Research Article

Application of *Streptomyces mutabilis* Derived Rhamnolipid to Enhance Microbial Activity and Nutrient Uptake in Barley Grown Under Saline Conditions

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Abstract | Soil salinization poses a significant global challenge, limiting land use efficiency and agricultural productivity. This study aimed to investigate the production and characterization of a rhamnolipid (RL) biosurfactant synthesized by a *Streptomyces* isolate obtained from arid soil, and evaluates its impact on barley (*Hordeum vulgare* cv. Giza 123) growth under saline conditions. The isolate, designated as NVC7, exhibited the highest emulsification index (up to 74%), oil displacement activity, hemolytic action, and foaming capacity, indicating superior biosurfactant productivity. Morphological, biochemical, and genotypic analyses confirmed its identity as *Streptomyces mutabilis*. Optimal biosurfactant production was achieved at 30 °C and pH 8.0, with moderate salinity boosting production by 20-30%. Fourier-transform infrared (FTIR) spectroscopy analysis verified the glycolipid nature of the produced biosurfactant. Additionally, *S. mutabilis* displayed key plant growth promoting traits, including indole-3-acetic acid (IAA) production (37.4 µg mL^{-1}), phosphate solubilization (96.3 µg mL^{-1}), and ammonia production, aiding stress alleviation through enhanced nutrient availability and root development. Field trials were conducted during the 2025 winter cropping season at two locations in El-Kharga Oasis, New Valley Governorate, Egypt, characterized by soil salinity levels of 8.3 and 10.82 dS m^{-1}. Inoculation with RL-producing *S. mutabilis* significantly ($p < 0.05$) improved barley growth parameters, biomass accumulation, photosynthetic pigment concentration, soil microbial activity, and nutrient uptake. *S. mutabilis* inoculation markedly enhanced positive trait correlations ($r > 0.7$) between grain yield, nutrient uptake, and microbial activity (DHA/FDA) versus controls. Principal component analysis (PCA) revealed that inoculated treatments under both salinity levels clustered strongly on the positive axis of PC1, reflecting consistent enhancement across all measured traits. These findings highlight RL-producing *S. mutabilis* as a promising bioinoculant for sustainable agriculture in saline environments.

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Introduction

Soil salinization represents a critical environmental challenge to global agricultural sustainability, affecting over 833 million hectares of land worldwide, equivalent to 8.7% of the Earth's surface, as reported by the Food and Agriculture Organization (FAO) (Canton, 2021). Driven by several abiotic factors

such as drought, seawater intrusion, and improper irrigation practices, the extent of saline-affected soils is expanding at an alarming rate of 1–2% annually (Wang *et al*., 2023). This degradation severely impairs soil fertility and productivity, posing substantial barriers to green agricultural development (Sultan *et al*., 2023). Salinity stress disrupts multiple plant physiological processes, including seed germination, root development, ion homeostasis, and photosynthetic efficiency, while inducing oxidative damage *via* reactive oxygen species (ROS) accumulation and altering phytohormone signaling (Ahmadzai *et al*., 2025). These effects manifest across all growth stages, from seedling establishment to maturity, leading to morphological abnormalities and significant yield reductions (Soltabayeva *et al*., 2021). Furthermore, salinity inhibits soil microbial respiration and enzymatic activities, exacerbating ecosystem imbalances and threatening food security (Kumar *et al*., 2020).

In response to these challenges, plant growth-promoting bacteria (PGPB) have emerged as eco-friendly agents for enhancing plant resilience to abiotic stresses. These microorganisms modulate root architecture, improve nutrient and water uptake, and produce bioactive metabolites that foster plant growth (Narayanan and Glick, 2022). Among PGPB, actinomycetes, particularly the genus *Streptomyces*, stand out for their biotechnological potential, constituting a significant portion of soil microbiota and excelling in root colonization even under adverse conditions due to their spore-forming capabilities (Vurukonda *et al*., 2021). *Streptomyces* spp. are prolific producers of secondary metabolites, including antibiotics, enzymes, and biosurfactants, contributing to approximately half of all known bioactive compounds (Khan *et al*., 2023). Biosurfactants are amphipathic molecules that reduce surface and interfacial tension, offer multiple advantages over synthetic counterparts, such as superior biodegradability, low toxicity, structural diversity, and stability across varying pH, temperature, and salinity levels (Sharma, 2021; Gayathiri *et al*., 2022). These compounds facilitate bacterial-plant interactions by enhancing root colonization, nutrient chelation, and the bioavailability of phytohormones and siderophores (Borah *et al*., 2021; Ayoib *et al*., 2024; Karimi *et al*., 2024). Biosurfactants are traditionally categorized into four principal groups according to their chemical composition; mainly lipoproteins or lipopeptides, phospholipids, glycolipids, and polymeric surfactants (Drakontis and Amin, 2020). Among these, glycolipids are characterized by the presence of one or more carbohydrate moieties forming the hydrophilic head and one or more fatty acid chains constituting the hydrophobic tail. Notable representatives of this class include rhamnolipids, sophorolipids, and trehalolipids.

Glycolipids, including rhamnolipids (RLs), represent a prominent class of biosurfactants with demonstrated efficacy in environmental remediation (Aqif *et al*., 2024). RLs are microbial-derived glycolipids featuring hydrophilic and hydrophobic moieties, exhibit low toxicity and high environmental compatibility. In saline-alkali soils, RLs can mitigate water evaporation by binding water molecules to their hydrophilic groups, improving soil wettability and water retention. Additionally, RLs chelate metal ions, enhancing their mobility and leaching, aiding in salt removal while potentially facilitating nutrient uptake by forming lipophilic complexes (Borah *et al*., 2021; Faccioli *et al*., 2024). Under alkaline conditions, RLs release H^+ ions, neutralizing hydroxide radicals and lowering soil pH (Li *et al*., 2023). Despite these promising attributes, the application of RL-producing *Streptomyces* for ameliorating salinity stress in crops remains underexplored.

Barley (*Hordeum vulgare* L.), a major cereal crop valued for its adaptability yet highly susceptible to salinity, serves as an excellent model for investigating such interventions (Soltabayeva *et al*., 2021). The objective of this study was to examine the effects of RL-producing *Streptomyces* on barley growth parameters grown under saline conditions, aiming to elucidate mechanisms of stress alleviation and contribute to sustainable agricultural strategies.

Materials and Methods

Isolation of biosurfactant-producing Streptomyces

Nineteen soil samples were collected in sterile polyethylene bags from multiple locations across New Valley Governorate, Egypt. Samples were air-dried at room temperature (~25 °C) for 7 d and stored in the same sterile bags until use. 1 g of dried soil was suspended in 10 mL sterile distilled water and agitated on an orbital shaker at 120 rpm for 30 min. The suspension was serially diluted (10^{-1} to 10^{-8}) in sterile water (Nandhini and Josephine,

2013). Aliquots of 100 µL from suitable dilutions were spread onto starch–nitrate agar plates (pH 7.0) and incubated at 28±2 °C for 7 d. After incubation, developed actinomycete colonies were selected on the basis of typical leathery, powdery, or pigmented morphology. Pure cultures were obtained by repeated streaking on starch–nitrate agar slants (Küster and Williams, 1964). Stock cultures were preserved in 20% (v/v) glycerol at 4 °C for subsequent biosurfactant screening.

Screening for biosurfactant-producing Streptomyces

Pure *Streptomyces* isolates were grown in 250 mL Erlenmeyer flasks containing 100 mL Kim's medium (Kim *et al*., 2000) composed of (g/ L): $NaNO_3$ 10.0, yeast extract 0.2, $MgSO_4 \cdot 7H_2O$ 0.1, KH_2PO_4 1.0, $CaCl_2$ 0.1, and 3% (v/v) olive oil as sole carbon source, adjusted to pH 6.0. Flasks were incubated at 30 °C on a reciprocal shaker (120 rpm) for 5 d. Following incubation, the obtained actinomycete cultures were centrifuged at 8 000 × g for 20 min at 4 °C to yield cell-free supernatant, which was immediately tested for extracellular biosurfactant activity. All fermentations were performed in triplicate, and active isolates were maintained on glycerol–yeast extract agar slants at 4 °C with long-term stocks preserved in 20% glycerol at –20 °C. The cell free filtrate was subjected to different screening methods, including the following:

Emulsification capacity (E24) assay

The emulsification index (E24) was determined following Satpute *et al*. (2008). Briefly, 2 mL of a cell-free fermentation supernatant were combined with 2 mL of olive oil in a screw-cap test tube, vortexed at maximum speed for 2 min, and allowed to stand undisturbed at room temperature (25 ± 2 °C) for 24 h. Sterile Kim's medium served as the negative control. All assays were performed in triplicate. The height of the stable emulsion layer (the creamy, opaque layer formed at the interface between the aqueous and oil phases) was measured using a ruler or caliper, and E24 was calculated according to the following equation (Satpute *et al*., 2008).

$$E_{24}(\%) = \frac{\text{Height of emulsion layer}}{\text{Total height of liquid column}} \times 100$$

Oil displacement assay

The oil-spreading assay was performed in 15 cm Petri plates, in reference to Morikawa *et al*. (2000). 40 mL of distilled water were added, followed by 2–3 mL of crude oil pre-stained with Sudan Black to enhance contrast. Once the oil film stabilized (~2 min), 500 µL of cell-free *Streptomyces* supernatant were gently dispensed onto the center. After 30 s, the developed clear halo diameter was measured using a digital caliper. Distilled water (500 µL) served as negative control. Assays were conducted in triplicate.

Hemolysis assay

The hemolysis test developed by Mulligan *et al*. (1984) is based on the principle that biosurfactants have the ability to lyse erythrocytes. In this assay, *Streptomyces* isolate was streaked on blood agar plates supplemented with 5% blood and incubated at 25 °C for 48 h. The appearance of clear hemolytic zones around the colonies, resulting from the lysis of red blood cells, served as an indicator of biosurfactant production.

Cetyltrimethylammonium bromide methylene blue agar plate assay

Extracellular anionic biosurfactants (especially rhamnolipids) were detected using the cetyltrimethylammonium bromide (CTAB) blue-agar method (Siegmund and Wagner, 1991; Pinzón, 2009). Mineral-salt agar was supplemented with 0.2 g L^{-1} CTAB and 0.005 g L^{-1} methylene blue, autoclaved and poured. Pure *Streptomyces* isolates were spot-inoculated and incubated at 30 °C for 48 h. Plates were subsequently refrigerated at 4 °C for 24–48 h to intensify the dark-blue background. Colonies secreting anionic surfactants formed distinct dark-blue halos against the opaque medium. Uninoculated plates served as blanks. All tests were performed in triplicate.

Foam formation assay

Foaming ability was assessed following the method conducted by El-Sheshtawy and Doheim (2014). 10 mL of cell-free supernatant were placed in a 50 mL graduated cylinder, shaken vigorously by hand for 1–2 min, and immediately observed. Foam and total liquid heights were recorded using the scale markings on the 50 mL graduated cylinder. Foaming capacity (FC) was calculated according to the following equation (El-Sheshtawy and Doheim, 2014):

$$\text{FC}(\%) = \frac{\text{Height of foam}}{\text{Total height}} \times 100$$

Sterile Kim's medium was used as a negative control.

Measurements were taken in triplicate and foam stability was observed after 10 min. Isolates producing ≥ 50 % foam with persistence >5 min were scored as foam high-producers.

Phenol–sulfuric acid assay for glycolipids

Glycolipid-type biosurfactants were detected and semi-quantified using the phenol–sulfuric acid method (Dubois *et al*., 1956). 1 mL of a cell-free supernatant was mixed with 1 mL of 5 % (w/v) aqueous phenol in a heat-resistant glass tube. 5 mL of concentrated H_2SO_4 (95–98 %) were added dropwise down the tube wall to form a lower acid layer. The tube was gently swirled, allowed to stand for 15 min at room temperature, and absorbance was read at 490 nm against a reagent blank using spectrophotometer (Thermoscientific, India). A rapid color change from pale yellow to deep orange confirmed the presence of carbohydrate-containing (glycolipid) surfactants. Rhamnolipid concentration was estimated from a standard curve prepared with L-rhamnose (0–100 µg mL^{-1}). All assays were performed in triplicate.

Identification of selected actinomycete isolate

The most potent surfactant-producing actinomycete isolate was identified based on its morphological, biochemical, and molecular characteristics. The methods described by Shirling and Gottlieb (1966) were used to assess the cultural and biochemical characteristics of the isolates. Observations of aerial and substrate mycelium coloration, along with diffusible pigment production, were conducted on 7-d-old cultures cultivated on starch nitrate agar medium.

The morphology of spore-bearing hyphae and complete spore chains, along with substrate and aerial mycelia, was examined using light and transmission electron microscopy (TEM) (JSM-5500 LV, JEOL Ltd., Tokyo, Japan). The used biochemical assays included starch hydrolysis, nitrate reduction, melanin production, gelatin liquefaction, and chitinase activity. Diaminopimelic acid (DAP) isomers were identified using the method described by Becker *et al*. (1964), and cell sugar composition was determined following Lechevalier and Lechevalier (1970).

Molecular identification involved DNA extraction from pure colonies using a bacterial genome isolation kit, followed by PCR amplification of the *16S rDNA* gene using two primers: F27 (5′-AGAGTTTGATCMTGGCTCAG-3′)andR1492 (5′-TACGGGYTACCTTGTTACGACTT-3′), as described by Edwards *et al*. (1989). Polymerase chain reaction (PCR) product was amplified under the following cycling conditions (94 ˚C for 5 min, 30 cycles of 94 ˚C for 30 s, 55 ˚C for 30 s, 72 ˚C for 1 min, and final extension at 72 ˚C for 10 min). PCR product was then purified, and sequenced by Sigma Company for Scientific Services (Cairo, Egypt) products were purified and sequenced by Sigma Company of Scientific Service (Cairo, Egypt). Sequence data were analyzed using the BLAST program on the NCBI website to determine DNA similarity. Phylogenetic analysis and multiple sequence alignment were conducted using BioEdit software (Hall, 1999). The sequences were deposited in NCBI GenBank.

Effect of environmental factors on biosurfactant production

The effect of temperature, pH and NaCl on biosurfactant production by selected *Streptomyces* was determined following the method of Elkhawaga (2018). The selected *Streptomyces* isolate was grown on a growth medium containing: NH_4NO_3 (1 g/L), KH_2PO_4 (0.2 g/L), $MgSO_4.7H_2O$ (0.2 g/L), glutamic acid (10 g/L), and olive oil (60 g/L). Production of a biosurfactant was studied at various temperature ranges (25–60), pH (5–10) and various NaCl concentrations (0– 30%). The inoculated media were incubated for 7 d under shaking conditions (120 rpm). Then the emulsification activity of the culture supernatant was determined as mentioned above.

Extraction of biosurfactant

Biosurfactant was recovered following Lourenço *et al*. (2018) methodology with minor modifications. Seven-day fermentation broth was centrifuged (10 000 × g, 5 min, 4 ˚C) to obtain a clear supernatant. The pH was lowered to 2.0 with 6 N HCl and the acidified supernatant was stored overnight at 4 ˚C to precipitate the biosurfactant. The turbid solution was extracted twice with an equal volume of ethyl acetate in a separatory funnel. The combined organic phase was evaporated under vacuum at 40 ˚C, yielding a brownish residue. This crude biosurfactant was either used directly or dissolved in 0.1 M sodium phosphate buffer (pH 7.2) for further characterization.

Fourier-transform infrared (FTIR) spectroscopy

The functional groups of the extracted biosurfactant were identified using a PerkinElmer Spectrum 400

fourier transform infrared (FTIR) spectrometer (USA). 2 mg sample were uniformly mixed with 200 mg KBr, pressed into a transparent pellet (10 mm diameter, 10 tons for 2 min), and scanned from 400 to 4000 cm^{-1} at 4 cm^{-1} resolution with 32 co-added scans. Background correction was performed against a blank KBr pellet. Peak assignments followed Luna *et al.* (2013). Spectra were baseline-corrected and smoothed using Spectrum 10 software; triplicate pellets yielded identical profiles.

In vitro assessment of PGP activities by the selected actinomycete isolate

The nitrogen-fixing and phosphate-solubilizing capabilities of the selected isolate were evaluated by culturing it on Jensen's and Pikovskaya's media, following the protocols reported by Subba Rao (1984); Gupta *et al.* (1994), respectively. Indole-3-acetic acid (IAA) production was quantified using a colorimetric assay as described by Gilbert *et al.* (2018). Ammonia generation was evaluated following the procedure of Cappuccino and Sherman (1992), while siderophore synthesis was assessed according to the method outlined by Rachid and Bensoltane (2005).

Field experiment

Field trials were carried out during the 2024/2025 winter season at two saline sites in El-Kharga Oasis, New Valley Governorate, Egypt, with soil salinity levels of 8.3 dS m^{-1} (Site 1) and 10.82 dS m^{-1} (Site 2). At each site, a randomized complete block design with three replicates was used to evaluate the effect of two inoculation treatments on growth of barley plant (*Hordeum vulgare* L. *cv.* Giza 123):

1. Soil inoculated with spore suspension (10^8 CFU/mL) of the rhamnolipid-producing isolate. This spore concentration of 10^8 CFU/mL was selected to ensure a high probability of successful colonization and maintain a robust metabolic profile.
2. Uninoculated control

Soil physicochemical properties, analyzed according to Page *et al.* (1982), are summarized in Table 1. Field temperatures during the trial ranged between 18–25 °C (day) and 8–12 °C (night). Following local agricultural practices (drip irrigation and conventional fertilization), each experimental plot covered an area of 11.2 m^2. The layout consisted of eight rows, each 4 m in length and spaced 35 cm apart. Barley seeds were sown in the upper third portion of each row at a seeding rate of 50 kg/feddan, equivalent to approximately 119 kg/ha. The soil was inoculated by evenly distributing the prepared spore suspension of isolated *Streptomyces* isolate directly into the planting rows at the time of sowing.

Agronomic data recoded

At harvest (150 days after sowing), all aboveground plant material within a 1 m^2 quadrat was clipped at ground level and immediately weighed. The fresh weight was first expressed as kg/feddan, then standardized to tons per hectare (t/ha) to calculate yield and growth-related traits, including spike length (cm), 1000-grain weight (g), grain yield (t/ha), straw yield (t/ha), biological yield (t/ha, sum of grain and straw yield) and harvest index percent (grain yield/total biomass × 100). Leaf chlorophyll was recorded in Soil Plant Analysis Development (SPAD) units using a Minolta SPAD-502 meter. SPAD units represent relative chlorophyll content, derived from leaf transmittance at red (650 nm) and near-infrared (940 nm) wavelengths, and are widely used as a nondestructive proxy for leaf greenness and nitrogen status. Dried, ground straw, and grain samples (0.1 g) were digested with 2 mL of 80% perchloric acid and 10 mL concentrated sulfuric acid for 12 h, total nitrogen was determined by the micro-Kjeldahl method (AOAC, 2000) and phosphorus content (P %) was estimated using the ascorbic acid method (Chapman and Parker, 1961). The total K and Na concentrations were determined in the digested plant materials using a flame photometer (Page *et al.*, 1982). Nutrient uptake for N, P, K, and Na was determined on a dry-weight basis. Calculations were performed using the following relationship reported by Havlin *et al.* (2016):

Table 1: *Analysis of experimental soil.*

Study site	physical analysis			Chemical analysis							
	Soil texture	pH	E.C $dS.m^{-1}$	Cation meq/l				Anion meq/l			
				Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO_3^{--}	HCO_3^{-}	Cl^{-}	SO_4^{--}
Site1	Sandy clay loam	8.41	8.3	18.9	2.9	42.4	1.8	0.9	2.5	49.3	12.2
Site2	Sandy clay loam	8.54	10.82	20.8	4.3	48.7	2.4	1.52	5.6	53.9	15.6

Nutrient uptake (kg/ha) = Yield (t/ha) × Concentration (%) × 100

Where; % refers to the elemental concentration (g of nutrient/100 g of dry plant tissue). For P and K, concentrations were converted from oxide forms to elemental forms prior to calculations.

Soil microbial activity assays

The soil microbial activity was determined by estimating the activity of two soil enzymes, mainly dehydrogenase and fluorescein diacetate hydrolase.

Dehydrogenase (DHA) activity was quantified by 2, 3, 5-triphenyltetrazolium chloride (TTC) reduction (Casida *et al*., 1964). 1 g of fresh sieved soil was incubated with 1 mL 3 % TTC and 2.5 mL 0.1 M Tris-HCl (pH 7.6) for 24 h at 37 ˚C in darkness. The formed triphenyl formazan (TPF) was extracted twice with 5 mL methanol, centrifuged (4000 × g, 5 min), and absorbance was read at 485 nm. DHA activity was expressed as µg TPF/ g dry soil/ h.

Fluorescein diacetate (FDA) hydrolase activity was measured according to Adam and Duncan (2001). 2 g of fresh soil were suspended in 15 mL 60 mM potassium phosphate buffer (pH 7.6), spiked with 0.2 mL FDA (1 mg/ mL in acetone), and orbitally shaken (200 rpm) at 30 ˚C for 20 min. The reaction was stopped by adding 15 mL chloroform: methanol (2:1 v/v). After centrifugation (2000 × g, 3 min), the aqueous phase was filtered using Whatman No. 42 filter paper and absorbance measured at 490 nm against a fluorescein calibration curve (0–50 µg/ mL). Activity was reported as µg fluorescein g dry soil/ min. All assays were performed in triplicate.

Statistical analysis

Data were subjected to two-way analysis of variance (ANOVA) (site × treatment) in SPSS v.27. Treatment means were separated by Duncan's multiple-range test ($p \leq 0.05$). Relationships among rhamnolipid-producing *Streptomyces* inoculation, soil salinity, enzyme activities, nutrient uptake, and barley performance were visualized *via* principal component analysis (PCA) and hierarchical clustering heat-map generated in XLSTAT 2019, with variables standardized and Euclidean distance/Ward's linkage applied.

Results and Discussion

Isolation and screening of actinomycetes for biosurfactant production

A total of 11 morphologically distinct actinomycete colonies were isolated from various soil samples collected across different locations in New Valley, Egypt. The initial selection was based on colony morphology, including pigmentation and color of both substrate and aerial mycelia, which are classical taxonomic features in actinomycete identification. These isolates were subjected to a comprehensive screening protocol to evaluate their biosurfactant-producing potential using a suite of complementary assays; mainly oil spreading, blood hemolysis, CTAB–methylene blue agar, and emulsification activity (summarized in Table 2). Among the 11 isolates, only four demonstrated positive results. Notably, isolate NVC7 emerged as the most promising candidate, exhibiting the highest emulsification index (74%), positive oil spreading (7.5 cm), dark-blue halo on CTAB–methylene blue plates, hemolytic activity, considerable foaming capacity (63.3%), and greatest rhamnolipid yield as confirmed by a positive rhamnose test (0.54 mg/mL). Based on this robust performance across multiple assays, NVC7 isolate was selected for further characterization and application studies.

For successful screening of a biosurfactant, a combination of different methods is required. The oil spreading assay is a rapid and sensitive method that exploits the surface activity of biosurfactants. When a drop of culture supernatant is placed on an oil-covered water surface, the biosurfactant reduces surface tension, displacing the oil and forming a clear zone. The diameter of this zone correlates with biosurfactant concentration and activity (Morikawa *et al*., 2000), making it a valuable qualitative indicator.

The hemolysis assay, conducted on blood agar plates, assesses the ability of microbial metabolites to lyse red blood cells. A clear halo surrounding the colony indicates hemolytic activity, often associated with biosurfactant production. However, this method has limitations. Hemolysis can result from various lytic agents, including enzymes unrelated to surface activity, and not all potent rhamnolipid producers exhibit complete hemolysis. In fact, previous studies have shown that up to 20% of high-yielding rhamnolipid strains may not display β-hemolysis, characterized by complete lysis of red blood cells and appearance of

Table 2: *Biosurfactant production by the selected actinomycete isolate.*

Isolate	Emulsification index %	Hemolysis	Foaming test %	Oil spreading assay (cm)	CTAB	RT (mg/ml)
NVC7	74 ± 1.5	β- hemolytic	63.3± 2.3	7.5± 0.5	+++	0.51±0.04

Where; RT: Rhamnose test; CTAB: Cetyl trimethyl ammonium bromide. Values are expressed as mean ± Standard deviation (±SD).

a clear, transparent zone around actinomycete olonies on blood agar due to the breakdown of hemoglobin's heme group (Kumar and Dubey, 2023). Additionally, the hemolytic potential can be influenced by the molecular structure of the biosurfactant, particularly the length of its hydrophobic chains (Bustelo *et al.*, 2017). Therefore, the hemolytic activity test should only be employed during the preliminary screening of rhamnolipids-producing strains.

The CTAB–methylene blue agar assay is a semi-quantitative method specifically designed to detect anionic biosurfactants such as rhamnolipids. In this assay, the interaction between the anionic biosurfactant and the cationic surfactant cetyltrimethylammonium bromide (CTAB) forms an insoluble complex. When methylene blue is added, this complex appears as a distinct dark blue halo around the colony (Siegmund and Wagner, 1991). The diameter of the halo provides an estimate of biosurfactant concentration. This method is particularly effective for identifying rhamnolipid-overproducing strains and is valued for its speed, simplicity, and specificity (Rani *et al.*, 2020; Kumar and Dubey, 2023).

The emulsification index (E24) is a quantitative measure of an isolate's ability to stabilize emulsions between hydrophobic and aqueous phases. Biosurfactants, due to their amphiphilic nature, reduce interfacial tension and enhance the solubility and bioavailability of hydrophobic compounds. Emulsification activity is expressed as a percentage, representing the height of the emulsion layer relative to the total liquid column after 24 h. This assay is widely used to screen for biosurfactant-producing microorganisms with potential applications in bioremediation, agriculture, and industry (Sumiardi *et al.*, 2018).

Foaming capacity is another indicative property of biosurfactants. These molecules stabilize air–liquid interfaces, leading to foam formation. The extent of foaming is directly related to the surfactant's ability to reduce surface tension and is often used as a supplementary screening tool (Gurkok and Ozdal, 2021). The presence of stable foam suggests the production of surface-active compounds capable of forming micelles and entrapping air.

Finally, the rhamnose test, based on the phenol–sulfuric acid reaction, is employed to detect glycolipid biosurfactants, particularly rhamnolipids (Dubois *et al.*, 1956). These molecules consist of one or two rhamnose sugar moieties linked to β-hydroxy fatty acids (Kumar and Dubey, 2023). The test quantifies rhamnose concentration, serving as a proxy for rhamnolipid content. A positive result confirms the presence of glycolipid-type biosurfactants and supports the biochemical identity of the compound produced.

Together, these assays provide a comprehensive framework for evaluating biosurfactant production, each contributing unique insights into the nature, quantity, and functionality of the surface-active compounds synthesized by the microbial isolates.

Polyphasic identification of Streptomyces isolate NVC7

Detailed morphological analysis of the *Streptomyces* isolate NVC7 was conducted using TEM (Figure 1). Micrographs (Figure 1A) revealed filamentous, branching hyphae with septation, typical features of the *Streptomyces* genus. The aerial hyphae formed spore chains of the spiral or *Retinaculiaperti* type, consisting of 5–10 mature, smooth-surfaced spores arranged in closed spirals (Figure 1B). These intact spore chains reflect strong sporulation capacity, indicative of the strain's resilience to environmental fluctuations (Bobek *et al.*, 2017).

Cultural and biochemical characteristics of NVC7, summarized in Table 3, showed that the isolate produced gray to grayish-white aerial mycelium. Whole-cell hydrolysate analysis revealed the presence of LL-diaminopimelic acid, with no detectable diagnostic sugars. The isolate demonstrated the ability to hydrolyze starch and chitin, and produced melanin, hydrogen cyanide, and gelatinase, but lacked nitrate reductase activity. It failed to grow at extreme temperatures (5 °C and 45 °C) or under acidic conditions, and exhibited only minimal growth in presence of 0.1% phenol or 0.0001% crystal violet.

Table 3: *Cultural and biochemical profile of isolate NVC7.*

	Color mycelium	Spore chain	Spore mass	Spore surface	Gram's reaction	Growth temperature	Growth at pH 4.3	Nitrate reduction test
Morphological and biochemical characteristics	Light grey	Retinaculiaperti type (looped)	White	Smooth	Gram-positive	28-37°C	-	-
	Gelatin liquefication	Growth capacity in the presence of crystal violet 0.0001%	Starch hydrolysis	Chitin degradation	Melanoid pigments	Growth capacity in the presence of phenol 0.1%	H2S production test	Milk coagulation
	+	+	+	+	+	+	-	+

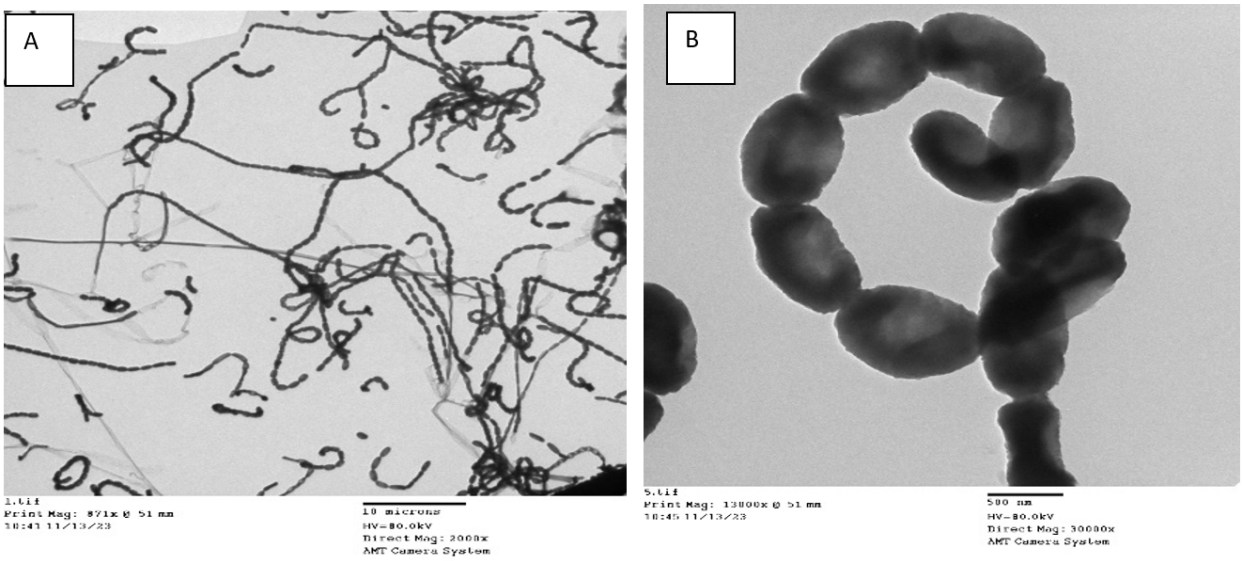

Figure 1: *Electron micrographs showing the morphology and spore chain structure of Streptomyces isolate NVC7. (A) Scanning Electron Micrograph (SEM) revealed sporulating mycelium showing spiral filamentous, branching hyphae (2000×). (B) TEM showed elongated spore chains of the spiral type with a smooth surface (30000×).*

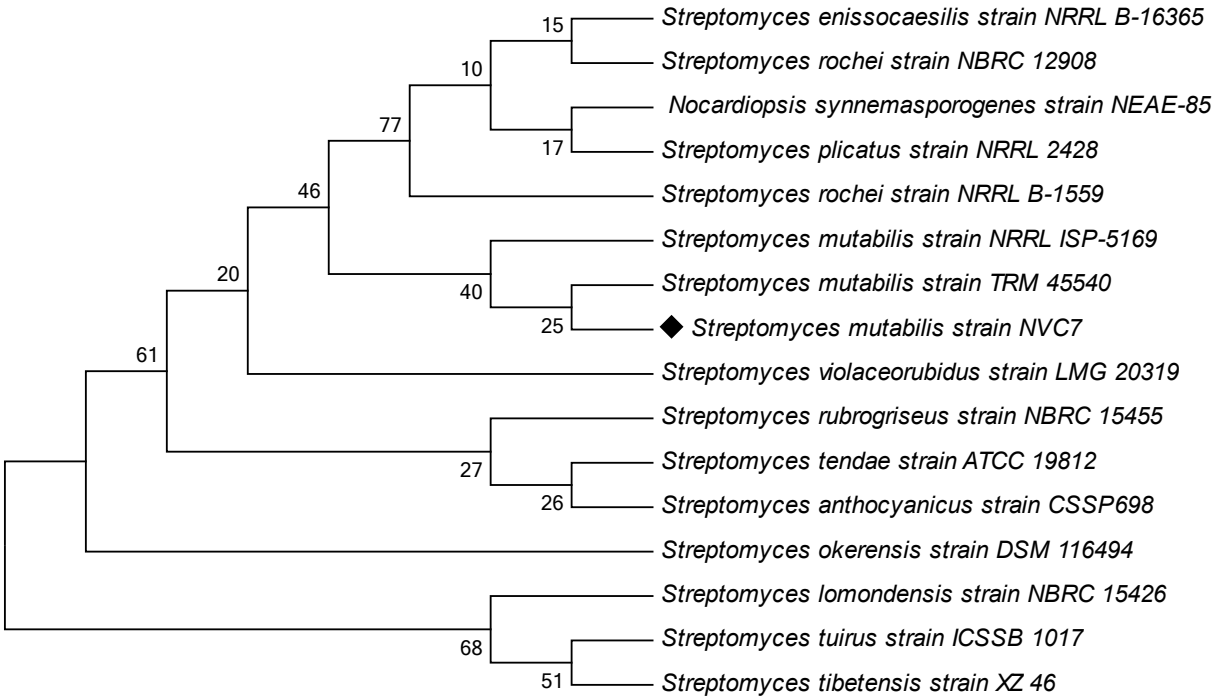

Figure 2: *Phylogenetic relationships of taxa closely related to Streptomyces mutabilis strain NVC7, inferred from 16S rDNA gene sequences using the neighbor-joining method. Numbers at nodes indicate bootstrap values expressed as percentages (%) based on 1,000 replications.*

For molecular identification, the 16S rRNA gene of isolate NVC7 was amplified and sequenced. Sequence alignment and phylogenetic analysis revealed 100% similarity with *S. mutabilis* NPRC 12800 (Figure 2), confirming its taxonomic identity. The sequence was deposited in NCBI GenBank and assigned an accession number of PX498040.

Based on its indistinguishable morphological features, chemotaxonomic profile, phenotypic traits, and complete 16S rRNA sequence identity, isolate NVC7 was formally identified as *S. mutabilis* NVC7. This species is well known for its ecological significance in soil environments and its ability to produce antibiotics. As highlighted by Vurukonda *et al.* (2021), the pronounced sporulation capacity of *S. mutabilis* serves as a vital survival mechanism, in consistence with the morphological characteristics observed in isolate NVC7.

Effect of environmental factors on biosurfactant production

The biosurfactant production by *S. mutabilis* was evaluated across a broad temperature range (25–60 °C) by cultivating the isolate at various temperatures and measuring the emulsification index (E24) of the culture supernatant. As shown in Figure 3a, the highest biosurfactant yield was recorded at 30 °C, with an E24 value of 75%. Production remained relatively stable up to 40 °C, indicating moderate thermal tolerance.

Regarding pH, the emulsification index was notably higher under slightly alkaline conditions (Figure 3b), with optimal biosurfactant production observed at pH 8.0. This trend suggests that alkaline environments favor both microbial biosurfactant synthesis and structural integrity of the biosurfactant molecule.

Salt concentration also played a remarkable role in emulsification activity. Maximum E24 values of 70%, 77%, and 72% were observed at NaCl concentrations of 0%, 3%, and 5% (w/v), respectively. However, a marked decline in activity was noted at 10% NaCl, where E24 dropped to 35% (Figure 3c). These results suggested that the isolated *S. mutabilis* was halotolerant, with moderate salinity enhancing biosurfactant production, likely by stimulating key metabolic pathways or improving nutrient uptake. In contrast, high salinity (≥10%) appears to inhibit microbial growth and biosurfactant synthesis, a phenomenon commonly reported among *Streptomyces* spp. and other actinobacteria.

These findings align with previous studies like that of Elkhawaga (2018) who identified a mesophilic temperature range of 28–35 °C as optimal for *Streptomyces* growth and biosurfactant production. Similarly, Sari *et al.* (2018) reported that alkaline pH conditions support both microbial biosurfactant production and molecular stability. Dabaghi *et al.* (2023) further demonstrated that rhamnolipid biosurfactants exhibit greater stability at basic pH, attributed to the precipitation of anionic biosurfactants at low pH and the enhanced stability of fatty acid micelles in alkaline environments.

Fourier transform infrared (FTIR) spectroscopic analysis of the extracted biosurfactant

Figure 4 presents the FTIR spectrum of the dried biosurfactant extract, recorded over the spectral range of 400–4000 cm^{-1}. FTIR spectroscopy is a commonly utilized analytical method for detecting functional groups and revealing chemical makeup of complex the mixtures. Kumar and Dubey (2023) emphasized the utility of FTIR to rapidly detecting hydroxyl, ester, and carboxylic functional groups, commonly found in biosurfactant molecules. The spectrum exhibited a broad, intense absorption band centered at 3283 cm^{-1}, assigned to O–H stretching vibrations from hydroxyl groups in the rhamnose moiety, overlapped with potential N–H stretching from amide linkages in di-rhamnolipid congeners. This feature indicated extensive hydrogen bonding, typical of a glycolipid biosurfactants.

Prominent peaks observed at 2923 cm^{-1} and 2856 cm^{-1} arised from asymmetric and symmetric C–H stretching of methylene ($–CH_2–$) groups in the aliphatic fatty acid chains, confirming the existence of hydrophobic lipid tail (likely β-hydroxydecanoic acid).

A sharp carbonyl stretch at 1639 cm^{-1} corresponded to the amide I band (C=O stretch coupled with N–H bending), verifying the ester-linked peptide bond between rhamnose and fatty acid moieties a hallmark of rhamnolipids. No significant protein contamination was evident, as amide II (~1540 cm^{-1}) was weak.

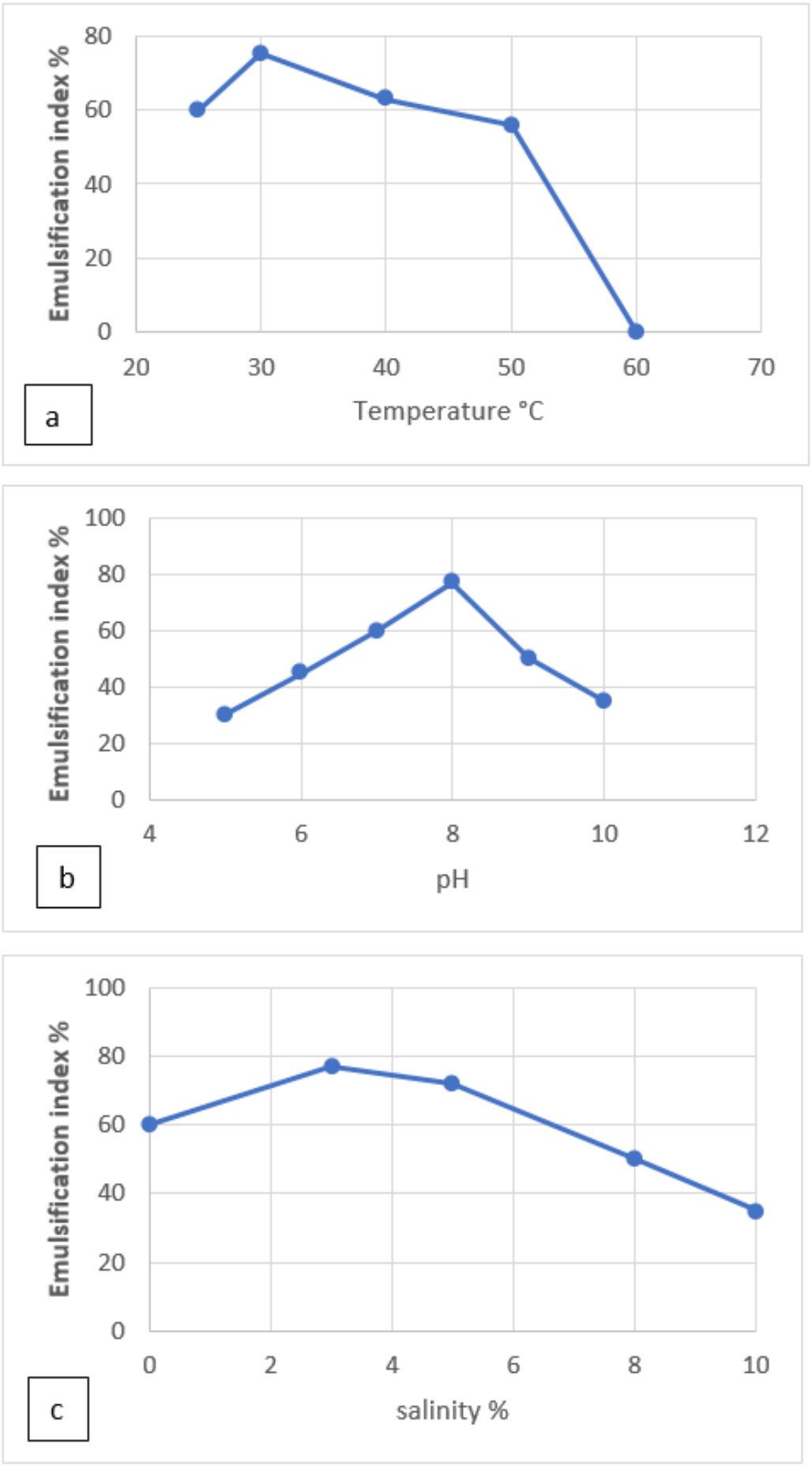

Figure 3: *Optimal conditions for biosurfactant production at various tested (a) temperature range, (b) pH, and (c) NaCl concentration.*

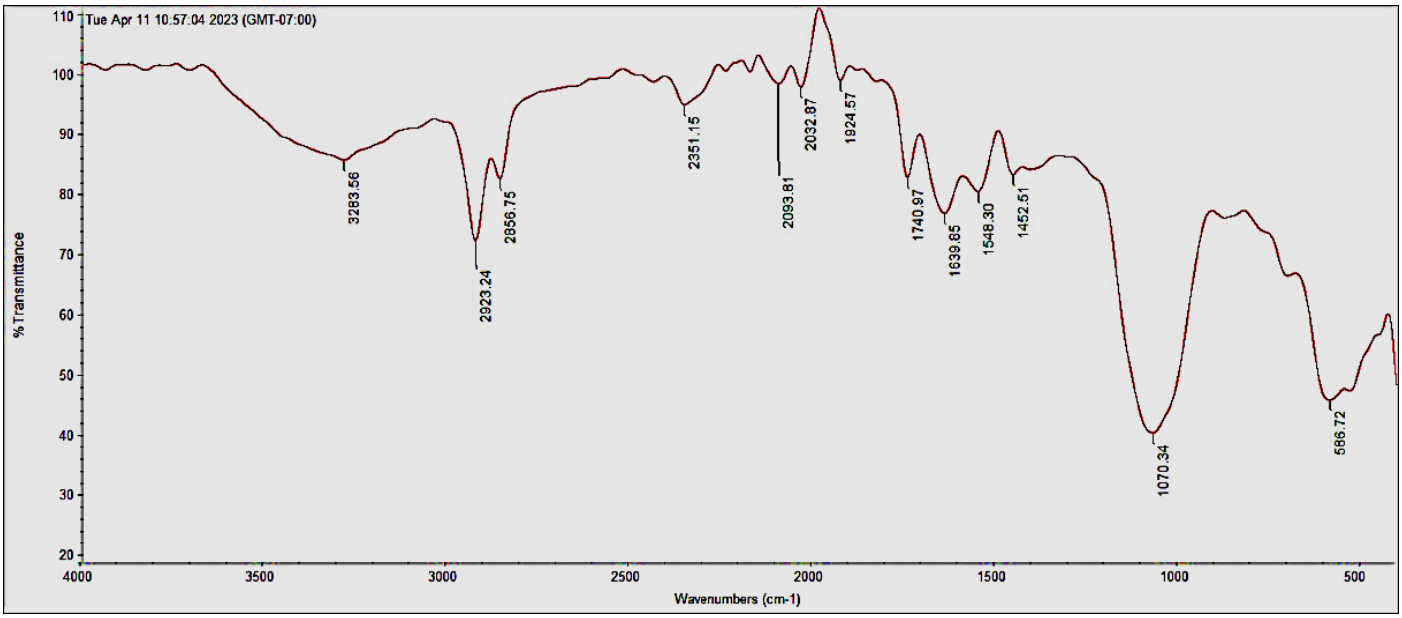

Figure 4: *FTIR analysis of a biosurfactant produced by Streptomyces mutabilis NVC7.*

The strong band at 1070 cm^{-1} was diagnostic of C–O–C glycosidic vibrations in the α-L-rhamnopyranosyl ring, further corroborated by weaker absorptions at 1456 cm^{-1} (CH_2 scissoring) and 1378 cm^{-1} (CH_3 symmetric bending). The FTIR profile aligns closely with reference spectra of mono- and di-rhamnolipids recorded in several previous studies (Tiwary and Dubey, 2018; Gharaei *et al*., 2022; Das *et al*., 2025). This clearly classified the extracted biosurfactant as an anionic glycolipid with a standard structure, composed of one or two rhamnose units esterified to one or two β-hydroxy fatty acids (chain length C_8–C_{14}). This structural confirmation supports its exceptional surface activity and environmental stability observed in saline field trials.

Plant growth-promoting (PGP) activities of Streptomyces mutabilis strain

The *S. mutabilis* strain demonstrated several plant growth-promoting (PGP) traits, including the production of indole-3-acetic acid (IAA), phosphate solubilization, and ammonia production, contributing to its potential as a biofertilizer. Quantitative analysis revealed IAA production at level of 37.25 ± 0.2 µg/mL and phosphate solubilization activity of 96.3 ± 0.1 µg/mL. However, it exhibited no detectable siderophore production or nitrogen fixation ability (Table 4). These findings are consistent with earlier reports highlighting the PGP capabilities of *Streptomyces* species. For instance, Kaur and Manhas (2022) reported that *Streptomyces hydrogenans* DH16 was able to solubilize phosphate and produce IAA, resulting in significant enhancement of plant biomass. Similarly, Chouyia *et al*. (2022) emphasized the diverse biochemical mechanisms employed by *Streptomyces* strains to mobilize insoluble phosphorus, including the secretion of organic acids and phosphatases. Such traits underscore the value of *Streptomyces* spp. as biofertilizer candidates in sustainable agriculture.

Table 4: *Plant growth-promoting attributes of Streptomyces mutabilis strain.*

PGP activities	IAA (µg ml^{-1})	Siderophores	NH_3 production	P Solubilization (µg ml^{-1})	N fixation
	37.25± 0.2	-	+	96.3± 0.1	-

Values are expressed as means ± Standard deviation (±SD).

Field experiment

Field experiments were conducted during the 2025 winter cropping season at two sites in El-Kharga Oasis, New Valley Governorate, Egypt, with soil salinity levels of 8.3 dS m^{-1} (Site 1) and 10.82 dS m^{-1} (Site 2) to evaluate the effect of an inoculation treatment on barley growth, soil microbial activities, and nutrient uptake.

Effect of rhamnolipid (RL)-producing Streptomyces mutabilis on barley growth criteria, chlorophyll content and yield and its components

Results in Figure 5 revealed that soil inoculation with RLs producing *S. mutabilis* had an appreciable impact on barely yield parameters, including morphological characteristics (*i.e*. spike length and plant height), physiological (chlorophyll concentration *via* SPAD readings), and yield-related traits (1000-grain weight, grain yield, straw yield, biological yield, and harvest

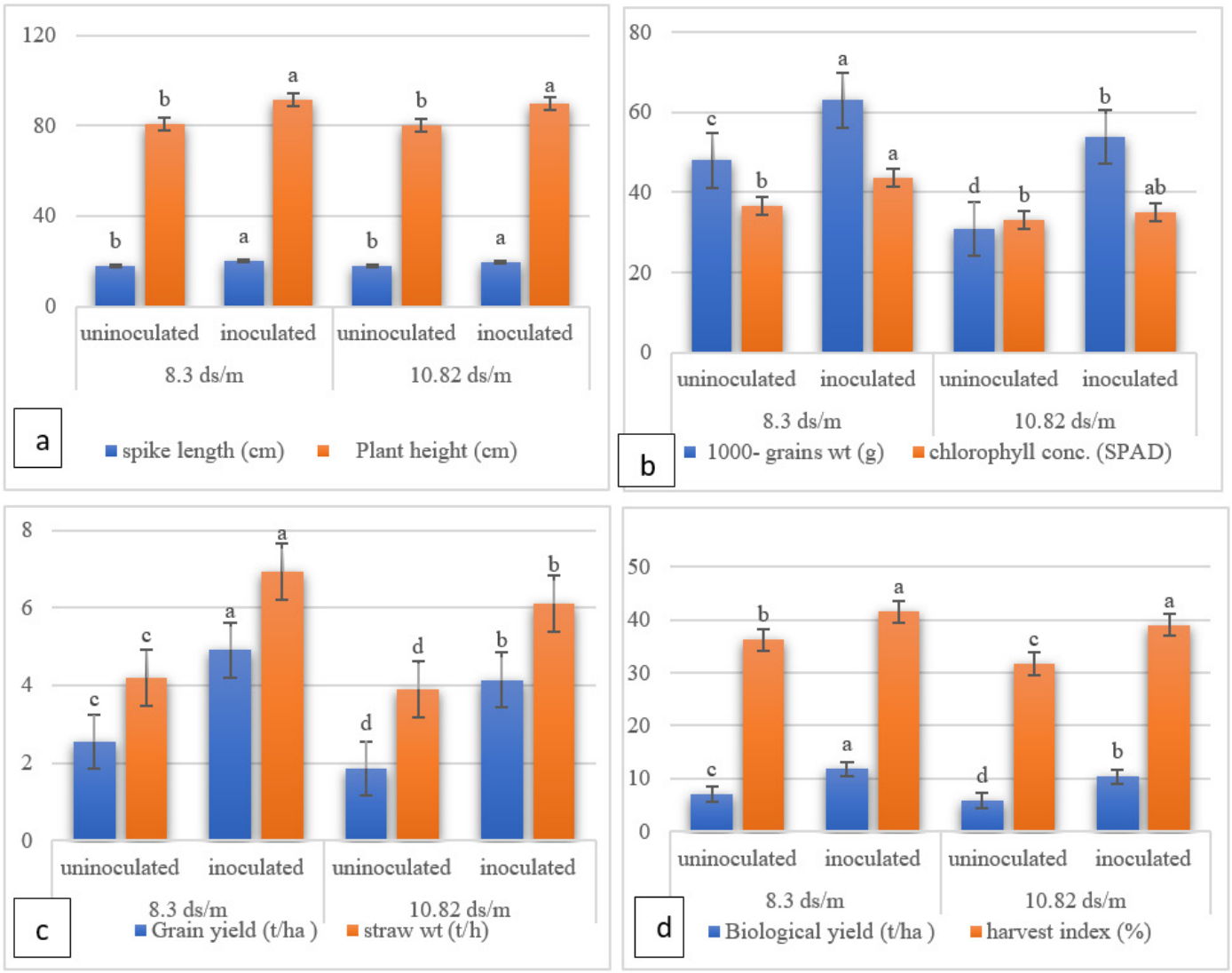

Figure 5: *Effects of rhamnolipid-producing Streptomyces mutabilis inoculation on growth of barely cv. Giza 123 grown under two varying salinity levels: (a) spike length and plant height, (b) 1000-grain weight and chlorophyll concentration, (c) grain and straw yield, and (d) biological yield and harvest index. Data are presented as mean ± SE (n = 3). Bars with different letters indicate statistically significant differences at p < 0.05.*

index). Data illustrated in Figure 5a, b highlighted that inoculation considerably increased spike length by 10.5% at 8.3 dS m^{-1} and 10.1% at 10.82 dS m^{-1}. Plant height showed similar increase in percent trend, recording 13.6 % at 8.3 dS m^{-1} and 12.05% at 10.82 dS m^{-1}. These improvements indicated enhanced vegetative vigor and tillering, possibly due to RLS-mediated root proliferation and better water/nutrient uptake in saline soils (Li *et al.*, 2023). Concerning 1000-grain weight trait, this reproductive trait exhibited the most dramatic response, with inoculation boosting weight by 31.3% at 8.3 dS m^{-1} and 74.2% at 10.82 dS m^{-1}. Additionally, inoculation improved photosynthetic pigment levels by 19.1% at lower salinity and 5.9% at higher salinity. The smaller gain observed under higher salinity may reflect the reduced capacity of actinomycete inoculation to counteract the damaging effects of ionic stress on chlorophyll stability. However, it demonstrated that inoculated plants maintained better photosynthetic efficiency compared to uninoculated controls.

Data in Figure 5c, d revealed that inoculation markedly increased grain yield by 92.2% at 8.3 dS m^{-1} and 121.6% at 10.82 dS m^{-1}. Uninoculated grain yield decreased by 27.5% from Site 1 to Site 2. Results regarding straw yield revealed that there was an increase in yield by 53.8% at lower salinity and 57.9% at higher salinity, contributing to biological yield gains by 66.2% and 77.6% at the two salinity levels respectively. This reflected an overall biomass accumulation, where inoculation prevented the 18.3% biological yield decline observed in the uninoculated treatments across both sites. Inoculation also,

improved harvest index (HI) by 14.5% at 8.3 dS m^{-1} and 23.1% at 10.82 dS m^{-1}. The highest recorded HI values of both inoculated treatments indicated that inoculation enhanced conversion of total biomass into economic yield, suggesting that *S. mutabilis* NVC7 helped the plant to prioritize grain filling over vegetative growth even under abiotic stress. Salinity stress exerts numerous detrimental effects on plant growth (Sultan *et al.*, 2023). It suppresses overall growth by disrupting key physiological functions, including photosynthesis and alters hormone balance and enzyme activities, collectively contributing to a marked decline in barley grain yield (Abdel-Ati and Eisa, 2015).

The obtained data demonstrated remarkable positive effects of bacterial inoculation on barley growth, physiological status, and yield parameters, particularly under salinity stress. Rhamnolipids, biosurfactants produced by *S. mutabilis,* likely enhance plant performance by improving soil structure, nutrient solubilization (e.g., phosphorus), root colonization, and stress tolerance through several mechanisms such as biofilm formation, osmotic adjustment, and reduction of Na^+ uptake (Zhang *et al.*, 2024). Similar results were obtained by Suralta *et al.* (2018) who found that rice inoculation with *S. mutabilis* under drought condition enhanced root system development and further contributed to the improvement of rice growth. Microbial inoculation with *S. mutabilis* established a self-sustaining system in the rhizosphere, providing continuous production of rhamnolipid and other beneficial metabolites, which supported long-term plant growth and soil health under saline stress. In addition, this approach enhanced ecological resilience and nutrient cycling (Reis *et al.*, 2024). Although its effectiveness varied based on the strain's survival among native microbes (Reis *et al.*, 2024), it offered a more sustainable alternative to purified rhamnolipid application. Since the purified rhamnolipids lacks the sustainability of living inoculants due to its rapid degradation and higher costs (Zhao *et al.*, 2022). Thus, microbial inoculation favors long-term resilience and ecological integration.

Determination of microbial growth and activity

Microorganisms are critical components of soil, contributing to decomposition, energy flow, and nutrient cycling (Neemisha and Sharma, 2022). The effects of rhamnolipid (RL)-producing *S. mutabilis* on microbial growth and activity in soil at different salinity levels (8.3 and 10.8 dS/m) were assessed by measuring dehydrogenase (DHA) and fluorescein diacetate (FDA) hydrolase activities. These methods were employed to provide a comprehensive evaluation of soil microbial health. DHA activity reflected the physiological state and biomass of the active microbial community, while FDA hydrolysis provided a broader measure of the soil's overall enzymatic potential. The results, presented in Figure 6, demonstrated that supplementation with RL-producing *S. mutabilis* appreciably enhanced microbial growth and

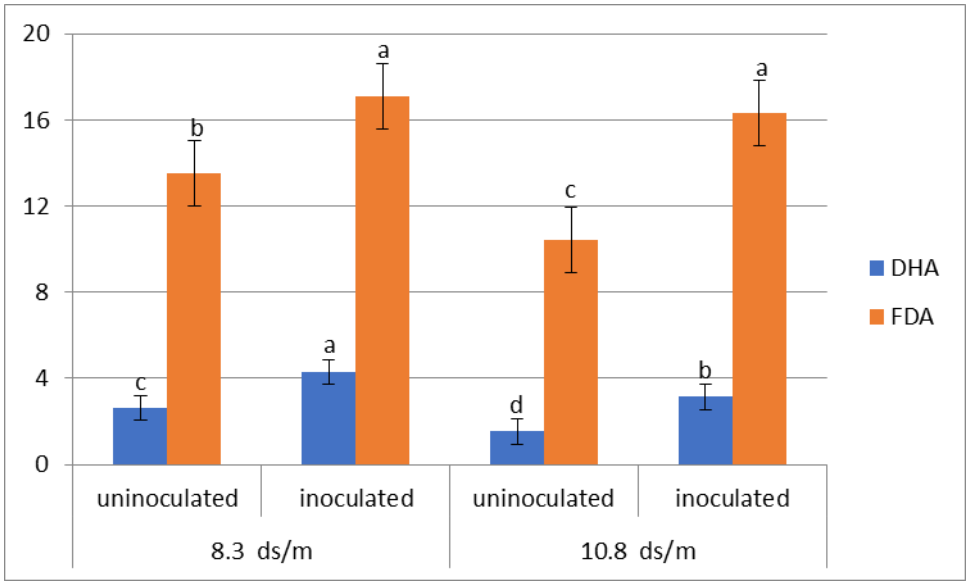

Figure 6: *Effects of rhamnolipid-producing Streptomyces mutabilis inoculation on microbial growth and activity in soil under two different salinity levels. DHA: dehydrogenase activity; FDA: fluorescein diacetate hydrolase activity. Data are presented as mean ± SE (n = 3). Bars with different letters indicate statistically significant differences at $p < 0.05$.*

activity at both salinity levels. DHA and FDA hydrolase activities in RL-supplemented treatments were 1.7–2.1 times higher for DHA and 1.3–1.7 times higher for FDA compared to the untreated control treatments at the two tested salinity levels, respectively. These findings indicate a strong growth-promoting effect of RLs on soil microorganisms. RLs considerably influence soil micro-ecology by acting as direct carbon source and as chelators that enhance the bioavailability of nutrients for microorganisms (Li *et al.*, 2023). The authors demonstrated that the addition of RLs favored the proliferation of bacterial species critical for improving soil properties and facilitating nutrients circulation. Moreover, RLs have been shown to remarkably increase the activity of some important soil enzymes, such as alkaline phosphatase, sucrase, and catalase (Wang *et al.*, 2023). Furthermore, multiple studies confirmed the stabilizing impact of rhamnolipids on rhizosphere microbial communities (Liu *et al.*, 2023; Chen *et al.*, 2024). This effect is often dose-dependent, as bacterial abundance in treated soil has increased in correlation with rising rhamnolipids concentration.

Effect of rhamnolipid (RL)-producing Streptomyces mutabilis on nutrients uptake

Results plotted in Figures 7a, b, c indicated that salinity inhibited nutrients uptake in control plants. Grain N, P, and K uptake reduced by 30.1%, 38.4%, and 26.0%, respectively, straw K/Na ratio reduced by 10.3 %, and straw K/Na ratio reduced by 10.3% at higher salinity level. Inoculation with RLs producing *S. mutabilis* appreciably enhanced nutrients uptake at both sites and mitigated Na^+ toxicity, substantially 10.82 dS m^{-1}. Data showed that inoculation had a significant effect on NPK uptake of barley grain and straw. Salinity in barley negatively impacted nutrients uptake by reducing overall growth, disrupting nutrient balance, and increasing the absorption of toxic ions like sodium and chloride while decreasing beneficial ones like potassium (Awais *et al.*, 2023). This may lead to a deficiency in essential nutrients, even if they were present in the soil, because of the physiological stress placed on the plant (Abbas *et al.*, 2021). Rhamnolipid mediated solubilization of nutrients and ion homeostasis, established *S. mutabilis* as an effective bioinoculant for sustainable barley growth in saline, desert environments. Based on the earlier study reported by Borah *et al.* (2021), ionic biosurfactants can considerably improve the availability of essential trace metals and nutrients in deficient soils, through forming strong complexes with metals, which are then released from the soil into the soil solution and hence they enhance metal mobility. This process is driven by the ability of a biosurfactant to drastically lower surface tension. In addition, biosurfactants are more effective and environmentally stable than synthetic alternatives, performing well under varying pH, (Ali *et al.*, 2022). Unlike chemical surfactants, these natural molecules continue to function properly across wide ranges of pH, temperature, and salinity, reducing soil pH and soil water- dissolved salts, and stimulating soil microorganisms to recycle carbon, nitrogen, phosphorus, and sulfur (Ali *et al.*, 2022; Wang *et al.*, 2023).

Interrelationship among plant growth promoting traits

To evaluate trait interrelationships, heat map and principal component analyses were performed. The correlation heatmap (Figure 8) demonstrated that inoculation with rhamnolipid-producing *S. mutabilis* markedly enhanced positive associations among grain yield, biological yield, straw yield, harvest index, grain nutrient uptake, and soil microbial activity (*i.e.*, DHA, FDA). Inoculated treatments displayed stronger red clusters ($r > 0.7$) compared to controls. At moderate salinity (8.3 dS/m), inoculation promoted coordinated traits integration, linking microbial activity with improved nutrients efficiency and yield stability. Under high salinity (10.8 dS/m), control plants showed a breakdown in trait coordination and increased negative correlations (e.g., straw nutrient uptake vs. harvest index and plant height vs. grain yield). In contrast, inoculated plants maintained robust positive networks, effectively mitigating stress-induced trade-offs.

Principal component analysis (PCA) based on the correlation matrix was illustrated using a biplot (Figure 9). In this plot, each variable is represented by an arrow, where arrow length indicates the variable's contribution to a principal component, the longer the arrow, the greater its influence. The angle between arrows reflects correlation strength: acute angles denote strong positive correlations, obtuse or right angles indicate weak or no correlation, and angles approaching 180° signify strong negative correlations. The PCA biplot (explaining 98.34% of total variance; F1: 94.51%) clearly distinguishes barley treatments under salinity stress. Inoculated samples (8.3 and 10.8 dS/m) clustered along the positive F1 axis are closely associated with high-yield traits, nutrient uptake, and

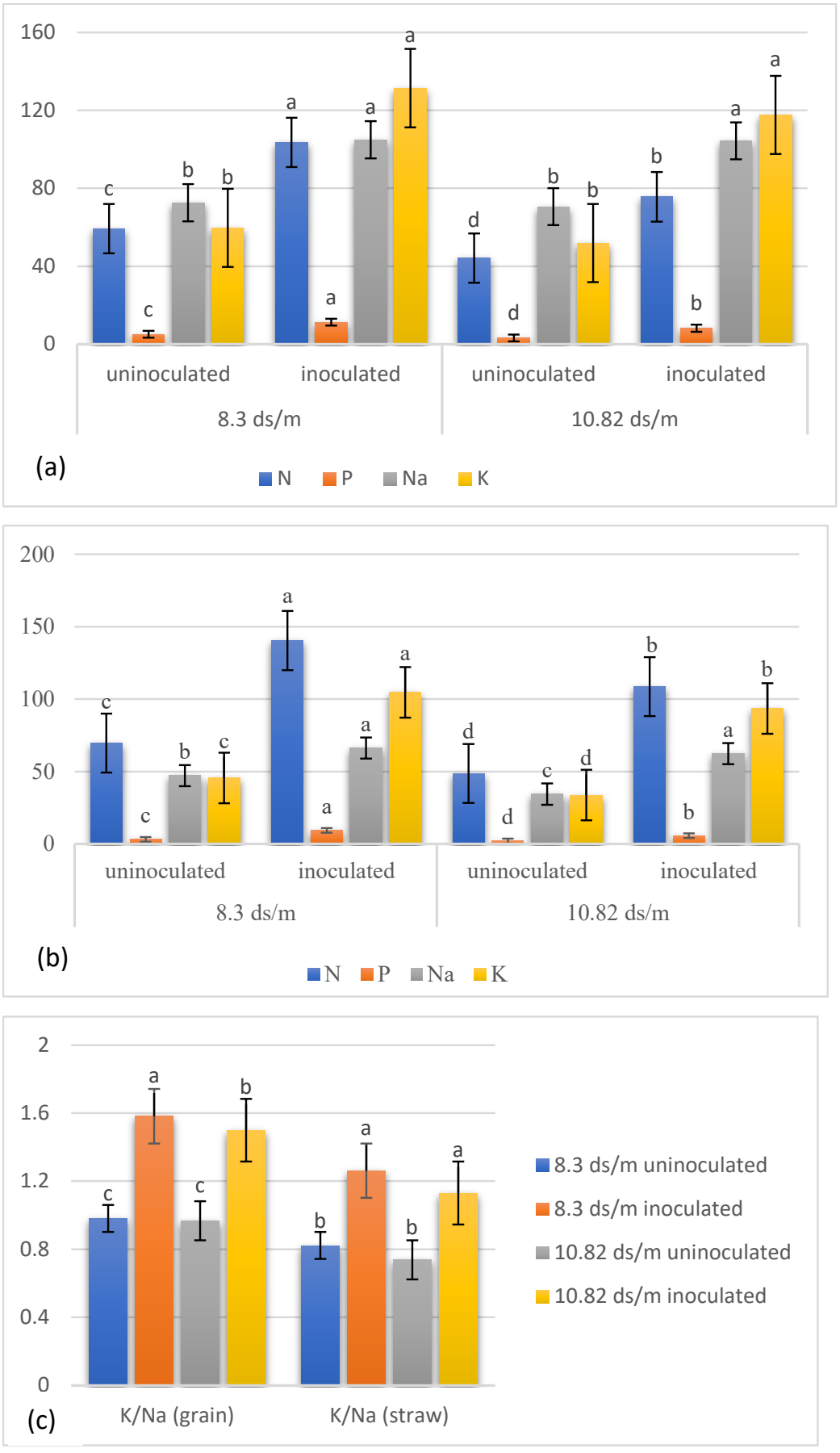

Figure 7: *Effects of rhamnolipid-producing Streptomyces mutabilis inoculation on nutrients uptake in barley cv. Giza 123 grown under varying salinity levels: (a) nutrients uptake (kg/ha) in grain, (b) nutrients uptake (kg/ha) in straw, and (c) K/Na ratio in grain and straw. Data are presented as mean ± SE (n = 3). Bars with different letters indicate statistically significant differences at p < 0.05.*

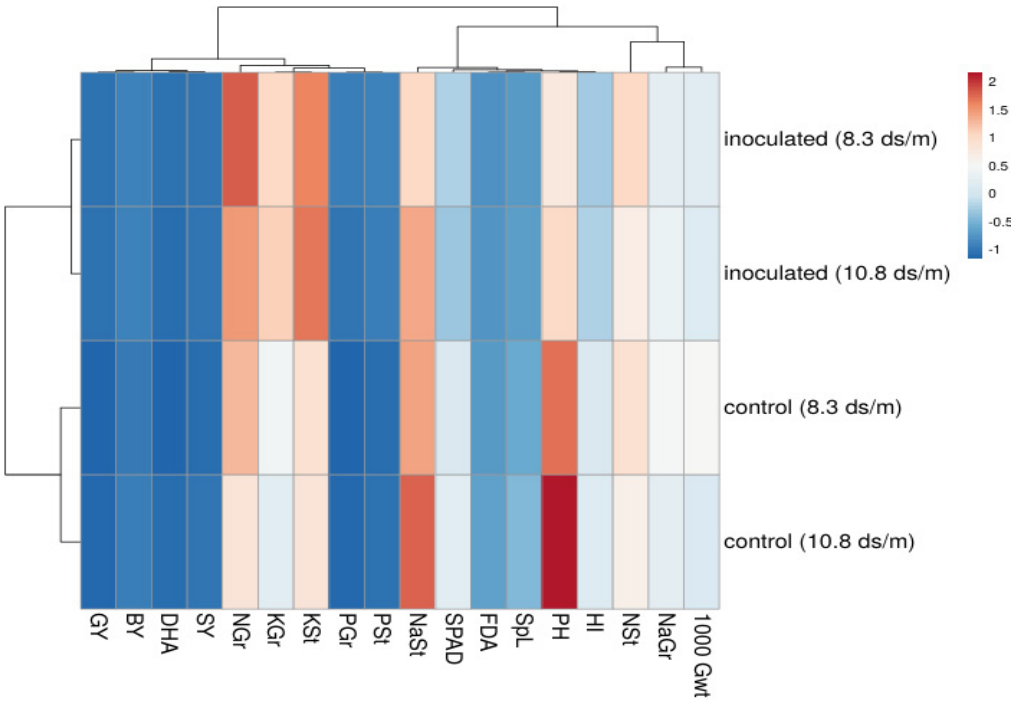

Figure 8: *A heatmap showing correlation patterns among barley growth traits, nutrients uptake, soil enzyme activities, and salinity responses following treatment with RLs producing Streptomyces mutabilis NVC7. Abbreviations: PH, plant height; SpL, spike length; 1000-GW, 1000-grain weight; GY, grain yield; SY, straw yield; BY, biological yield; HI, harvest index; DHA, dehydrogenase activity; FDA, fluorescein diacetate hydrolase activity; NGr, nitrogen uptake in grains; NSt, nitrogen uptake in straw; PGr, phosphorus uptake in grains; PSt, phosphorus uptake in straw; and KGr, potassium uptake in grains; KSt, potassium uptake in straw.*

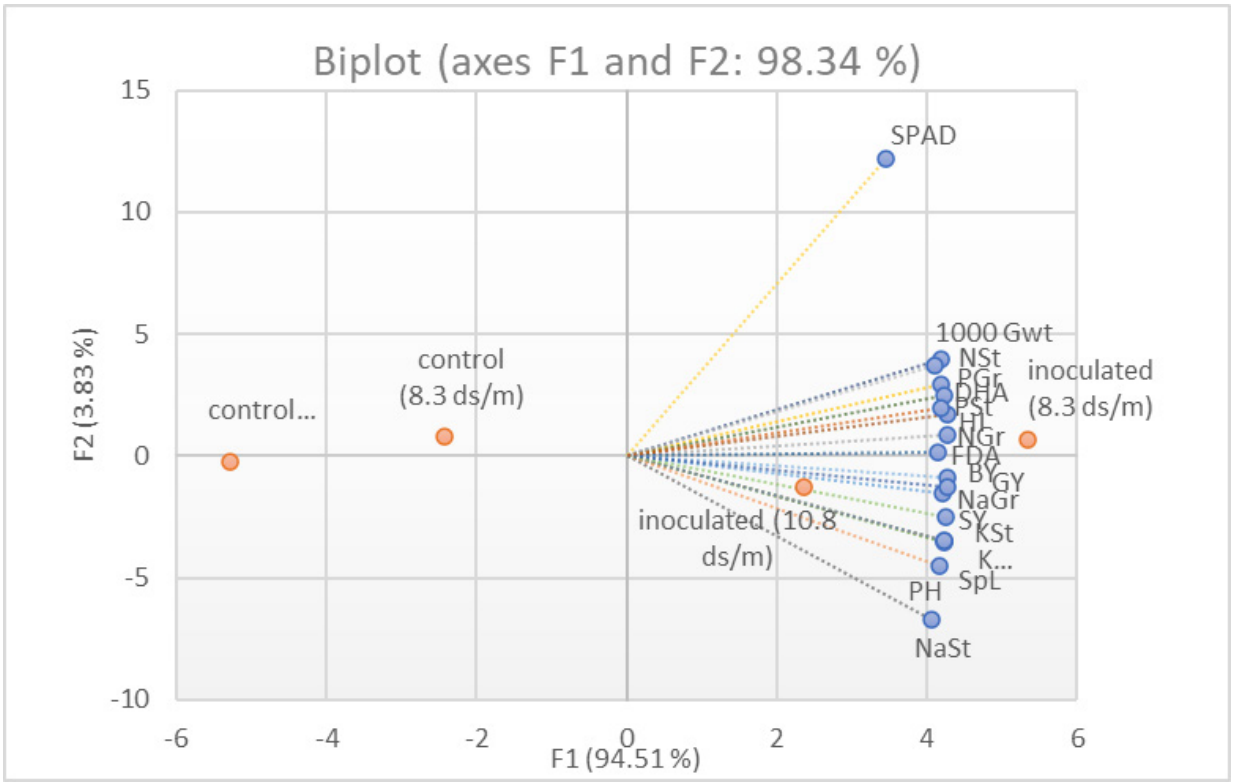

Figure 9: *A biplot of principal component analysis based on the correlation matrix applied to plant growth traits, yield, and its components, in addition to biochemical traits. Abbreviations: PH, plant height; SpL, spike length; 1000-grain weight; GY, grain yield; SY, straw yield; BY, biological yield; HI, harvest index; DHA, dehydrogenase activity; FDA, fluorescein diacetate hydrolase activity; NGr, N uptake in grains; NSt, N uptake in straw; PGr, P uptake in grains; PSt, P uptake in straw; and KGr, K uptake in grains and KSt, K uptake in straw.*

increased microbial activity. In contrast, control samples, particularly those under high salinity (10.8 dS/m), grouped on the negative F1 axis, characterized by reduced overall performance and being specifically linked to nitrogen uptake in the straw rather than the grain. These patterns mirror the heatmap results: inoculated treatments display strong positive correlations (r > 0.7) among yield, grain nutrients uptake, and activities of soil microflora, underscoring coordinated enhancement mediated by rhamnolipids. Conversely, controls under high salinity exhibit trait decoupling and negative associations (e.g., straw nitrogen uptake vs. grain yield and plant height vs. yield). Together, both analyses confirm that *S. mutabilis* inoculation integrated biochemical, nutritional, and growth promoting traits into a resilient, high-yielding system, effectively counteracting salinity-induced stress.

Rhamnolipids, glycolipid biosurfactants, act by improving soil aggregation, increasing nutrient bioavailability (especially P and K), reducing Na^+ uptake *via* ion selectivity, and stimulating rhizosphere microbiota (Li *et al*., 2023; Zhang *et al*., 2024; Silva *et al*., 2024). Similar yield gains (15–28%) and stress mitigation occured in cotton, tea, and tomato under salinity/drought, with enhanced N/P use efficiency and root exudation (Chen *et al*., 2023; Hu *et al*., 2023; Xu *et al*., 2025). Additionally, rhamnolipids function as elicitors of plant immunity by upregulating defense-related genes and inducing the synthesis of signaling hormones that activate key immune pathways (Pierre *et al*., 2023; Yang *et al*., 2024; Kuźniak and Gajewska, 2024).

Conclusions and Recommendations

This study demonstrated that *S. mutabilis* NVC7; a rhamnolipid-producing isolate, serves as a highly effective bioinoculant for mitigating salinity stress in barley. Through optimized biosurfactant synthesis, robust plant growth-promoting traits, and enhanced rhizosphere functionality, actinomycete strain inoculation substantially improved growth, biomass, photosynthetic efficiency, nutrients (N, P, K) uptake, and soil microbial activity under moderate (8.3 dS m^{-1}) and severe (10.8 dS m^{-1}) salinity levels. Multivariate analyses correlation matrices showing reinforced positive trait associations (r > 0.7) and PCA explaining 98.3% of variance collectively confirmed that rhamnolipids drived system-level integration, transforming stress-induced trait decoupling into a resilient, high-yielding phenotype. These findings validate *S. mutabilis* as a sustainable, multifunctional microbial solution for enhancing crop performance in salt-affected soils, with strong potential for scalable application in marginal agroecosystems. Future studies are recommended to explore rhamnolipids-producing *S. mutabilis* long-term field persistence, multi-season efficacy, and compatibility with integrated nutrients management, to fully harness its agronomic and environmental benefits.

Novelty Statement

This study represents the first report on the production and characterization of rhamnolipid biosurfactants production by *Streptomyces mutabilis* isolated from arid soil, and its subsequent application in enhancing barley growth under saline field conditions. The findings underscore the dual capacity of *S. mutabilis* for biosurfactant production and plant growth promotion, positioning this strain as a highly promising bioinoculant for sustainable agriculture in salt-affected environments.

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Generative AI and AI-assisted technology statement
The author has declared that generative artificial intelligence (AI) and AI-assisted technologies were not used in the generation of data, analysis, or interpretation of results presented in this manuscript. AI tools were employed for language editing and grammar refinement during manuscript preparation.

Conflict of interests
The author has declared no conflicts of interest.

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