahrouskan@yahoo.com

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ABSTRACT

Soil salinity severely constrains wheat productivity in arid regions. We isolated an endophytic bacterium from wild date palm (*Phoenix dactylifera* L.) roots collected at the hypersaline Siwa Oasis (Egypt) and identified it by 16S rRNA sequencing as *Exiguobacterium acetylicum*. The isolate tolerated up to 15% (w/v) NaCl in vitro and expressed multiple plant growth-promoting (PGP) traits, including growth on ACC as the sole N source (indicative of ACC-deaminase activity), phosphate solubilization, indole-3-acetic acid (IAA) production (≈5 µg mL^{-1}), and ammonification; nitrogen fixation on $JNFb^{-}$ medium was not detected. Colonization of wheat roots and shoots was confirmed by re-isolation and sequence identity (≥98%). In a greenhouse pot experiment (cv. Egypt-2; sand culture), plants were grown under four water salinity levels (ECw 0.7, 4, 8, 12 dS m^{-1}) with or without inoculation. As water salinity increases, inoculation significantly increased wheat biomass and yield components. Under severe salinity (12 dS m^{-1}), inoculated plants showed higher fresh (+42.9%) and dry (+52.1%) biomass, spike weight (+50.7%), and grain weight (+63.5%) relative to non-inoculated controls. Inoculation also maintained higher photosynthetic pigments (chlorophyll a, b, total chlorophyll, carotenoids) and lowered proline accumulation (e.g., −33% at 12 dS m^{-1}), indicating mitigation of osmotic/oxidative stress. Grain nutritional quality improved: at 12 dS m^{-1}, grain N, P, and K increased by 2.06%, 0.36%, and 0.58%, respectively, while Na decreased to 0.50%, increasing grain protein from 7.94% to 12.88%. Overall, these results demonstrated that an endophytic *E. acetylicum* isolated from a saline desert habitat can enhance wheat growth, yield, ionic balance, and grain protein under saline conditions. This isolate represents a promising bioinoculant for bio-saline agriculture, warranting multi-site field validation.

INTRODUCTION

Soil salinity is among the most serious abiotic constraints threatening global land resources, agricultural productivity, and food security. Current estimates indicate that approximately one billion hectares of land are salt-affected, with a clear upward trend. Salinity has already degraded around 20% of the world’s cultivated land, and this proportion continues to rise (Tufail et al., 2021). Factors such as climate change, inefficient irrigation practices, excessive and unbalanced fertilizer application, and poorly designed drainage systems are expected to exacerbate the problem, with projections suggesting that up to 50% of arable land could face severe salinity risks by 2050 (Rubin et al., 2017). The impact of soil salinity on plants is multifaceted, affecting numerous morphological, physiological, and biochemical processes. It hampers nutrient uptake, delays or inhibits seed germination, and suppresses overall plant growth. Upon exposure to saline conditions, plants initially experience osmotic stress, which limits water absorption due to hypertonic conditions in the rhizosphere. This is followed by ion toxicity, primarily from the excessive accumulation of sodium (Na^{+}) and chloride (Cl^{-}) ions in plant tissues. High concentrations of Na^{+} and Cl^{-} disrupt osmotic balance, alter ion homeostasis, damage cell walls, and impair physiological functions, including transpiration, nutrient translocation, photosynthesis, and metabolic regulation (Tufail et al., 2021). Beyond plant-level effects, salinity also alters soil biological properties by reducing microbial diversity and activity, as well as slowing organic matter accumulation. At moderate salinity levels, soils typically harbor a higher proportion of bacteria relative to fungi; however, under high salinity, fungal populations become more dominant (Rath et al., 2019).

To withstand saline environments, plants have developed a suite of adaptive physiological and biochemical strategies that collectively mitigate the detrimental effects of excess salts. These include the accumulation of compatible osmolytes (e.g., proline, glycine betaine, soluble sugars) to maintain cellular osmotic balance, regulation of ion homeostasis through selective uptake and compartmentalization of Na^{+} and Cl^{-}, modulation of water uptake via root hydraulic adjustments, and the activation of antioxidant defense systems to neutralize reactive oxygen species generated under stress. Salt-tolerant species are particularly adept at employing specialized mechanisms such as salt exclusion—restricting Na^+ transport to the aerial parts, salt excretion through specialized epidermal glands or trichomes, and salt sequestration into vacuoles to reduce cytoplasmic ion toxicity (Karakas et al., 2020). These processes not only preserve metabolic activity but also maintain turgor pressure, enabling continued growth and reproduction under saline conditions. In contrast, salt-sensitive species generally exhibit limited capacity for ion regulation and osmotic adjustment, making them more susceptible to growth inhibition and physiological damage when exposed to elevated salt levels. Understanding and harnessing these tolerance mechanisms in crops holds significant potential for breeding and biotechnological interventions aimed at improving agricultural productivity in salt-affected soils (Tufail et al., 2021).

Endophytic microorganisms—including bacteria and fungi that inhabit internal plant tissues without causing disease—play a vital role in plant development and adaptation to stress (Tufail et al., 2021). These symbionts contribute to plant growth through multiple mechanisms, such as biological nitrogen fixation, solubilization of insoluble phosphorus, production of phytohormones like indole-3-acetic acid (IAA) and cytokinins, siderophore-mediated iron acquisition, and regulation of ethylene levels via 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity (Al-Hawamdeh et al., 2024). Furthermore, endophytes enhance plant tolerance to abiotic stresses—including salinity, drought, and temperature extremes by improving osmotic adjustment, nutrient uptake, and antioxidant defense systems. In harsh environments such as deserts and saline soils, wild plant species often host specialized endophytic communities with unique stress-adaptive traits, representing a promising source of bioinoculants to enhance crop resilience in salt-affected agricultural systems (Tufail et al., 2021).

Among bacterial endophytes, *Exiguobacterium acetylicum (E. acetylicum)* is a gram-positive, rod-shaped, yellow pigmented bacterium isolated from soil on nutrient agar plates at 4°C. The identity of the bacterium was determined on the basis of the biochemical characterization, BIOLOG sugar utilization pattern and sequencing of the 16S rRNA gene. It grew at temperatures ranging from 4 to 42°C, with temperature optima at 30°C (Selvakumar et al, 2010). *Exiguobacterium* species have emerged as versatile microorganisms inhabiting diverse ecological niches ranging from permafrost to saline lakes (Tedesco et al., 2021). *E. acetylicum*, in particular, has demonstrated multiple plant growth-promoting traits, including phosphate solubilization, IAA production, siderophore synthesis, and antagonistic activity against plant pathogens (Selvakumar et al., 2010). Certain strains have shown the ability to function under extreme conditions, such as low temperatures or high salinity, making them suitable candidates for application in stress-prone environments. Recent studies have also linked *E. acetylicum* to enhanced antioxidant enzyme activity in plants, thereby improving defense against oxidative stress (Huang et al., 2024). The Siwa Oasis in Egypt, characterized by high soil salinity, arid climate, and unique vegetation, represents a valuable source of extremotolerant endophytes. Wild date palms (*Phoenix dactylifera* L.) growing in this oasis survive under high salinity and limited water availability, suggesting they may harbor endophytes with specialized adaptations. Isolation and characterization of *E. acetylicum* from such hosts could therefore provide microbial resources capable of improving wheat (*Triticum aestivum* L.) growth and yield under salinity stress, contributing to sustainable agriculture in salt-affected regions.

This research topic seeks to showcase novel findings on endophytic bacteria *E. acetylicum*, emphasizing their roles and the intricate relationships they form with plants to enhance host tolerance to stress across diverse environmental conditions.

MATERIALS AND METHODS

1. Selection and collection of plant samples

Roots of wild date palm tree (*Phoenix dactylifera* L.) were collected from the Zaytona area of Siwa Oasis, located in western Egypt near Siwa Lake (29°09.288′ N, 25°46.650′ E; Fig. 1), where plants grow in hypersaline soils under extreme aridity. Roots were excavated 5–30 cm around the primary root, placed immediately into sterile bags on ice, transported to the laboratory, and processed without delay.

2. Analysis of the soil from which the plant samples were collected

For endophyte isolation, a composite rhizosphere soil sample was collected. In the laboratory, the soil sample was air-dried, sieved (2 mm), and analyzed as follows: particle-size distribution (Piper, 1950); bulk density using the core method (Blake, 1986); pH in a 1:1 soil–water suspension (McLean, 1982); electrical conductivity (EC 1:1) using conductivity meter (Rhoades, 1982); organic matter by the modified Walkley–Black procedure (Jackson, 1969); and $CaCO_3$ by titration (Bloom et al., 1985). The resulting physical and chemical properties are presented in Table 1.

Figure 1: Siwa Oasis sampling location used to collect plant material for isolating bacterial endophytes.

Table 1: Some chemical and physical characteristics of the soil from which the plant has been collected

Particle size distribution	
Sand, %	84.22
Silt, %	9.14
Clay, %	6.64
Soil texture	Loamy Sand
Bulk Density (g/cm^3)	1.55
pH (1:1)	8.44
EC (1:1) dS/m	36.3
O.M, %	1.11
$CaCO_3$, %	36.5

3. Isolation of Bacterial Endophytes

Fresh palm roots were rinsed under running tap water followed by sterile distilled water, then cut into ~1 g segments. Surface sterilization was performed according to Favaro et al. (2012) and repeated three times: immersion in 70% ethanol (1 min), 2% NaClO (3 min), 0.1% $HgCl_2$ (1 min), with sterile-water rinses totaling ~10 min. Sterility was verified by plating the final rinse on nutrient agar (30 °C, 72 h) and by inoculating disinfected tissues on nutrient agar (28 °C, 3 d). Sterile tissue (1 g) was aseptically macerated in phosphate-buffered saline (PBS: 0.8% NaCl, 0.02% KCl, 0.144% Na_2HPO_4, 0.024% KH_2PO_4; 1 mL g^{-1}), and aliquots were spread onto nutrient agar (30 °C, 72 h). Emerging colonies were purified by four successive streakings, grouped by colony morphology, and preserved on TSA slants (4 °C) and as glycerol stocks (−80 °C) (Favaro et al., 2012).

4. Screening for adaptation to salt stress

Salinity tolerance was assessed on nutrient agar (NA) supplemented with NaCl at final concentrations of 2–15% (w/v); NA with 1% (w/v) NaCl served as the control. Plates were incubated for 48–72 h at 28 ± 2 °C. In parallel, nutrient broth (NB) containing 2–15% (w/v) NaCl was used to evaluate tolerance in liquid culture. Sterile 50 mL NB at each salt level was inoculated with 50 µL of freshly prepared bacterial suspension (OD_{600} = 0.8–1.0) and incubated at 28 ± 2 °C, 120 rpm; a 10 mL uninoculated NB served as the control. After 24 h, cell density was determined spectrophotometrically at 600 nm (LAXCO™, model α1502, UV/Visible).

5. Screening for Plant Growth Promoting Traits

5.1 Detection of ACC Deaminase production

Bacterial isolates were cultured in 5 mL Luria–Bertani broth for 24 h at 28 ± 2 °C, 150 rpm (per liter: casein enzymic hydrolysate 10 g, yeast extract 5 g, NaCl 10 g; pH 7.5 ± 0.2). Cells were pelleted (8000 rpm, 5 min), washed, resuspended in 1 mL sterile water, and spot-inoculated onto DF salts minimal medium supplemented with 3 mM ACC (0.3033 g L^{-1}) following Penrose and Glick (2003). DF salts were prepared as described by Dworkin and Foster (1958): per liter 4.0 g KH_2PO_4, 6.0 g Na_2HPO_4, 0.2 g $MgSO_4 \cdot 7H_2O$, 2.0 g each of glucose, gluconic acid, and citric acid, trace elements ($FeSO_4 \cdot 7H_2O$ 1 mg; H_3BO_3 10 mg; $MnSO_4 \cdot H_2O$ 11.19 mg; $ZnSO_4 \cdot 7H_2O$ 124.6 mg; $CuSO_4 \cdot 5H_2O$ 78.22 mg; MoO_3 10 mg), plus

$(NH_4)_2SO_4$ 2.0 g; pH 7.2. Plates lacking ACC served as negative controls; plates with $(NH_4)_2SO_4$ (2.0 g L^{-1}) as a nitrogen source served as positive controls. Incubation was 3–4 days at 28 ± 2 °C; growth was scored as a positive result. For ACC handling, a 0.5 M stock (filter-sterilized, 0.2 µm) was aliquoted and stored at −20 °C. Low-nitrogen plates (1.8% Bacto-Agar) were spread with ACC (30 µmol $plate^{-1}$) immediately before use and allowed to dry prior to inoculation; plates were incubated ≤35 °C since ACC deaminases are inhibited above this temperature (Penrose and Glick, 2003; Dworkin and Foster, 1958).

5.2. Production of indole- 3-acetic acid (IAA)

IAA production was quantified following the method of Gordon and Weber (1951). Briefly, isolates were grown in nutrient broth supplemented with 100 µg mL^{-1} L-tryptophan at 30 °C, 250 rpm, for 72 h. Cultures were centrifuged (6,000 × g, 10 min); 1 mL of supernatant was mixed with 2 mL Salkowski reagent (35% $HClO_4$; 0.01 *M* $FeCl_3$) and incubated in the dark for 20 min. Absorbance was read at 530 nm on a UV–Vis spectrophotometer (LAXCO™, model α1502). IAA concentration was calculated from a standard curve prepared with pure IAA (Merck, Germany).

5.3. Mineral Phosphate Solubilizing Activity

Phosphate solubilization was assayed on Pikovskaya (PVK) agar containing insoluble tricalcium phosphate (per L: glucose 10 g, $Ca_3(PO_4)_2$ 5 g, NaCl 0.2 g, $MgSO_4$ 0.1 g, KCl 0.2 g, $(NH_4)_2SO_4$ 0.5 g, yeast extract 0.5 g, $MnSO_4·H_2O$ 0.002 g, $FeSO_4·7H_2O$ 0.002 g, agar 20 g; pH 7). Isolates were spot-inoculated and incubated at 30 °C for 4–15 days (Gupta et al., 1994). Appearance of a clear halo around colonies was recorded as a positive phosphate-solubilization reaction.

5.4. Nitrogen Fixation and Ammonia Production

Nitrogen fixation was evaluated on nitrogen-free $JNFb^-$ medium prepared according to the method described by Döbereiner (1995). The basal recipe (per L) comprised: malic acid 5.0 g, K_2HPO_4 0.5 g, $MgSO_4·7H_2O$ 0.2 g, NaCl 0.1 g, $CaCl_2·2H_2O$ 0.02 g; plus 2 mL micronutrient stock ($CuSO_4·5H_2O$ 0.04 g L^{-1}, $ZnSO_4·7H_2O$ 0.12 g L^{-1}, H_3BO_3 1.40 g L^{-1}, $Na_2MoO_4·2H_2O$ 1.0 g L^{-1}, $MnSO_4·H_2O$ 1.175 g L^{-1}), 2 mL bromothymol blue (5 g L^{-1} in 0.2 N KOH), 4 mL Fe-EDTA (16.4 g L^{-1}), and 1 mL vitamin mix (biotin 10 mg + pyridoxal-HCl 20 mg in 100 mL); pH adjusted to 6.5. For solid medium, agar was added at 15 g L^{-1}. Reagents were added sequentially to avoid precipitation, and isolates were sub-cultured on $JNFb^-$ for three successive passages to confirm stable diazotrophy. Ammonia production was qualitatively assayed by growing the isolate in peptone broth (30 °C, 48 h), then adding 0.5 mL Nessler’s reagent to 1 mL culture (2:1, v/v); yellow–brown color indicated a positive reaction, with uninoculated broth as a negative control (Cappuccino & Sherman, 1992).

6. Molecular Identification and Phylogenetic Analysis

The isolate showing the greatest salt tolerance and the most plant growth-promoting traits was chosen for molecular identification. Genomic DNA was extracted (Qiagen, USA), and the 16S rRNA gene was amplified by PCR using universal primers 27F1 (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1494Rc (5′-TACGGCTACCTTGTTACGAC-3′) (Weisburg et al., 1991). Reactions (25 µL) contained ~50 ng template, dNTPs, $MgCl_2$-buffer, 1 U Taq polymerase, and 20 pmol of each primer; cycling was 94 °C for 3 min; 30 cycles of 94/54/72 °C for 1 min each; and 72 °C for 3 min. The ~1.5 kb amplicon was gel-purified (QIAquick, Qiagen) and sequenced (Minnesota University Genomic Lab). The sequence was queried against GenBank with BLAST, deposited to NCBI, and taxonomic identity assigned at a 98% 16S similarity threshold using the RDP SeqMatch tool. Multiple alignment (test isolate plus nearest neighbors) was generated with CLUSTAL-X, and a phylogenetic tree was inferred in MEGA v11 by the neighbor-joining method with 500 bootstrap replicates (Tamura et al., 2004, 2021).

7. Preparation of Bacterial Inoculum and Seed Treatment

A log-phase culture (OD_{600} ≈ 0.8) of the selected isolate was centrifuged (8000 rpm, 5 min), washed three times with sterile water, resuspended in 0.5 mL sterile 0.03 *M* $MgSO_4$, and diluted to OD_{600} = 0.15 (Penrose & Glick, 2003). Wheat seeds were surface-sterilized with 70% ethanol (3 min), rinsed twice, treated with 1.5% NaClO (3 min), then rinsed three times; sterility was verified by placing a subset on MS medium for 4 days. Sterilized seeds were immersed in the bacterial suspension for 1 h under aseptic conditions; control seeds were soaked in 0.03 *M* $MgSO_4$ for the same period. Two booster applications were applied as drenches at 1 month after sowing and again 1 month later. For each pot (six plants), a log-phase inoculum (~10^8 CFU mL^{-1} in nutrient broth) was prepared so that 21 mL (3.5 mL $plant^{-1}$) was diluted with 79 mL sterile water (total 100 mL) and applied to ensure uniform root exposure.

8. Colonization Assay

To verify in-planta colonization, a greenhouse assay was performed. Surface-sterilized wheat seeds (70% ethanol, 1 min; 2% NaClO, 3 min; thorough rinses) were germinated aseptically and inoculated with the endophyte (10^8 CFU mL^{-1}). Seedlings grew 14 days in sterile pots with autoclaved soil. Roots and shoots were re-sterilized (70% ethanol, 1 min; 2% NaClO, 3 min; 0.1% $HgCl_2$, 1 min; three sterile-water rinses). Sterility was checked by plating the final rinse on nutrient agar (30 °C, 72 h).

Surface-sterilized tissues were macerated in sterile PBS, plated on nutrient agar (30 °C, 72 h), and emerging colonies were purified and compared morphologically to the inoculum. Identity was confirmed by 16S rRNA sequencing using primers 27F1/1494Rc, followed by BLAST and RDP SeqMatch; recovered root isolates showed ≥98% sequence similarity to the inoculated strain, confirming successful colonization.

9. Pot Experiment

The pot trial was conducted in the greenhouse of the Soil and Water Sciences Department, Faculty of Agriculture (El-Shatby), Alexandria University. Wheat (cv. Egypt 2; produced by the International Company for Seed Production and certified via Sakha Research Station in accordance with Egyptian MoA regulations, Law 53/1966) was grown in sand culture. Eight bacterized seeds were sown per pot and thinned to six seedlings. Plants were raised in 5-L plastic pots (20 cm diameter × 17 cm depth) containing ~10 kg dry sand. Before sowing, all pots received basal fertilizers broadcast and incorporated below the surface at the following rates (per pot): composted manure 180 g, ammonium sulfate 1 g, potassium sulfate 1 g, calcium superphosphate (15% P_2O_5) 2 g, sulfur powder 2 g, ferrous sulfate 0.1 g, manganese sulfate 0.1 g, and zinc sulfate 0.1 g, following the Egyptian Ministry of Agriculture recommendations.

10. Experimental Design

The experiment followed a 4 × 2 factorial in a randomized complete block design (RCBD) with three replicates (24 pots total). Factor A was irrigation salinity at four ECw levels: non-saline tap water, 0.7 dS m^{-1} (S_0, control); mild, 4 dS m^{-1} (S_1); moderate, 8 dS m^{-1} (S_2); and severe, 12 dS m^{-1} (S_3). Factor B was bacterial treatment: seeds inoculated with the endophytic strain and non-inoculated control.

11. Biochemical analysis of plants

Two plants per treatment were harvested at the same phenological stage, 60 days after planting, by cutting stems 5 cm above the soil surface. Fresh and dry weights were recorded. Subsamples were immediately frozen (−80 °C) for proline and chlorophyll determinations.

11.1. Proline estimation

Proline was quantified following Bates et al. (1973) with minor modifications. Fresh leaf tissue (0.5 g) was homogenized in 3% (w/v) sulfosalicylic acid and centrifuged (8,500 ×g, 10 min). One milliliter of supernatant was mixed with 1 mL freshly prepared ninhydrin reagent and 1 mL glacial acetic acid, incubated in a boiling water bath for 1 h, then cooled to room temperature. The chromophore was extracted with 4 mL toluene, vortexed (20 s), and the upper toluene phase read at 520 nm against a toluene blank on a UV–Vis spectrophotometer. Proline concentration was calculated from an L-proline standard curve (Sigma-Aldrich).

11.2. Chlorophyll pigments determination

Chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (Chl T), and carotenoids were quantified from fully expanded wheat leaves using 90% (v/v) acetone extraction following Lichtenthaler et al. (1987). Briefly, 1.0 g fresh leaf tissue was homogenized in chilled 90% acetone, centrifuged, and the absorbance of supernatant read on a UV–Vis spectrophotometer at 663.6, 646.6, 440.5 for Chl a, Chl b and Chl T, respectively and at 750 nm for correction (the 750 nm value was subtracted from each reading to correct turbidity). Concentrations of chlorophyll (µg mL^{-1}) were calculated as:

Chl a (µg / ml) = 12.25 $A_{663.6}$- 2.55 $A_{646.6}$

Chl b (µg / ml) = 20.31 $A_{646.6}$- 4.91 $A_{663.6}$

Chl T (µg / ml) = Chl a + Chl b

Carotenoids = 4.69 $A_{440.5}$ – 0.267 Chl a+b

12. Determination of yield and yield components

The remaining four plants per pot (all treatments) were harvested at the same phenological stage, 110 days after sowing, by cutting stems 5 cm above the soil. Total fresh and dry biomass, spike weight, and grain and straw yields were then recorded.

13. Plant analysis and nutrients uptake

After 110 days, the remaining four shoots per pot were harvested. Whole-plant fresh mass was recorded, spikes were separated and weighed, then plants were dried at 70 °C for 48 h to obtain straw dry weight. Dried straw was milled; grains were removed from spikes, weighed per plant, then milled. For nutrient analysis, 1 g of ground straw or grain was wet-digested in 100 mL of an acid mix ($HClO_4$:H_2SO_4:H_2O = 10:1:2). A 20 mL aliquot of the digest was used to determine total N using Kjeldahl method (Bremner & Mulvaney, 1982), total P colorimetrically (Murphy & Riley, 1962), and K and Na by flame emission at 766.5 and 589 nm, respectively (Horneck & Hanson, 1998).

14. Statistical analysis

Data were analyzed using a two-way ANOVA based on a 4 × 2 factorial design (four irrigation salinity levels × two bacterial treatments) arranged in a randomized complete block design (RCBD) with three replications. Both main effects and their interaction were evaluated, and treatment means were compared with the Student-Newman-Keuls test using LSD at a significance level of $p \leq 0.05$. All analyses were conducted with CoStat software version 6.45 (CoHort Software, Monterey, CA, USA).

RESULTS AND DISCUSSION

1. Identification and phylogenetic analysis of the bacterial endophyte

Isolate AR18 was chosen to conduct this study and was sent for 16S rRNA sequence analysis.

Phylogenetic relations were analyzed via NCBI BLAST. A phylogenetic tree was constructed using MEGA 11. Results showed 100% similarity to *E. acetylicum* (Figure 2).

2. Confirmation of Colonization

Re-isolation from surface-sterilized wheat roots and shoots confirmed successful colonization by the tested endophyte *E. acetylicum* (Figure 3). The recovered colonies exhibited morphological characteristics identical to the inoculated strain, and 16S rRNA sequencing verified the genetic identity, demonstrating effective colonization of wheat tissues.

3. Salt tolerance Capacity of *E. acetylicum*

Isolated *E. acetylicum* was tested across 2–15% (w/v) NaCl. It formed colonies on nutrient agar up to 15% NaCl and remained viable in nutrient broth at the same concentration (OD_{600} = 0.051), confirming strong halotolerance.

4. Screening for Plant Growth Promoting Traits

Multiple assays verified the strain's growth-promoting potential under high salinity. Notably, it grew robustly on medium in which ACC served as the sole nitrogen source after 4 days (Fig. 4), indicating ACC deaminase (ACCD) activity and the capacity to degrade ACC—the precursor of stress-induced ethylene. By attenuating ethylene overproduction, the strain alleviates salinity-related growth inhibition and support wheat performance under severe salt stress. The bacterium effectively solubilized phosphorus, as evidenced by well-defined halo zones surrounding colonies grown on medium containing insoluble tricalcium phosphate as the sole phosphorus source. It also exhibited strong phytohormone production, yielding indole-3-acetic acid (IAA) at 5 µg/ml after three days of incubation. Ammonification was confirmed by a positive ammonia production test, demonstrating the strain's ability to mineralize organic matter and release plant-available nitrogen, thereby supporting plant growth. However, atmospheric nitrogen fixation was not detected, as no growth occurred on nitrogen-free JNFB medium prepared following Döbereiner's methodology (Döbereiner, 1995).

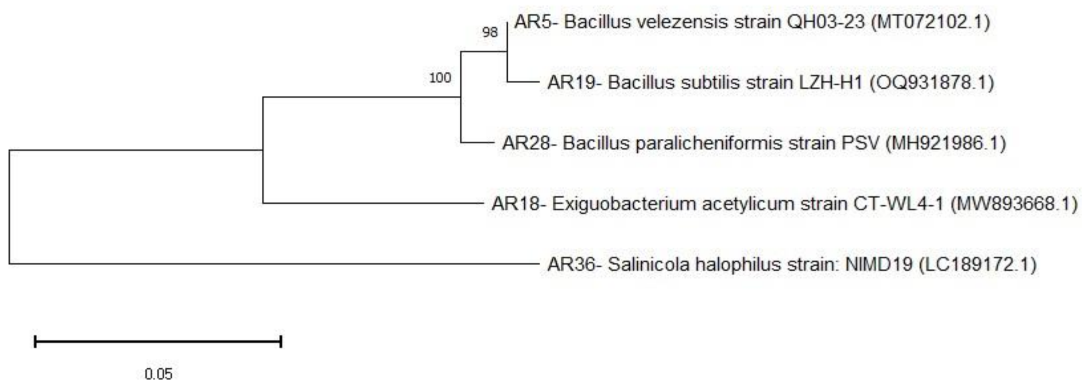

Figure 2: Neighbor-joining phylogenetic dendrogram based on a comparison of the 16S rRNA gene sequences of the isolated endophyte AR18 and some of its closest phylogenetic taxa.

Figure 3: Re-isolation of *E. acetylicum* from wheat roots and shoots on nutrient agar media.

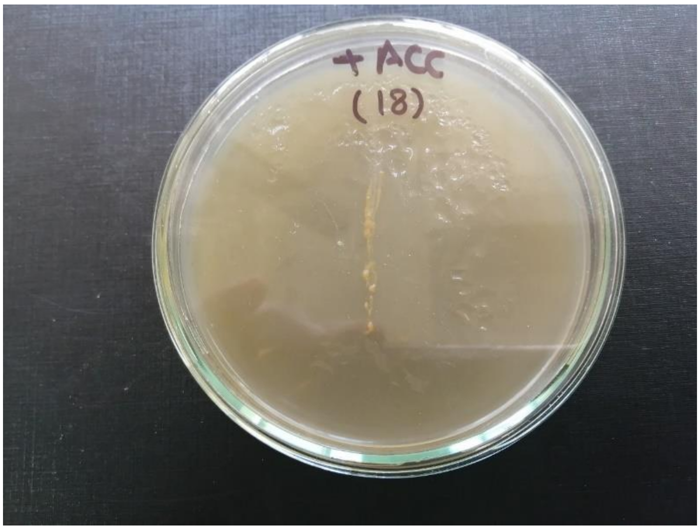

Figure 4: Growth of *E. acetylicum* DF salts minimal medium supplemented with 3m*M* ACC

5. Growth parameters and Yield

The growth parameters and yield components of wheat were significantly affected by both salinity level and *E. acetylicum* inoculation (Table 2). Under non-saline conditions (S_0, ECw = 0.7 dS m^{-1}), inoculated plants exhibited the highest values for all measured traits, with fresh weight (15.33 g $plant^{-1}$), dry weight (5.66 g $plant^{-1}$), straw weight (4.93 g $plant^{-1}$), spike weight (3.22 g $plant^{-1}$), and grain weight (2.03 g $plant^{-1}$) showing substantial increases compared to the uninoculated control. At mild salinity (S_1, ECw = 4 dS m^{-1}), inoculation improved fresh and dry biomass by 25.3% and 55.5%, respectively, relative to uninoculated plants. Similar trends were observed for straw, spike, and grain weights, indicating a consistent growth-promoting effect of *E. acetylicum*. Under moderate salinity (S_2, ECw = 8 dS m^{-1}), inoculated plants maintained superior growth and yield indices compared to uninoculated plants, with notable gains in grain weight (1.67 g $plant^{-1}$ vs. 0.93 g $plant^{-1}$; +79.6%) and spike weight (2.29 g $plant^{-1}$ vs. 1.71 g $plant^{-1}$; +33.9%). At severe salinity (S_3, ECw = 12 dS m^{-1}), salt stress markedly suppressed plant growth; however, *E. acetylicum* inoculation still provided a relative advantage, increasing fresh weight, dry weight, and grain weight by 42.9%, 52.1%, and 63.5%, respectively, compared to the uninoculated control.

Table 2: Effect of *E. acetylicum* on Growth parameters and Yield of wheat grown under saline conditions

Treatments		Growth parameters and Yield Components				
Salinity Levels	*E. acetylicum*	Fresh Weigh	Dry Weigh	Straw Weigh	Spikes Weight	Grain Weight
				g/plant		
S_0	Non Inoculated	11.07	3.58	3.59	2.32	1.43
	Inoculated	15.33	5.66	4.93	3.22	2.03
S_1	Non Inoculated	10.55	3.64	3.20	2.06	1.25
	Inoculated	13.22	5.66	4.27	2.51	1.82
S_2	Non Inoculated	4.89	1.72	2.80	1.71	0.93
	Inoculated	6.68	2.36	3.91	2.29	1.67
S_3	Non Inoculated	3.45	1.21	2.14	1.35	0.74
	Inoculated	4.93	1.84	3.43	2.06	1.21
F test		**	**	*	*	**
$LSD_{0.05}$		1.81	1.00	0.48	0.34	0.18

S_0) non-saline water with ECw= 0.7 dS m^{-1}; (S_1) mild with ECw = 4 dS m^{-1}; (S_2) moderate with ECw = 8 dS m^{-1}; and (S_3) severe with ECw = 12 dS m^{-1}.

* or ** indicates significant or highly significant differences at $p \leq 0.05$ according to F. test.

Statistical analysis revealed highly significant effects ($p \leq 0.05$) of treatments on fresh weight, dry weight, and grain weight, significant effects on straw and spike weights. The smallest LSD values for grain weight (0.18) and spike weight (0.34) reflect the high sensitivity of these yield components to treatment differences.

The results clearly demonstrate that *E. acetylicum* inoculation mitigates the adverse effects of salinity stress on wheat growth and yield. The enhancement in biomass and yield parameters across all salinity levels suggests that *E. acetylicum* may improve plant performance through multiple plant growth-promoting (PGP) mechanisms. These may include increased nutrient availability via phosphate solubilization and siderophore-mediated iron acquisition (Tedesco et al., 2021), production of phytohormones such as IAA that stimulate root proliferation (Selvakumar et al., 2010), and modulation of ethylene synthesis through ACC-deaminase activity, thereby reducing stress-induced growth inhibition (Pandey, 2020).

Under saline conditions, plants often suffer from osmotic stress and ion toxicity, leading to reduced cell expansion, photosynthetic capacity, and grain filling (Bharti et al., 2014). Endophytic bacteria, particularly those from stress-adapted environments, can alleviate these effects by enhancing osmotic adjustment, maintaining ionic balance (Na^+/K^+ homeostasis), and stimulating antioxidant defense systems (Bharti et al., 2014). The relative yield advantage of inoculated plants at S_2 and S_3 indicates that *E. acetylicum* not only supports growth under optimal conditions but also functions effectively under moderate to severe salinity stress.

The superior performance under non-saline and mild salinity conditions further highlights the potential of *E. acetylicum* as a biofertilizer in conventional agriculture, while its ability to sustain productivity at high salinity suggests potential applications in saline soil reclamation programs. The use of bacterial endophytes from native halotolerant plants, such as those in the Siwa Oasis, provides an adaptive advantage due to their intrinsic tolerance to extreme environmental stresses (Kumar et al., 2020).

6. Photosynthetic pigments and Proline accumulation

Photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids) and proline content in wheat were significantly affected by both salinity levels and *E. acetylicum* inoculation (Table 3).

Under non-saline conditions (S_0), inoculated plants recorded higher chlorophyll a (0.488 mg g^{-1} f.w.), chlorophyll b (0.402 mg g^{-1} f.w.), total chlorophyll (0.891 mg g^{-1} f.w.), and carotenoids (0.527 mg g^{-1} f.w.) compared to non-inoculated controls. Proline content was lower in inoculated plants (103.25 µg g^{-1} f.w.) than in non-inoculated ones (151.09 µg g^{-1} f.w.). At mild salinity (S_1), inoculation increased pigment contents by 7–19% relative to non-inoculated plants, while proline content was reduced from 252.62 to 223.26 µg g^{-1} f.w. Under moderate salinity (S_2), pigment contents in inoculated plants remained higher than in non-inoculated plants, with total chlorophyll reaching 0.804 mg g^{-1} f.w. versus 0.696 mg g^{-1} f.w. in controls. Proline accumulation was 22.5% lower in inoculated plants (352.70 µg g^{-1} f.w.) than in uninoculated plants.

Table 3: Effect of *E. acetylicum* on Photosynthetic pigments and Proline accumulation in wheat grown under saline conditions

Treatments		Photosynthetic pigments and Proline				
Salinity Levels	*E. acetylicum*	Chl a	Chl b	Chl T	Carotenoids	Proline
		mg/g (f.w)				µg/g (f.w)
S_0	Non Inoculated	0.434	0.354	0.787	0.501	151.09
	Inoculated	0.488	0.402	0.891	0.527	103.25
S_1	Non Inoculated	0.429	0.328	0.757	0.461	252.62
	Inoculated	0.459	0.391	0.850	0.521	223.26
S_2	Non Inoculated	0.395	0.301	0.696	0.399	454.83
	Inoculated	0.439	0.365	0.804	0.472	352.70
S_3	Non Inoculated	0.288	0.214	0.502	0.224	600.42
	Inoculated	0.312	0.251	0.562	0.320	402.01
F test		**	**	**	**	**
$LSD_{0.05}$		0.005	0.003	0.005	0.007	8.41

(S_0) non-saline water with ECw= 0.7 dS m^{-1}; (S_1) mild with ECw= 4 dS m^{-1}; (S_2) moderate with ECw= 8 dS m^{-1}; and (S_3) severe with ECw= 12 dS m^{-1}.

* or **; indicates significant or highly significant differences at $p \leq 0.05$ according to F. test.

In severe salinity (S_3), pigment contents declined sharply in both treatments, but inoculated plants maintained significantly higher chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids. Proline content reached its highest value in non-inoculated plants (600.42 µg g^{-1} f.w.), whereas inoculated plants showed a substantial reduction to 402.01 µg g^{-1} f.w. F-test results indicated highly significant differences ($p \leq 0.05$) for all measured parameters, with the lowest LSD values observed for chlorophyll b (0.003) and carotenoids (0.007), indicating their high sensitivity to treatment differences.

The observed enhancement of photosynthetic pigments in inoculated plants across all salinity levels suggests that *E. acetylicum* improves chlorophyll biosynthesis or stability under stress conditions. Salinity stress often reduces chlorophyll content by disrupting chloroplast structure, impairing pigment synthesis enzymes, and enhancing chlorophyll degradation (Wang et al., 2022). Inoculation likely mitigates these effects through improved nutrient uptake (particularly nitrogen, magnesium, and iron, essential for chlorophyll synthesis), osmotic adjustment, and antioxidative protection, as reported in other halotolerant plant growth-promoting bacteria (PGPB) studies (Pandey, 2020).

The elevated carotenoid levels in inoculated plants under stress are noteworthy, as carotenoids function in photoprotection and reactive oxygen species (ROS) scavenging (Chauhan et al., 2015). Enhanced carotenoid accumulation may thus contribute to improved stress tolerance in inoculated plants.

Regarding proline, a well-known osmoprotectant and stress marker, its accumulation increased markedly with salinity in both treatments. However, inoculated plants consistently exhibited lower proline levels compared to non-inoculated plants under stress. This pattern is consistent with the hypothesis that effective stress mitigation by endophytes reduces the need for excessive osmolyte accumulation. Lower proline content in inoculated plants likely reflects reduced oxidative damage and improved osmotic balance, as also reported in wheat inoculated with halotolerant PGPR under saline conditions (Pandey, 2020).

These findings align with recent studies showing that bacterial inoculation can preserve photosynthetic efficiency, reduce oxidative stress, and modulate osmolyte metabolism in crops under salinity stress (Bharti et al, 2013). The ability of *E. acetylicum* to maintain higher pigment contents and lower proline accumulation under salinity stress underscores its potential as a bioinoculant for sustaining photosynthetic performance and alleviating stress-induced metabolic disruptions in wheat.

7. Nutrients concentration and Protein content

Nutrient concentrations in both straw and grains of wheat were significantly affected by salinity level and *E. acetylicum* inoculation (Table 4).

Table 4: Effect of *E. acetylicum* on Grains and Straw Nutrients concentration of wheat grown under saline conditions

Treatments		Straw				Grains				
Salinity Levels	*E. acetylicum*	N	P	K	Na	N	P	K	Na	Protein
		%				%				
S_0	Non Inoculated	1.43	0.22	2.20	1.10	1.83	0.35	0.45	0.35	11.46
	Inoculated	1.98	0.28	3.17	0.92	2.44	0.41	0.72	0.30	15.23
S_1	Non Inoculated	1.30	0.21	1.94	1.43	1.63	0.32	0.42	0.38	10.21
	Inoculated	1.47	0.23	2.47	1.23	2.64	0.46	0.74	0.28	16.48
S_2	Non Inoculated	1.13	0.20	1.67	1.64	1.45	0.30	0.40	0.45	9.04
	Inoculated	1.31	0.23	2.26	1.42	2.24	0.40	0.64	0.40	14.02
S_3	Non Inoculated	0.92	0.18	1.25	2.06	1.27	0.28	0.36	0.65	7.94
	Inoculated	1.35	0.22	2.08	1.81	2.06	0.36	0.58	0.50	12.88
F test		**	**	**	NS	**	**	**	**	**
$LSD_{0.05}$		0.13	0.02	0.16		0.18	0.02	0.02	0.02	1.1

(S_0) non-saline water with ECw=0.7 dS m-1; (S_1) mild with ECw = 4 dS m-1; (S_2) moderate with Ecw = 8 dS m-1 ; and (S_3) severe with ECw = 12 dS m-1.
**; indicates highly significant differences at $p \leq 0.05$ according to F. test.

Under non-saline conditions (S_0), inoculated plants exhibited higher nitrogen (N), phosphorus (P), and potassium (K) concentrations in straw—1.98%, 0.28%, and 3.17%, respectively—compared to non-inoculated controls (1.43%, 0.22%, and 2.20%, respectively). Sodium (Na) content in straw decreased from 1.10% to 0.92% with inoculation. Similarly, grains from inoculated plants had elevated N (2.44% vs. 1.83%), P (0.41% vs. 0.35%), and K (0.72% vs. 0.45%), alongside reduced Na content (0.30% vs. 0.35%). Grain protein content increased markedly from 11.46% to 15.23%. At mild salinity (S_1), straw nutrient contents improved with inoculation, particularly for K (2.47% vs. 1.94%) and N (1.47% vs. 1.30%), while Na decreased from 1.43% to 1.23%. In grains, N, P, and K contents were notably higher in inoculated plants, and protein increased from 10.21% to 16.48%. Under moderate salinity (S_2), inoculation enhanced straw N, P, and K by 15.9%, 15%, and 35.3%, respectively, and reduced Na by 13.4%. Grain N rose from 1.45% to 2.24%, and protein content increased from 9.04% to 14.02%. In severe salinity (S_3), nutrient concentrations in both straw and grains declined overall, but inoculated plants still maintained significantly higher N, P, and K, alongside reduced Na compared to controls. Grain protein improved from 7.94% to 12.88%, representing a 62.2% increase relative to non-inoculated plants. The F-test indicated highly significant differences ($p \leq 0.05$) for all parameters except straw Na. The smallest LSD values were observed for grain P, K, and Na (0.02), highlighting the sensitivity of these parameters to treatment differences. The enhancement of macro-nutrient (N, P, K) concentrations and reduction of Na in both straw and grains of inoculated wheat across all salinity levels indicates that *E. acetylicum* improves nutrient acquisition and ion homeostasis under saline conditions. Similar trends have been reported for halotolerant plant growth-promoting bacteria (PGPB) that improve nutrient uptake efficiency and reduce toxic ion accumulation (Kumar et al. 2020).

Nitrogen enhancement in grains is particularly important for improving protein content, as observed here, and may be attributed to bacterial facilitation of nitrogen assimilation pathways and modulation of nitrate reductase activity (Pandey, 2020). Increased phosphorus levels could result from bacterial phosphate solubilization, a well-documented trait in *Exiguobacterium* spp., which enhances energy metabolism and stress resilience in plants. Potassium accumulation, essential for osmotic regulation and enzyme activation, has been shown to be promoted by endophytic inoculation under salinity stress (Tedesco et al., 2021).

Reduced concentration of Na^+ levels in both straw and grains of inoculated plants suggest improved Na^+ exclusion or compartmentalization, thereby protecting metabolic processes and photosynthetic machinery. Maintaining a high K^+/Na^+ ratio is a known mechanism for salinity tolerance in cereals (Wang et al., 2022). The substantial increases in protein content, especially under moderate and severe salinity, indicate that *E. acetylicum* not only enhances biomass but also improves grain quality—a critical factor for food security in saline-prone regions. This aligns with recent findings that bioinoculants from stress-adapted environments can improve both yield and nutritional quality of cereal crops under abiotic stress (Al-Hawamdeh et al., 2024).

Overall, the results support the potential of *E. acetylicum* as a multifunctional bioinoculant capable of enhancing nutrient status, maintaining ionic balance, and improving grain quality under a wide range of salinity conditions.

CONCLUSION

This study highlights the potential of an endophytic *E. acetylicum* strain, isolated from desert wild palm, to enhance wheat performance under salinity stress. The isolate exhibited multiple plant growth-promoting traits: indole-3-acetic acid (IAA) production, phosphate solubilization, ammonia release, and putative nitrogen fixation. Inoculated wheat plants demonstrated significant increases in biomass, chlorophyll content, and nutrient acquisition, along with reduced levels of stress indicators (proline) and toxic ion accumulation (Na^+). Collectively, these findings underscore the biotechnological value of native endophytes from arid habitats for sustainable crop production on salt-affected soils. Multi-site field trials are warranted to validate these greenhouse results under on-farm conditions.

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الملخص العربي

عزل وتوصيف البكتيريا الداخلية *Exiguobacterium acetylicum* من جذور نخيل البلح البري من واحة سيوة وتطبيقها في تحسين نمو القمح تحت الإجهاد الملحي العالي

أحمد رمضان[١]؛ محمد الحلفاوي[١]؛ عصام قريش[١]؛ نيفين عثمان فتحي[٢]؛ محروس قنديل[١]

[١] قسم علوم الأراضي والمياه، كلية الزراعة، الشاطبي، جامعة الإسكندرية، مصر

[٢]معمل ملوحة وقلوية التربة، وزارة الزراعة، الإسكندرية، مصر

من المعروف جيداً أن ملوحة المياه والتربة تؤثر سلباً على نمو النباتات بشدةُ، خاصةً إنتاجية القمح في المناطق الجافة. في هذه التجربة البحثية تم عزل بكتيريا داخلية من جذور نخيل البلح البري (*Phoenix dactylifera* L.) جمعت من واحة سيوة ذات الملوحة العالية (مصر)، وتم تعريفها باستخدام تسلسل جين 16S rRNA على أنها *Exiguobacterium acetylicum*. وقد أظهرت السلالة المعزولة قدرة كبيرة على تحمل ما يصل إلى ١٥٪ (وزن/حجم) من NaCl في المختبر على بيئة JNFb. كما أظهرت هذه السلالة عدة صفات محفزة لنمو النبات (PGP) ، بما في ذلك النمو على بيئة المحتوية على ACC كمصدر وحيد للنيتروجين دلالة على نشاط إنزيم ACC-deaminase وإذابة الفوسفات و إنتاج إندول-٣-أسيتيك أسيد (IAA) بتركيز يقارب (٥ ميكروجرام/مل) وإنتاج الأمونيا. بينما لم يُلاحظ قدرة هذه السلالة لتثبيت النيتروجين الجوي على بيئة JNFb. وقد تم التأكد من وجود البكتيريا في جذور وأفرع القمح من خلال إعادة العزل والتطابق التسلسلي (≥98%) .

في تجربة أصص داخل الصوبة، تم زراعة القمح (الصنف Egypt-2) في بيئة رملية تحت أربع مستويات من ملوحة مياه الري ٠.٧، ٤، ٨، ١٢ dS m^{-1} مع أو بدون التلقيح بهذه السلالة المعزولة. أظهرت النتائج عبر جميع

مستويات الملوحة، أن التلقيح بهذه السلالة المعزولة أدى إلى زيادة معنوية في المجموع الخضري لنبات القمح ومكونات المحصول. على سبيل المثال، تحت الملوحة الشديدة (١٢ ديسيسيمين/م)، أظهرت النباتات الملقحة زيادات في الوزن الطازج (+٤٢.٩٪) والجاف (+٥٢.١٪)، ووزن السنبلة (+٥٠.٧٪)، ووزن الحبوب (+٦٣.٥٪) مقارنة بالكنترول غير الملقح. كما حافظ التلقيح على مستويات أعلى من الأصباغ الضوئية (الكلوروفيلa ، الكلوروفيلb ، الكلوروفيل الكلي، الكاروتينات) وخفض تراكم البرولين (-٣٣٪ عند ١٢ ديسيسيمين/م)، مما يشير إلى تخفيف الإجهاد الأسموزي/التأكسدي على النباتات الملقحة .كما تحسنت الجودة الغذائية للحبوب: عند ١٢ ديسيسيمين/م حيث ارتفعت تركيزات عناصر N ، P، K في الحبوب إلى ٢.٠٦٪، ٠.٣٦٪، ٠.٥٨٪ على التوالي، بينما انخفض Na إلى ٠.٥٠٪، مما رفع محتوى البروتين من ٧.٩٤٪ إلى ١٢.٨٨%.

بشكل عام، أظهرت هذه النتائج أن البكتيريا الداخلية *Exiguobacterium acetylicum* المعزولة من بيئة صحراوية مالحة قادرة على تعزيز نمو القمح ومحصوله والتوازن الأيوني ومحتوى البروتين في الحبوب تحت ظروف الملوحة. وتمثل هذا العزلة لقاحاً حيوياً واعداً للزراعة في الأراضي المالحة، مع ضرورة إجراء تجارب تأكيدية حقلية متعددة المواقع للتحقق من أن النتائج قابلة للتطبيق.