



The Impact of Synthetic Osmoprotectants and *Salsola vermiculata* on Growth of *Streptomyces* sp. AH1 Isolated from an Arid-Soil

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Abstract: Soil salinity is a major environmental constraint that adversely affects microbial growth and plant productivity. Actinomycetes are recognized as important producers of phytohormones, particularly indole-3-acetic acid (IAA), while halophytes possess the ability to produce and accumulate osmoprotective compounds. This study aimed, first, to isolate actinomycetes from arid soil and screen the isolates for high IAA production and, second, to evaluate the osmoprotective effects of the halophyte *Salsola vermiculata* and the synthetic osmoprotectants glycine betaine, proline, and trehalose on the growth of an actinomycete strain isolated under saline conditions. Eleven actinomycete strains were isolated from soil collected in southern Setif, Algeria, using starch-casein agar medium. Based on cultural characteristics, strain AH1 was identified as *Streptomyces* sp. AH1 and produced a high level of IAA ($84.30 \pm 0.3 \mu\text{g mL}^{-1}$). Neutral pH and temperatures of 25 and 30°C were found to be favorable for IAA production, yielding 64.00 ± 0.4 , 81.18 ± 0.4 , and $85.25 \pm 0.2 \mu\text{g mL}^{-1}$, respectively. The growth of *Streptomyces* sp. AH1 under high NaCl concentrations was significantly enhanced by the addition of glycine betaine, proline, and trehalose. Similarly, *Salsola vermiculata* exhibited a pronounced osmoprotective effect, significantly increasing actinomycete growth under saline stress. Growth rates increased by 10.69 times and 10.01 times at 0.5 and 0.8 M NaCl, respectively. Overall, *S. vermiculata* significantly improved the growth of *Streptomyces* sp. AH1 in the presence of NaCl, demonstrating its strong osmoprotective potential. The combined use of beneficial actinomycetes and halophytes as biofertilizers or biostimulants could help mitigate the detrimental effects of soil salinity and represents a promising approach for improving plant growth and agricultural productivity in saline soils.

Key words: Arid soil, actinomycetes, indole acetic acid, salinity, *Salsola vermiculata*.

Soil microorganisms play a crucial role in agricultural ecosystems by maintaining soil health and enhancing crop productivity. They contribute to nutrient cycling, improve soil structure, and help plants tolerate environmental

stresses. Among soil microbial communities, actinomycetes are particularly important and are widely distributed in dry, saline, and alkaline soils (Jeffrey, 2008). In arid ecosystems, actinomycetes capable of producing bioactive molecules represent a promising source of valuable metabolites (Smati *et al.*, 2025). They are particularly abundant in soils rich in organic matter and produce a wide range of bioactive compounds with important applications in agriculture and medicine. In addition to their role in nutrient cycling, actinomycetes contribute to nitrogen fixation, nutrient solubilization and immobilization, siderophore production, and the biological control of soil-borne pathogens (Helmi, 2025). They can also promote plant growth through the production of phytohormones such as indole-3-acetic acid (IAA), phosphate solubilization, siderophore synthesis, and inhibition of phytopathogenic microorganisms (Minakshi *et al.*, 2016). Furthermore, actinomycetes are well known for producing bioactive substances with antibacterial, antifungal, and antiviral properties (Manivasagan *et al.*, 2014).

Halophilic and halotolerant actinomycetes are particularly well adapted to saline environments. Genera such as *Micromonospora*, *Rhodococcus*, and *Streptomyces* have been isolated from saline soils (Javad *et al.*, 2013). Actinobacteria adapted to high salt concentrations have also been recovered from highly saline ecological habitats, where they produce diverse and potentially novel bioactive compounds and enzymes (Mohamed *et al.*, 2018). Among these microorganisms, *Streptomyces* is recognized as one of the most prolific producers of bioactive compounds (Tatar, 2021). These characteristics make halophilic and halotolerant actinobacteria particularly promising candidates for agricultural applications, especially in arid and saline soils.

The increasing world population has resulted in a growing demand for food production, highlighting the need for sustainable approaches to improve crop productivity, particularly in salt-affected agricultural soils. The use of beneficial microorganisms as bioinoculants or biofertilizers represents a promising strategy for improving plant growth and productivity under adverse environmental conditions. In this context, halophilic actinobacteria, particularly *Streptomyces* spp., may serve as

effective agricultural bioinoculants through their ability to produce bioactive metabolites and phytohormones under arid and saline conditions. This approach is particularly relevant because soil salinity deteriorates soil properties, reduces microbial activity, and ultimately inhibits plant growth through nutritional stress, ionic imbalance, and disruption of enzymatic processes (Goszcz *et al.*, 2025).

Microorganisms inhabiting extreme environments have developed diverse mechanisms to survive and function under stressful conditions, including high temperature, salinity, alkalinity, and acidity (Binayke *et al.*, 2018). One of the major mechanisms used by bacteria to withstand salinity stress is the accumulation of compatible solutes or osmoprotectants. These low-molecular-weight compounds include trehalose, glycine betaine, glycerol, mannitol, L-proline, L-glutamate, L-glutamine, ectoine, and 5-hydroxyectoine, among others (Jayaranjan and Singhal, 2015). Trehalose, proline, and glycine betaine, in particular, function effectively as osmolytes in microorganisms exposed to high-salinity environments (Wood *et al.*, 2001). Their accumulation helps maintain cellular osmotic balance and protects essential cellular components from the damaging effects of salt stress.

Halophytes provide another important biological strategy for coping with salinity. These plants are naturally adapted to saline soils and can accumulate substantial amounts of osmolytes and compatible solutes, including glycine betaine, proline, and trehalose (Guendouz *et al.*, 2022). In addition to their ability to tolerate salinity, halophytes can contribute significantly to soil ecosystem functioning by improving soil quality, promoting carbon sequestration and nutrient cycling, and supporting biodiversity conservation (Hameed *et al.*, 2024). Consequently, halophytes may play an important role in improving soil health, particularly in salt-affected environments (Goszcz *et al.*, 2025). They can effectively contribute to the remediation of saline soils through cellular, physiological, biochemical, and molecular mechanisms, thereby improving soil physicochemical properties and influencing soil microbial communities (Hameed *et al.*, 2024).

The demand for bioinoculants and biofertilizers has increased considerably in recent years because of their potential as effective and sustainable tools for improving crop productivity (Da Cruz Silva *et al.*, 2022). Combining beneficial microorganisms with salt-tolerant plants may therefore provide an innovative approach for managing saline soils. In particular, the interaction between halophilic actinomycetes and halophytes could provide complementary benefits: actinomycetes may supply phytohormones and other growth-promoting metabolites, whereas halophytes may contribute to osmoprotection and nutrient availability under saline conditions.

Accordingly, the first objective of the present study was to isolate actinomycetes from arid soil, screen the isolates for their ability to produce IAA, and optimize the conditions for IAA production. The second objective was to investigate the effect of NaCl stress on the growth of the selected high-IAA-producing actinomycete strain in the presence of the synthetic osmoprotectants glycine betaine, proline, and trehalose, as well as the natural osmoprotective source represented by the halophyte *Salsola vermiculata*. Particular attention was given to determining whether *S. vermiculata* could promote bacterial growth under saline conditions through both osmoprotective and nutritional effects. Thus, this study focuses on the selection of an actinomycete capable of producing substantial amounts of IAA and on exploring the beneficial effects of *S. vermiculata* on its growth under salt stress. Such an association could have potential applications in saline and arid regions, where the use of halophytes and beneficial actinomycetes may contribute to improving agricultural productivity. In their natural habitats, actinomycetes may serve as sources of phytohormones such as IAA, while *S. vermiculata* may help alleviate the deleterious effects of salinity through its osmoprotective properties, providing a promising basis for developing sustainable microbial-plant strategies for saline agriculture.

Material and Methods

Isolation of actinomycetes

The arid soil was collected from Ouled Chebel, located in the southern region of Setif, Algeria, near Sebkhet Melloul (LAT: 36.00572°N

(36°0'21" N); LONG: 5.33584 E (5°20'9" E), at a depth of 12-15 cm. After collection, the samples, were tightly sealed in sterile polythene bags and transported to the laboratory.

Starch casein agar was used for the isolation of actinomycetes. The composition of the starch casein agar medium was as follows: starch (10 g L⁻¹), K₂HPO₄ (2 g L⁻¹), KNO₃ (2 g L⁻¹), casein (0.3 g L⁻¹), MgSO₄·7H₂O (0.05 g L⁻¹), FeSO₄·7H₂O (0.01 g L⁻¹), CaCO₃ (0.02 g L⁻¹), and agar (15 g L⁻¹). The medium was supplemented with griseofulvin, nystatin, and nalidixic acid at concentrations of 25, 10, and 10 µg mL⁻¹, respectively. The plates were incubated at 30°C, and the development of actinomycete colonies was observed after 7 days, following the method described by Vijayakumar *et al.* (2007).

Screening of actinomycetes for Indole-3-Acetic acid activity

YM medium (g L⁻¹) was used for the growth of the isolated strains, its composition is the following: peptone (5 g L⁻¹), malt extract (3 g L⁻¹), glucose (5 g L⁻¹), and agar (18 g L⁻¹). The isolates were incubated at 30°C for 3 days. Agar discs containing actinomycete mycelia were then transferred to YM broth supplemented with L-tryptophan at 2 mg mL⁻¹ and incubated at 30°C with shaking at 125 rpm for 4 days. The cultures were subsequently centrifuged at 10,000 rpm for 15 min, and 1 mL of the supernatant was mixed with 2 mL of Salkowski reagent. The mixture was incubated at 20°C for 20 min, after which the absorbance of the developed pink colour was measured at 530nm. The IAA concentration in the culture was determined using a calibration curve prepared with pure IAA as the standard, followed by linear regression analysis. The IAA concentration was expressed as µg mL⁻¹, according to Bano and Musarrat (2013).

Optimization of IAA production parameters

The actinomycete strain showing high levels of IAA was tested to determine the best conditions for the IAA production by one factor at a time. The effect of L-tryptophan concentrations (2, 5 and 7 mg mL⁻¹); the optimum NaCl level (0.1, 0.2, 0.3 and 0.5 M), the effect of temperature (25, 30 and 37°C) and the effect pH level (5, 6, 7, 8 and 9) were studied using ISP2 medium (Glycerol Asparagine Agar) added with L-tryptophan (2 mg mL⁻¹). The

cultures were incubated for 7 days at 30°C in a shaker at 120 rpm (Ameur and Ghoul, 2012).

Effect of NaCl concentrations on actinomycete strain growth

Actinomycete strain was cultivated aerobically in YM medium for 3 days at 30°C. Cells were centrifuged, and 1 mL of cell supernatant was used as an inoculum supplemented to ISP5 medium of composition described by Shirling and Gottlieb (1966) added of different concentrations of NaCl (0, 0.1, 0.5, 0.8 and 1 M).

Impact of osmoprotectants and Salsola vermiculata on actinomycete growth: Actinomycete strain was grown in ISP5 medium (Glycerol Asparagine Agar) supplemented with G.B. (Glycine Betaine), proline, and trehalose, 1 mM each. *Salsola vermiculata* (Chenopodiaceae) was taken from the Sebkhet of Ain Ouelmen (Ouled Chebel, south of Setif, Algeria). The halophyte is rinsed abundantly with distilled water, dried in the open air and ground finely. The aqueous extract is the result of sterilization of the halophyte at 110°C for 30 min. Heat promotes the release of osmoprotective molecules. The halophyte is used to a content of 2% in ISP5 medium supplemented with concentrations of NaCl (0. 0.1, 0.5, 0.8 and 1 M). actinomycetes growth was determined at 540 nm for 8 days. Values were given as means $\pm$ SD for triplicate samples (Ameur *et al.*, 2011).

Results and Discussion

Actinomycetes isolation

Eleven strains of actinomycetes were isolated from the arid soil of Setif (Algeria); their count was 2.75×10^4 CFU g⁻¹ soil. After seven days of incubation at 30°C, all of the isolates produced well-grown colonies (2-9 mm). The colonies are dry and have different coloured aerial and substrate mycelia (Table 1). Microscopic exam shows branched filaments of all the isolates, they were Gram positive.

Screening of actinomycetes for indole acetic acid activity

Indole acetic acid is produced by all strains except stains AH8 and AH9. The actinomycete strain AH1 is the most efficient; it produced high level of IAA ($84.30 \pm 0.3 \mu\text{g mL}^{-1}$) (Table 2).

Table 1. Colours of aerial mycelium and substrate mycelium of the isolated actinomycetes

Actinomycete strain	Aerial mycelium	Substrate mycelium
AH1	White	Olive
AH2	Olive	Olive
AH3	White	Yellow
AH4	Beige	Green
AH5	White	Black
AH6	Beige	Beige
AH7	Yellow	Beige
AH8	Grey	Green
AH9	White	Beige
AH10	Grey	Black
AH11	White	Yellow

Table 2. Indole acetic acid levels produced by actinomycete strains

Actinomycete strains	Indole acetic acid ($\mu\text{g mL}^{-1}$)
AH1	84.3 ± 0.3
AH2	62.28 ± 0.4
AH3	37.46 ± 0.4
AH4	43.97 ± 0.5
AH5	9.99 ± 0.2
AH6	81.85 ± 0.4
AH7	30.44 ± 0.5
AH8	-
AH9	-
AH10	32.54 ± 0.4
AH11	45.74 ± 0.4

Optimization of IAA production parameters

Our results showed that strain AH1 produced the highest amount of IAA ($84.3 \pm 0.3 \mu\text{g mL}^{-1}$) compared with the other strains. It production touched $164.86 \pm 0.4 \mu\text{g mL}^{-1}$ in the medium supplemented with $5 \mu\text{g mL}^{-1}$ of L-tryptophan and $74.18 \pm 0.5 \mu\text{g mL}^{-1}$ with $2 \mu\text{g mL}^{-1}$ of T-tryptophan. pH levels (6 and 7) seem be optimum for the IAA production with IAA concentrations touching $62.94 \pm 0.2 \mu\text{g mL}^{-1}$ and $64 \pm 0.4 \mu\text{g mL}^{-1}$ respectively. AH1 actinomycete strain produced $85.25 \pm 0.2 \mu\text{g mL}^{-1}$ at 30°C and $81.18 \pm 0.4 \mu\text{g mL}^{-1}$ at 25°C. An inhibitory effect on IAA production was observed at 37°C (Fig. 1). At 0.1M of NaCl, the actinomycete strain AH1 produced $60.27 \pm 0.5 \mu\text{g mL}^{-1}$. IAA production is strongly inhibited at 0.5M of NaCl ($32.84 \pm 0.3 \mu\text{g mL}^{-1}$) (Fig. 2).

Impact of NaCl concentrations and osmoprotectants on actinomycete strain AH1

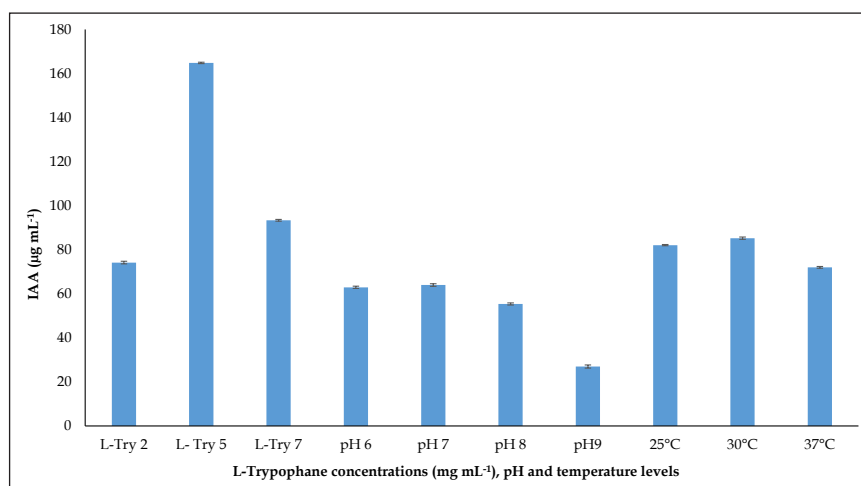


Fig. 1. Effect of pH, temperature and L-tryptophane concentrations on IAA production in actinomycete strain AH1.

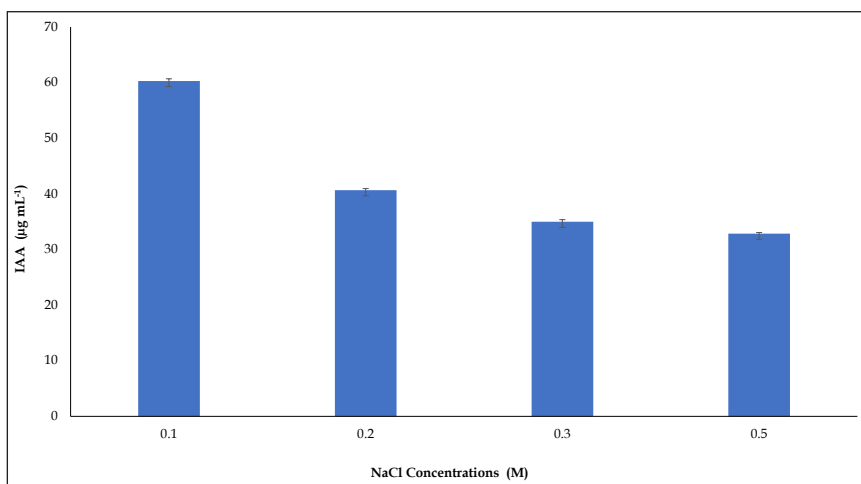


Fig. 2. Effect of NaCl concentrations on IAA production in actinomycete strain AH1

growth (Figure 3): In the absence of NaCl and at 0.1 M NaCl, glycine betaine stimulates actinomycetes growth beside proline and trehalose; however, *Salsola vermiculata* was the most efficient. Actinomycetes growth is strongly affected at NaCl (0.5 and 0.8 M NaCl). Natural osmoprotectants restore the actinomycete stain growth. The osmoprotective effect of *S. vermiculata* was observed at inhibitory concentration mainly at 1 M NaCl.

Actinomycetes represent over 30% of total soil microbial communities, with populations ranging from to CFU g⁻¹ depending on parameters like soil pH, aeration, moisture, and organic content (Barka *et al.*, 2016). Geographic sampling across diverse habitats highlights this numerical heterogeneity (Vieira and Nahas, 2005), yielding 2.75×10^4 CFU g⁻¹ in Algerian arid soil, 3.5×10^5 CFU g⁻¹ in Nigerian sandy soil (Agadagba, 2014), and lower yields in hyper-

arid Chilean environments (to 19.1×10^3 CFU g⁻¹) (Okoro *et al.*, 2009). In our study, mature colonies were dry present different colours of aerial and substrate mycelia, smelled earthy, and stuck to agar very firmly. Actinomycetes colonies isolated from different habitats were pigmented, dry, sticky, and distinctly on optimal media such as Starch Casein Agar and Bennett's Agar (Labeda, 1987; Muiru *et al.*, 2008; Zamir *et al.*, 2023). Mature colonies of the actinomycete strain AH1 were dry, with olive substrate mycelia and white aerial mycelium. This isolate may be referred to *Streptomyces* sp. AH1. *Streptomyces* exhibits smooth, dry colonies with varying coloured aerial mycelia and substrate. (Locci, 1989). Morphologically, these Gram positive, branched filamentous bacteria predominantly belonging to the genus *Streptomyces* (Küster and Williams, 1964; Locci, 1989; Sharief Dar and Ahmad,

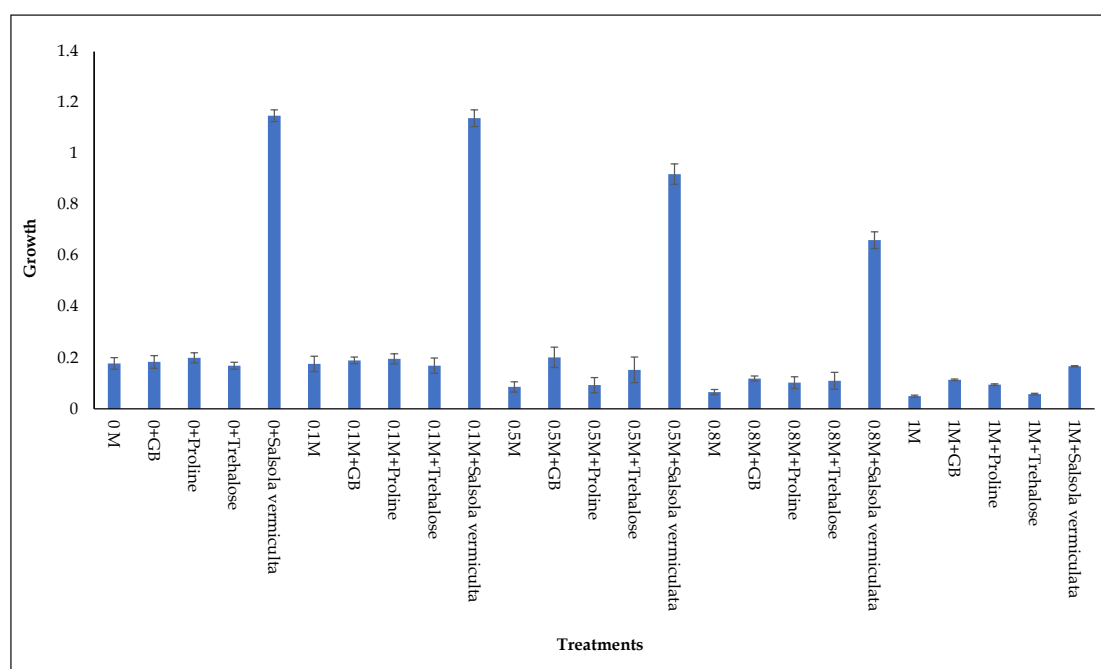


Fig. 3. Effect of G.B, proline, trehalose and *Salsola vermiculata* on actinomycete strain AH1 growth at 0, 0.1, 0.5, 0.8 and 1 M of NaCl.

2025; Dahbi *et al.*, 2025). Soils are dominated by actinomycetes and Streptomycetes are the most dominant group among them (Meenakshi *et al.*, 2024; Dahbi *et al.*, 2025). These isolates, such as *Streptomyces* sp. AH1, synthesize plant growth-stimulating phytohormones like Indole-3-Acetic Acid (IAA), which governs crucial processes such as embryogenesis and root, shoot, and fruit development (Benizri *et al.*, 2001; Khamna *et al.*, 2008; Anwar *et al.*, 2016). Strains AH1 and AH6 produced large amounts of IAA, they are respectively ($84.30 \pm 0.3 \mu\text{g mL}^{-1}$ and $81.85 \pm 0.4 \mu\text{g mL}^{-1}$) supplemented with $2 \mu\text{g mL}^{-1}$ of tryptophan. Actinomycetes are known to produce IAA with large amounts in different locations. IAA levels ranging from 17.36 to $109 \mu\text{g mL}^{-1}$, depending to the species and the soil type (Khamna *et al.*, 2008; Khamna *et al.*, 2010; Abd-Alla *et al.*, 2013; Sameera *et al.*, 2018; Myo *et al.*, 2019; Fatmawati *et al.*, 2019; Nafis *et al.*, 2019; Ali *et al.*, 2021). *Saccharothrix texasensis* MB15 isolated from Algerian hypersaline soil produces more than $148 \mu\text{g mL}^{-1}$ of IAA (Benadjila *et al.*, 2022). *Streptomyces* sp. AH1 reaches high IAA yields of $164.86 \mu\text{g mL}^{-1}$ with $5 \mu\text{g mL}^{-1}$ of L-tryptophan, under optimal neutral pH and temperatures between 25°C and 30°C . This finding is coherent with those of Aldesuquy *et al.* (1998), Khamna *et al.* (2010) and Ghoudjal *et al.* (2013). According to Yurekli *et al.* (2003) and Shirokikh *et al.* (2007), the

abundance of *Streptomyces* spp. is depending on pH soil, actinomycetes counts were very low in acidic and highly alkaline soils. Acidic and alkaline pH inhibited an affected strongly the function of enzyme systems and the solubility of substances required in bacterial growth and IAA production. The maximum IAA production was found at 0.1 M NaCl ($60.27 \mu\text{g mL}^{-1}$). IAA decreased at 0.5 M NaCl ($34 \mu\text{g mL}^{-1}$). Similar results showed that IAA production was declined under salt stress (Zhang *et al.*, 2019a; Saleem *et al.*, 2021).

Glycine betaine, proline and trehalose, enhance growth of *Streptomyces* sp. AH1 effectively, at 0 and 0.1 M of NaCl, however, *S. vermiculata* was the most efficient ($\times 6.44$ and $\times 7.6$ respectively), this is can be explained by the fact that halophytes are sources of mineral and organic matter, which stimulate strongly actinomycete growth. Nutritional analyses showed that halophytes contain high levels of vitamins, minerals, proteins, lipids, carbohydrates, phenolic compounds, fatty acids, amino acids and fibers (Lu *et al.*, 2010; Barreira *et al.*, 2017; Lopes *et al.*, 2023; El-Amier *et al.*, 2022; Hawas *et al.*, 2022). NaCl mainly at 1 M inhibit strongly *Streptomyces* sp. AH1 growth. Microbes are killed by the effect of soluble salts, which cause plasmolysis, nutritional imbalance, enzyme inactivity and production

of Reactive Oxygen Species (ROS) destroying cellular macromolecules, damaging membrane and losing protein function (Maganhotto de Souza Silva and Francisconi Fa, 2012; Yan *et al.*, 2015; Zhang *et al.*, 2019b; Johnson and Hug, 2019). *Streptomyces* sp. AH1 tolerate concentrations of 0.5 and 0.8 M of NaCl, it probably produces osmolytes in the presence of extreme conditions. Bacteria have elaborated mechanisms allowing adaption to live optimally in high salinity environments, they synthesized large amounts of betaines sugars, polyols, amino acids,...etc. To maintain turgor pressure, cell volume, stabilize the native conformations of biological macromolecules (Wood *et al.*, 2001; Grover *et al.*, 2016). Actinomycetes accumulate compatible solutes in order to alleviate salinity and drought stress (Killham and Firestone, 1984; Niu *et al.* 2022; Gao *et al.*, 2022; Sharma *et al.*, 2022).

Actinomycete growth is stimulated with glycine betaine, proline and trehalose; however, *S. vermiculata* was the most efficient. Growth rates were significantly increased respectively by 10.69 times and 10.01 times at 0.5 and 0.8 M NaCl. Halophytes use a variety of strategies to adapt to salinity soil by synthesizing enzymes required in their production; synthesizing osmolytes; controlling ion absorption, increasing soil organic matter, improving soil quality and synthesizing phenolics, flavonoids, alkaloids, saponins (Siddiqui *et al.*, 2021; El-Amier *et al.*, 2022; Hameed *et al.*, 2024). Osmoprotectants prevent strongly membrane injury and stabilize effectively proteins/enzymes under osmotic stress (Surówka and Hura, 2020). In many halophytic species, osmotic potential of the Na⁺ and Cl⁻ is adjusted by proline and glycine betaine. (Slama *et al.*, 2015; Pardo-Domènech *et al.*, 2016). Halophytes are known to destroy toxic ROS by synthesizing phenolics that directly scavenge ROS, protecting microbial cells from oxidative damage and improving overall habitat quality (Ksouri *et al.*, 2012; Giordano *et al.*, 2021; Hawas *et al.*, 2022). The main results proved the effectiveness of *Streptomyces* sp. AH1 isolated from an Algerian arid soil to produce important levels of IAA. Our findings prove and confirm the osmoprotective effect of the synthetic osmoprotectants tested on the actinomycete growth, however *Salsola vermiculata* was the most efficient and the most powerful in term of osmoprotection.

Conclusions

Telluric actinomycetes and halophytes may be used in arid soils or those affected by salinity to eradicate the salinity problem. Hence, both *Salsola* and *Streptomyces* sp. AH1 can be used to improve agricultural yield mainly in saline soils. *Salsola* by its contribution to alleviate the inhibiting salt stress, by the synthesis of osmolytes and compatibles solutes, which alleviate effectively the damage caused by salinity stress. Furthermore, it is a potential source of mineral and organic matter, which participates sensibly in the growth of plants and bacteria on the other hand; *Streptomyces* sp. AH1 can ameliorate plant productivity by IAA synthesis, which improves strongly different physiological mechanisms in plants. This will be very advantageous for soils suffering from salinity, which is considered the major problem, which acts negatively on plants growth. Application of actinomycetes as inoculants and halophytes as an exponentially source of osmolytes in sustainable agricultural, mainly in arid and saline soils will be a great solution to cope the deleterious effects of salinity. This study will be useful for further studies because there is little informations for researches about actinomycetes and halophytes mainly in arid soils.

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