***Pak. J. Bot.*, 56(3): 1163-1170, 2024.** **DOI: http://dx.doi.org/10.30848/PJB2024-3(19)**

PROPERTIES OF CELLULOLYTIC *STREPTOMYCES* STRAINS FROM SALINE-SODIC SOIL AND THEIR EFFECTS ON RICE GROWTH UNDER SALINE CONDITIONS

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Abstract

Rice (*Oriza sativa*) is one of the most important candidate crops that can be used to increase crop yield in the saline region of China. Cellulolytic microorganisms are considered to be the major drivers of litter decomposition in saline-sodic soils. This work presents an evaluation of the effects of cellulolytic *Streptomyces* strains on the growth of rice plants under saline conditions.

In this study, three cellulolytic bacterial isolates were obtained from saline-sodic grassland soils and identified as *Streptomyces* by 16S rDNA sequence analysis. These *Streptomyces* isolates were found to be positive for the production of carboxymethyl cellulase, xylanase, and nitrogenase activities under normal and saline conditions. All three isolates produced indole-3-acetic acid under saline (150 mM NaCl) and non-saline conditions. Furthermore, the three *Streptomyces* isolates were tested for their plant growth-promoting traits in pot trials using saline-sodic soil.

Inoculation of rice plants with each of these *Streptomyces* strains resulted in significant increases in growth performance, including fresh and dry weights, plant height, and leaf width, compared to the control rice plants. This is the first known report of cellulolytic *Streptomyces* strains from saline-sodic soils improving plant growth under saline conditions.

Results from this study suggested that these cellulolytic *Streptomyces* strains could be used to increase plant growth under saline-sodic conditions.

Key words: Cellulolytic bacteria; Nitrogen-fixing activities; IAA producing; Plant growth; Saline soils.

Introduction

Particularly in arid and semiarid regions, soil deterioration brought on by salt and/or sodicity is a substantial environmental obstacle with severe negative effects on agricultural production and productivity. (Ado *et al*., 2022). Worldwide, approximately 932 million ha of soil are estimated to be salt affected. According to the database of China's second national soil survey, soil salinization affects an estimated 35 million ha in China, of which 29.3 million ha are in grassland. Salinity results in soil degradation and severe decreases in land potential productivity (Ren *et al*., 2022).

In salt-affected soils, salinity not only reduces plant growth and damages the soil structure but also affects the microorganisms present in the soil by reducing their activities, thus reducing the fertility of the soils (Haj-Amor *et al*., 2022). In soils, microorganisms are the main components of the biogeochemical cycles for all nutrients, and the processes they control affect the properties and quality of the soils (Orhan & Gulluce, 2015). Plant litter is the main source of soil organic matter, and soil decomposer microorganisms play a vital role in soil nutrient cycling and energy transformation (Giweta, 2020). Cellulose is the most abundant polymer in plant litter, and soil cellulolytic microorganisms are considered to be the major drivers of litter decomposition (Behera *et al*., 2017). Evidence from previous studies indicated that the microorganisms occurring in naturally saline habitats have developed multiple adaptations to cope with such extreme environments (Gupta *et al*., 2014; Rath & Rousk, 2014). So the salt-adapted cellulolytic microorganisms might have plant growth-promoting effects through plant-bacteria interactions and can be used to increase crop growth under saline-sodic conditions.

The Songnen Plain, located between N 42°30′–51°20′N and E 121°40′–128°30′, covers an area of about 17.0 9 106 ha. It is the largest plain in northeast China and one of the five largest salt-affected soil regions in China (Zhai *et al*., 2021). Salinization and alkalization have substantially changed the properties of the soil in the Songnen Plain. The saline sodic soils are characterized by a high pH and a large exchangeable sodium percentage (ESP), and their permeability is very low (wang *et al*., 2021). In recent decades, to meet the increasing demands for food from a rapidly growing population, grassland has been converted to cropland in areas with soils with lower salt and alkali content. Rice (*O. sativa*) is moderately sensitive to salinity and sodicity and has proved to be a promising crop for cultivation on saline-sodic soils (Khatab *et al*., 2022; Zhao *et al*., 2020; van Asten *et al*., 2004). In order to effectively utilize these saline-sodic soils, rice (*O. sativa*) was planted in this plain. For improving rice growth, environmentally friendly methods such as the application of microorganisms have become crucial (Chataldi *et al*., 2020). Previous studies had shown that plant growth-promoting microorganisms could enhance the growth and yield of inoculated plants (Lopes *et al*., 2021; Nautiyal *et al*., 2013). The direct plant growth-promoting mechanisms may involve providing plants with fixed nitrogen, phytohormones, iron chelators, and soluble phosphate, and the indirect stimulation of plant growth may include reducing attack by phytopathogens (Ajijah *et al*., 2023). In recent years, some plant growth-promoting bacteria have been reported to increase crop growth and grain yield through the secretion of plant growth-promoting substances under salt-stress conditions (Kumawat *et al.*, 2023; Sarkar *et al.*, 2018). These studies indicated that plant growth-promoting bacteria could help plants overcome salt stress and provide a significant benefit to plant growth in the saline soil ecosystem. Plant growth-promoting microorganisms that affect crop growth in a saline environment have received much research attention, but, to the best of our knowledge, there are no reports of cellulolytic bacteria from a saline-sodic soil improving plant growth under saline soil conditions.

In the present study, three cellulolytic *Streptomyces* isolates were isolated from saline-sodic grassland soil in Jilin, China. The cellulolytic, nitrogen-fixing, and IAA-producing activities of the *Streptomyces* isolates were assessed, and their effects on inoculated rice under saline soil conditions were also evaluated.

Material and Methods

Soil samples collected: The soil samples were collected from a saline-sodic grassland soil, which lies within the Grassland Ecological Research Station of Northeast Normal University, Jilin Province, China (44° 45′N, 123° 45′E). The climate is semi-arid continental with a mean annual temperature of 4–7°C, and annual precipitation of 280–400 mm. The soils are mixed saline and alkaline (pH 8.0–10.5) and the vegetation is dominated by the perennial grass false wheatgrass *Leymus chinensis* (Trin.) Tzvel.

In August 2019, soil samples were collected from five randomly selected locations within a 20 m × 20 m area. About 200 g of soil in each location was extracted from a depth of 0–15 cm underground. The samples were placed in a cool box and transported to our laboratory within 6 hours of sampling. Each sample was separated into two sub-samples: one 10 g sub-sample was used for isolating cellulolytic microbes, while the other 190 g was air-dried to a constant weight for determining soil chemo-physical properties at room temperature.

Soil chemo-physical properties: Wet oxidation with K2CrO7 in the presence of sulfuric acid was used to determine soil organic carbon at 170–180°C. Soil total C and total N contents were determined by a dry combustion method using a C/N analyzer (Vario Macro, Elementar, Germany). Total P content was determined using the $HClO_4$-H_2SO_4 heating digestion method and measurements with an inductively coupled plasma emission spectrometer (ICP) (Qu *et al.*, 2016). Soil-available nitrogen was measured using determination methods as previously described (Zhang *et al.* 2013). Soil pH was measured in a 1:2.5 (v/v) soil: water suspension with a digital pH meter (PHS-3C, Shanghai Lida Instrument Company, China). The electrical conductivity of saturated-soil extract (ECe) was determined using a conductivity meter (DDS-11A, REX, Shanghai).

Isolation of cellulolytic bacteria: The cellulolytic strains were isolated from the collected soil sample (10 g) on a CMC-Na (carboxymethyl-cellulose, sodium salt) agar plate, which was prepared with nutrient salts medium, 1.0% CMC-Na, and 2.0% agar (w/v). The nutrient salt medium contained $NaNO_3$, 2.0 g l^{-1}; K_2HPO_4, 1.5 g l^{-1}; $MgSO_4$, 0.3 g l^{-1}; $FeSO_4$7H2O, 5 mg l^{-1}; $MnSO_4·H_2O$, 1.6 mg l^{-1}; ZnSO4·7H_2O, 1.4 mg l^{-1} and $CoCl_2$, 0.5 mg l^{-1}. The pH was adjusted to 8.5.

Morphologically different colonies were picked up and transferred to fresh CMC-Na agar plates, which were then incubated at 30°C for 120 h. The pure cultures were obtained through repeated streaking on CMC-Na agar plates. After being cultured at 30°C for 120 h, the plates were stained with 0.1% (w/v) Congo red solution for 10 min, and then washed with 0.1 M NaCl solution on each individual colony (Xu *et al.*, 2015). A clear zone on the culture plate, indicating cellulolytic activity, was used to identify the cellulase-producing strains. The NaCl, temperature, and pH ranges for growth were determined according to the methods described by Ren & Zhou, (2005).

Phylogenetic analysis of bacterial isolates: The cellulolytic isolates were identified based on analyses of 16S ribosomal genes (16S rRNA). The amplified gene fragment was sequenced by Comate Bioscience Co. Ltd. (Changchun, China). The BLASTN procedure was used to search for a sequence homology. The tree topologies were evaluated by the neighbor-joining tree method using the original data set and 1000 bootstrap data sets (Ulrich *et al*., 2008).

Cellulase, xylanase and nitrogenase assays: Each cellulolytic isolate was inoculated into 100 ml of liquid CMC-Na or corn straw media (nutrient salts solution 100 ml, 1.0 % (w/v) 1-cm long corn straw 5.0 g, pH 8.5), each under non-saline (without NaCl) and saline (150 mM NaCl) conditions, and incubated at 30°C with 120 rpm rotation for 5 d. The cultures were centrifuged at 14000 x g for 20 min at 4°C. The supernatants were used as the source of the extracellular crude enzyme preparation for measuring the activities of CMCase (from CMC-Na medium) or xylanase (from corn straw medium).

CMCase activity was measured by mixing 1 ml of crude enzyme solution with 1 ml of 1.0% (w/v) low-viscosity CMC-Na solution in 50 mM phosphate buffer, pH 8.5, and incubating at 50°C for 30 min. The reducing sugar generated by cellulase activity was determined using the dinitrosalicylic acid procedure (Miller 1959). One unit of CMCase activity (IU) was defined as the amount of enzyme releasing 1 μmol reducing sugars per minute under the assay conditions. Xylanase activity was assayed by the same method with birch wood xylan as substrate (Sigma-Aldrich Co. Ltd., St. Louis, USA). One unit of xylanase activity (IU) was defined as the amount of enzyme releasing 1 μmol reducing sugars per minute under the assay conditions (Schinner & Mersi, 1990). The nitrogenase activity (associated with nitrogen fixation) was determined in nitrogen-free medium using the acetylene reduction assay (Hardy *et al*., 1973). The nitrogenase activity of each of the isolates was investigated under non-saline (without extra NaCl) and saline (extra 150 mM NaCl) conditions. Nitrogenase activity was defined as nmol C_2H_4 ml^{-1} h^{-1}.

IAA production: IAA production by the isolates was determined according to the method of Glickmann & Dessaux, (1995). The IAA producing activity of each of the isolates was investigated under non-saline (without extra added NaCl) and saline (extra added 150 mM NaCl) conditions.

Evaluation of rice growth promotion: To study the effects of each of the three isolates on rice growth, pot experiments were conducted from July 18 to August 10, 2019. There were five treatments replicated four times (20 pots) for each treatment. The treatments were as follows: Control (non-inoculated soil); Sn-1 (inoculated with isolate Sn-1); Sn-3 (inoculated with isolate Sn-3); Sn-23 (inoculated with isolate Sn-23); mixed strains (inoculated with a combination of isolates Sn-1, Sn-3, and Sn-23). The rice seeds (*O. sativa*, Jn138) were surface disinfected by soaking in 70% ethanol for 5 minutes and then in 1% sodium hypochlorite for 10 minutes. Once drained, the seeds were rinsed five times with sterile distilled water. Twenty seeds were sown per pot (diameter 15 cm × height 20 cm) containing 4200 g of soil. The soil used for the experiment was sterilized by autoclaving (120ºC, 0.1 MPa for 60 min), and the main characteristics were pH 8.71, electrical conductivity 178.4 μS cm^{-1}; organic carbon 6.87 g kg^{-1}; total nitrogen 0.84 g kg^{-1}; available nitrogen 0.107 g kg^{-1}; available potassium 143.0 mg kg^{-1} and available P 3.20 mg kg^{-1}. For the treated groups, *Streptomyces* spore suspension was added to autoclaved soil, and the final cfu g^{-1} was adjusted to 10^6. This pot experiment was carried out in a greenhouse with natural daylight at 29/19 ± 4ºC (day/night) with a 15-h photoperiod.

Plant growth parameters measured on day 21 after sowing were dry weight, fresh weight, plant height, number of leaves, leaf width, and root length. The dry weight of plants was determined after drying to a constant weight at 80°C for 48 h. The chlorophyll concentration in plant leaves was measured by colorimetry (Moran 1982). Statistical differences between treatments and the control were assessed by one-way parametric ANOVA ($p<0.05$), with multiple pairwise comparisons being carried out with Fisher's Protected LSD Test using SPSS version 19.0 (IBM, USA).

Results

Phenotypic characteristics of cellulolytic isolates

Molecular identification of cellulolytic isolates: Nineteen cellulolytic isolates isolated from the saline-sodic soil were studied. The actinomycete isolates were the most dominant members of the cellulolytic microflora, making up more than 90 % of the isolates. Three actinomycete isolates (Sn-1, Sn-3, and Sn-23), which exhibited relatively high enzymic and growth-promoting activities compared to other isolates, were selected for further investigation. Morphological and biochemical characteristics of the three selected isolates are given in Table 1. The spore cells of the three isolates were gram-positive and had a globular or slender rod shape. All three isolates hydrolyze starch and gelatin. Except for isolate Sn-23, which was unable to utilize xylose, mannitol, or galactose, all three isolates were able to use xylose, dextrin, mannitol, saccharose, galactose, or maltose as carbon sources Our results showed that the three isolates could grow on medium with pH ranging from 7.0 to 10.5, and with NaCl concentrations in the range 0–4 % (Table 1). All three isolates were able to produce antibacterial antibiotics (Table 1).

Table 1. Morphological and biochemical characteristics of the *Streptomyces* strains Sn-1, Sn-3 and Sn-23.

Characteristic	Isolates		
	Sn-1	Sn-3	Sn-23
Gram reaction	**G$^+$**	**G$^+$**	**G$^+$**
Shape of cell	**Globular**	**Slender rod**	**Slender rod**
Cell Length (μm)	1.2	0.9	0.5-2.0
Colony Color	Grey	White	Pink
Citrate utilization	—	—	—
Gelatinase activity	+	+	+
Degradation of starch	+	—	+
Catalase activity	+	+	+
Xylose	+	+	—
Dextrin	+	+	+
Mannitol	+	+	—
Saccharose	+	+	+
Galactose	+	+	—
Maltose	+	+	+
NaCl 0-8％	+	+	+
pH7.0-10.5	+	+	+

＋: Positive reaction. －: Negative reaction

The partial 16S rRNA gene sequences obtained from each of the three isolates were aligned, the affiliations deduced using BLAST analysis, and submitted to GenBank. All three isolates showed 99 % similarity with the *Streptomyces* strains and have been deposited under the GenBank accession numbers KJ716528.1, KJ716530.1 and KJ742904.1 for *Streptomyces* Sn-1, Sn-3 and Sn-23 strains, respectively (Fig. 1).

Cellulolytic and nitrogen-fixing activities: The three isolates exhibited CMCase activities (3.33–6.57 × 10^{-2} IU ml^{-1}) in CMC medium and xylanase activities (42.88–67.89 × 10^{-2} IU ml^{-1}) in corn straw medium (Table 2). When the cellulolytic *Streptomyces* strains were inoculated onto nitrogen-free Ashby or CMC agar plates, colonies were clearly observed after incubation for three days, indicating that these isolates were nitrogen-fixing cellulolytic *Streptomyces* strains. The Biological nitrogen-fixing activities of the isolates were determined as nitrogenase enzyme activity measurements, which ranged from 18.60–24.81 nmol C_2H_4 ml^{-1} h^{-1} culture. The effects of NaCl on the activities of the CMCase, xylanase, and nitrogenase enzymes are described in Table 2. The results showed that the 150 mM NaCl treatment had no significant effects on the CMCase, xylanase and nitrogenase activities in any of the three isolates.

Plant growth-promoting activities: The plant growth-promoting activities of the three cellulolytic *Streptomyces* strains are summarized in Table 2. All three *Streptomyces* strains produced IAA, with the amount ranging from 1.25 to 2.05 μg ml^{-1}. The amount of auxin produced by strain Sn-3 increased after NaCl was added, from 1.65 to 8.89 μg ml^{-1} in 150 mM NaCl (Table 2). Production of siderophores in CAS medium was exhibited by only one strain, *Streptomyces* strain Sn-23 (Table 2), which also exhibited clear zones of solubilization around the colonies on NBRIP medium, indicating that this strain was able to solubilize phosphate.

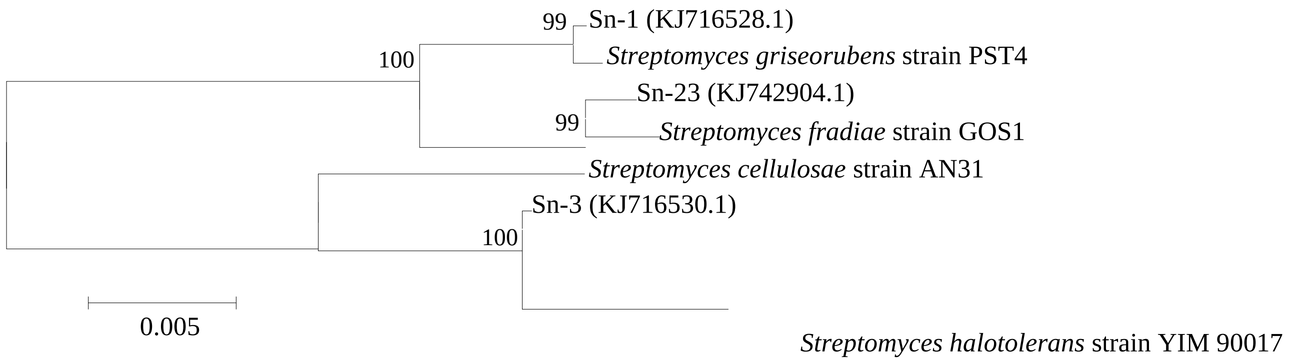

Fig. 1. The phylogenetic tree of *Streptomyces* strains Sn-1, Sn-3 and Sn-23 constructed on the basis of 16S rDNA sequences. The bar represents 0.005 substitutions per site. Bootstrap values are based on 1000 replications.

Table 2. Enzymic activities and plant growth-promoting characteristics of the isolates.

Characteristics	Sn-1	Sn-3	Sn-23
CMCase ($\times10^{-2}$ IU) (without NaCl)	6.57	3.92	3.33
CMCase ($\times10^{-2}$ IU) (150 mM NaCl)	4.13	4.09	4.23
Xylanase ($\times10^{-2}$ IU) (without NaCl)	42.88	63.15	67.89
Xylanase ($\times10^{-2}$ IU) (150 mM NaCl)	28.47	61.12	78.65
Nitrogenase (nmol C_2H_4/ml·h)	23.38	24.81	18.60
Nitrogenase (nmol C_2H_4/ml·h) (150 mM NaCl)	21.33	22.15	17.38
IAA production (mg/L) (without NaCl)	2.05	1.65	1.25
IAA production (mg/L) (150 mM NaCl)	2.91	8.89	0.65
Siderophore production	—	—	+
Phosphate solubilization	—	—	+
Antibacterial activity	+	+	+

＋: Positive reaction. —: Negative reaction

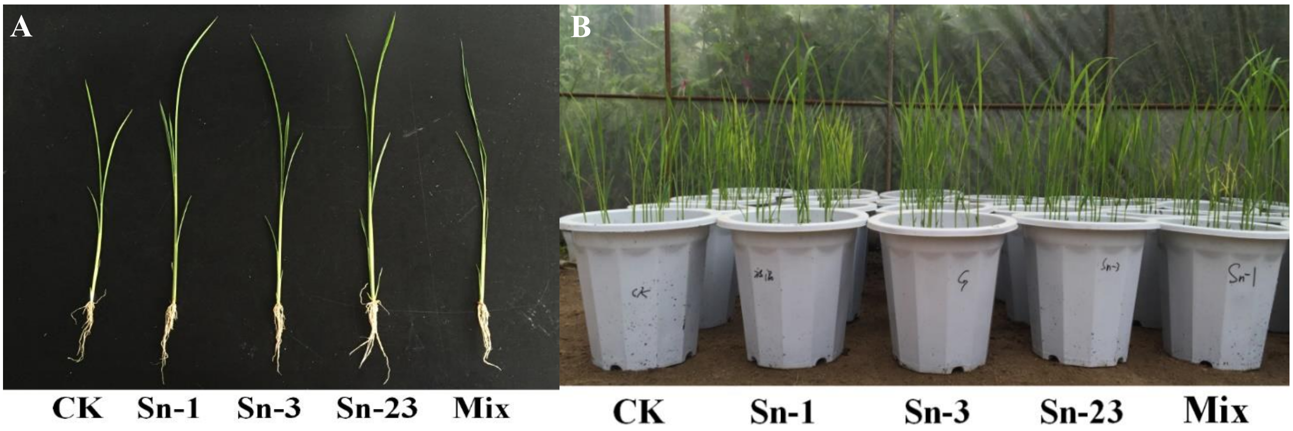

Fig. 2. A: Effect of the application of cellulolytic *Streptomyces* isolates on growth of 21-day-old rice plants: (a) control (non-inoculated soil), (b) treated with Sn-1, (c) treated with Sn-3, (d) treated with Sn-23, (e) treated with strains Sn-1+Sn-3 + Sn-23. B: Effects of individual and co-inoculation of cellulolytic *Streptomyces* strains on growth promotion of rice plants at 21 days after inoculation.

Rice growth-promoting effects: In this study, plant growth parameters were measured 21 days after inoculation. The effect of inoculation with the isolated *Streptomyces* strains on rice growth is summarized in (Fig. 2). Rice seedlings treated with the single strains Sn-1, Sn-3 or Sn-23 significantly ($p<0.05$) enhanced plant height (Fig. 3) (by 56.7%, 42.0 % and 37.7%, respectively) as compared to the uninoculated control seedlings. All treatments increased fresh and dry weight (Fig. 4) in comparison with the control, with inoculation with strain Sn-1 (136.8% increase in dry weight; $p<0.05$) resulting in a significant increase. Moreover, all treatments were found to significantly ($p<0.05$) increase test leaf width (Fig. 5) by 23.7% to 34.2%, compared to the control seedlings. Root length (Fig. 6) and leaf number (Fig. 7) significantly increased by 10.7%, 14.9%, and 9.1%, 13.5%, respectively, by different inoculation treatments. The chlorophyll concentration increased significantly following inoculation with each of the strains, compared to the value in the uninoculated control seedlings. However, there were no statistical differences between seedlings in the control and any of the different inoculation treatments in terms of chlorophyll a (Fig. 8) (Chl a), chlorophyll b (Chl b) (Fig. 9), or chlorophyll (a+b) (Fig. 10) concentrations.

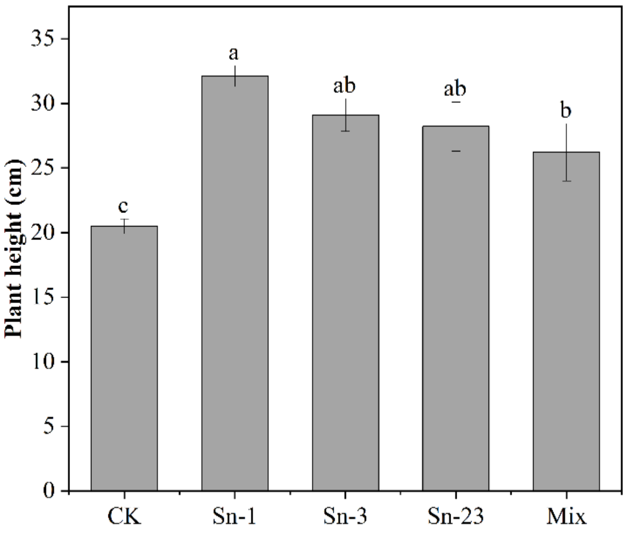

Fig. 3. Effect of the application of *Streptomyces* isolates on height of rice plants grown in saline soil.

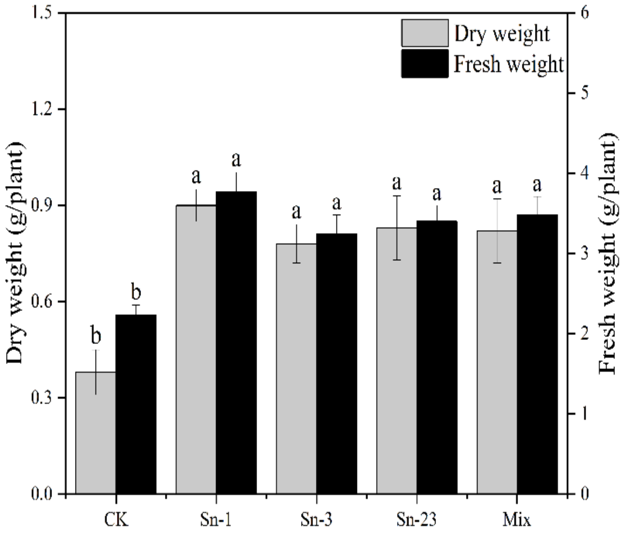

Fig. 4. Effect of the application of *Streptomyces* isolates on growth characteristics of rice plants grown in saline soil.

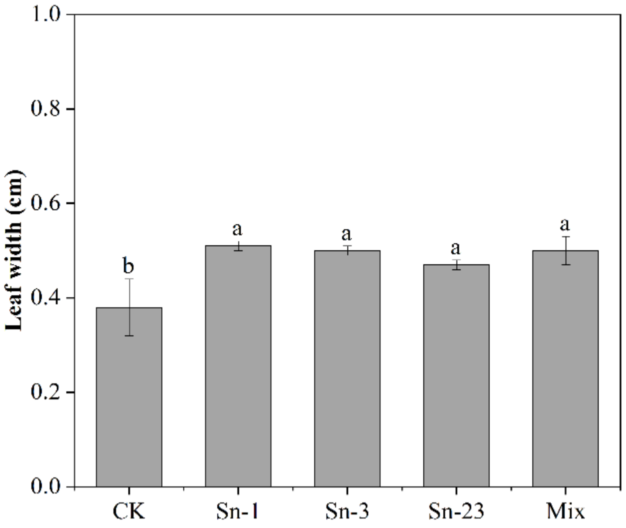

Fig. 5. Effect of the application of *Streptomyces* isolates on leaf width of rice plants grown in saline soil.

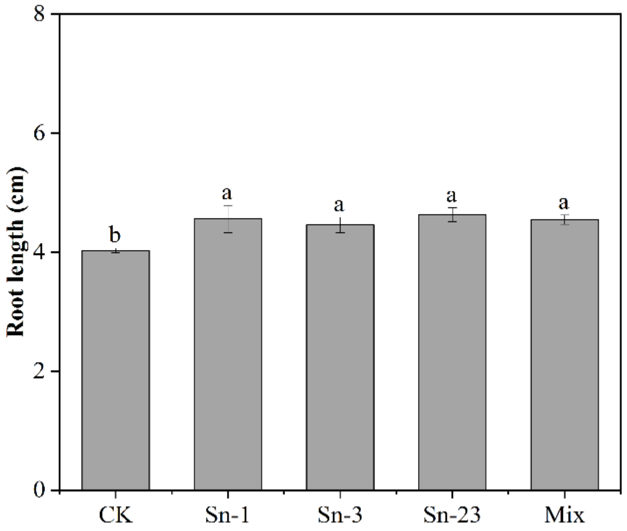

Fig. 6. Effect of the application of *Streptomyces* isolates on Root length of rice plants grown in saline soil.

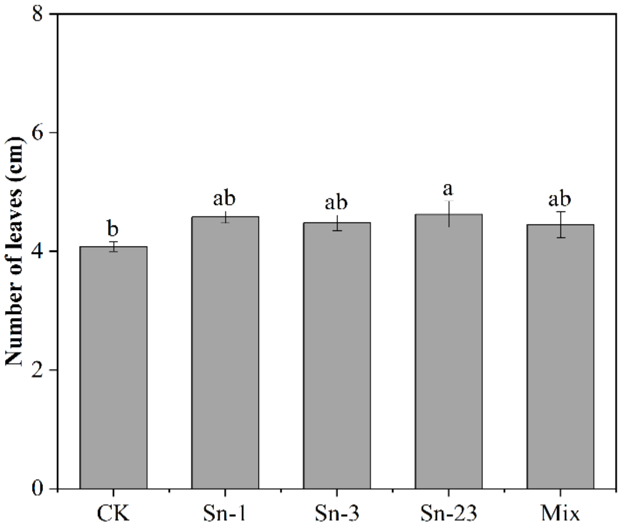

Fig. 7. Effect of the application *of Streptomyces* isolates on leaf number of rice plants grown in Saline soil.

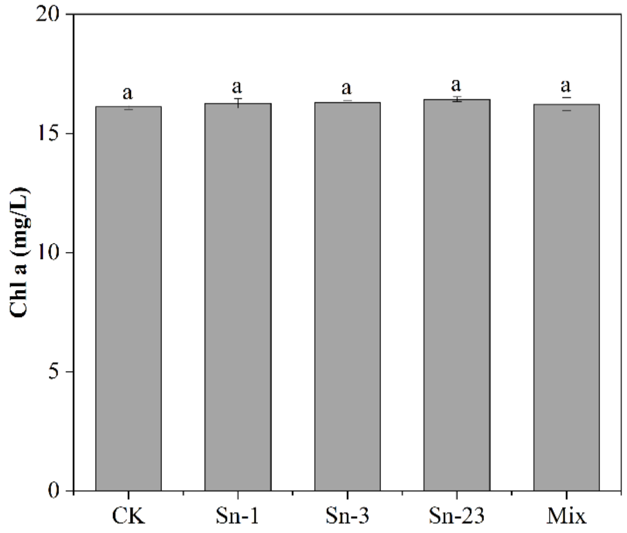

Fig. 8. Effect of the application *of Streptomyces* isolates on chlorophyll a of rice plants grown in saline soil.

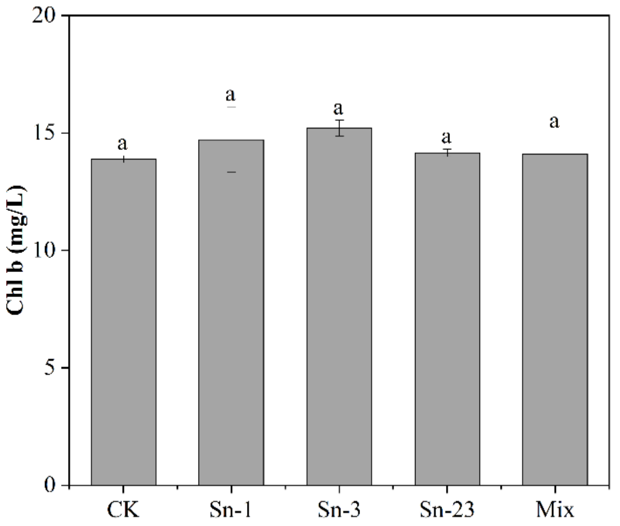

Fig. 9. Effect of the application of Streptomyces isolates on chlorophyll b of rice plants grown in saline soil.

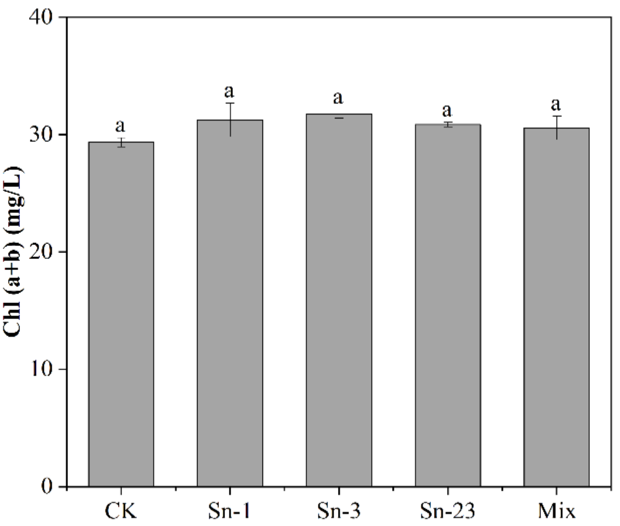

Fig. 10. Effect of the application *of Streptomyces* isolates on chlorophyll a & b of rice plants grown in saline soil.

Discussion

In the present study, cellulolytic bacteria from saline-sodic grassland soils were isolated and identified, and the capacity of some isolated cellulolytic *Streptomyces* strains to promote rice growth was evaluated under saline conditions. Our data revealed that *Streptomyces* was clearly the most common genus of cellulolytic microflora in the studied soil. *Streptomyces* are gram-positive bacteria that have a great capacity to survive in adverse environments, such as saline soils (Nozari *et al*., 2022). As one of the dominant microbial genus in different unfavorable growth conditions, *Streptomyces* isolates have developed multiple adaptive characteristics such as producing filamentous structures, formatting spores, secreting antibiotics, and hydrolyzing a wide range of polysaccharides (Lertcanawanichakul & Sahabuddeen, 2023; Alam *et al*., 2022; Viaene *et al*., 2016), and particularly the capacity to hydrolyze organic matter into simpler forms, improving nutrient cycling in the soil (Grzyb *et al*., 2020; Chater *et al*., 2010). In saline-sodic soils, these physiologically adaptive characteristics of cellulolytic *Streptomyces* strains clearly conferred on them a competitive advantage over other soil microorganisms.

Our data showed that the selected isolates could not only decompose cellulose with the aid of CMCase and xylanase activities, but also fix nitrogen, with nitrogen-fixing activities ranging from 18.60 to 24.81 nmol C_2H_4 ml^{-1} h^{-1} (Table 2), indicating that the three cellulolytic *Streptomyces* strains were nitrogen-fixing cellulolytic isolates. Up to now, the only cellulolytic nitrogen-fixing bacteria reported have been one aerobic cellulolytic species isolated from a gland of marine shipworms and four anaerobic cellulolytic nitrogen-fixing bacterial species from freshwater mud and soil (Waterbury *et al*., 1983; Leschine *et al*., 1988). There have been no reports of cellulolytic nitrogen-fixing *Streptomyces* isolates. Our results suggested that nitrogen fixation by these cellulolytic *Streptomyces* strains may enable them to meet their own nitrogen requirements when growing on a nitrogen-poor diet in such an extreme environment as saline-sodic soils. These nitrogen-fixing *Streptomyces* strains may play important roles in maintaining the nutrient cycles of saline soils.

In addition, the *Streptomyces* strains *Streptomyces atrovirens*, *Streptomyces lydicus*, *Streptomyces rochei*, and *Setaria viridis* also showed crucial plant growth-promoting traits, namely IAA production, siderophore production, and phosphate solubilization (Table 2). Production of IAA has been implicated in virtually all aspects of plant growth and development (Al-Tammar *et al*., 2023; William *et al*., 2006). It has been reported that some *Streptomyces* strains synthesize and export phytohormones. Orozco-Mosqueda *et al*., (2023) reported IAA production by *Streptomyces* isolates in the range 11–144 µg ml^{-1} when the production medium was supplemented with the IAA precursor, 2 mg ml^{-1} tryptophan. Another report indicated that inoculation with *Streptomyces* sp. En-1, an IAA-producing strain, significantly increased the total plant biomass of *Arabidopsis* (Worsley *et al*., 2020). Several studies have revealed that inoculation of wheat and rice with *Streptomyces* strains that produce IAA stimulates plant growth under saline conditions (Sadeghi *et al*., 2012; Nautiyal *et al*., 2013). In the current study, all three *Streptomyces* strains showed the ability to produce IAA in the presence of the precursor tryptophan under saline conditions. In particular, the amount of auxin produced by strain Sn-23 increased by 439% in the presence of salt, reaching up to 8.09 µg ml^{-1} in 150 mM NaCl (Fig. 3). This result agreed with those of Sadeghi *et al*., (2012), who reported that NaCl had a positive effect on the level of IAA synthesis by *Streptomyces*, revealing their adaptive nature in saline-sodic soil conditions.

The ability of PGPR to solubilize precipitated phosphates and to enhance phosphate availability to crops represents a possible mechanism of plant growth promotion (de Andrade *et al*., 2023; Hamdali *et al*., (2008) demonstrated that a phosphate-solubilizing strain of *Streptomyces griseus* could promote the growth of wheat plants. Sadeghi *et al*., (2012) reported that *Streptomyces* strains solubilized inorganic phosphate and promoted wheat growth. In the present study, *Streptomyces* strain Sn-23 was able to solubilize phosphate, as visualized by the formation of clear zones on NBRIP agar plates. Siderophores are low-molecular-weight molecules that are secreted by many microorganisms under iron-limiting conditions (Ahmed & Holmström, 2014). There are several reports that show that siderophores are involved in both plant growth promotion and health protection by chelating iron. Singh *et al*., (2022) reported that a siderophore-producing *Streptomyces* strain increased the root and shoot lengths and biomass of rice and mung bean compared to the siderophore-deficient *Streptomyces* mutant. In this study, siderophore production by strain Sn-23 was detectable in CAS medium (Table 2), which may contribute to the increased plant growth following inoculation with Sn-23 under saline-sodic conditions. All the results showed that these cellulolytic *Streptomyces* strains isolated from saline-sodic grassland soils had multiple adaptive characteristics, including cellulose decomposition, nitrogen fixation, IAA production, phosphate solubilization, siderophore synthesis, and antibiotic secretion, and may play critical roles in sustaining nutrient recycling, improving plant growth, and supporting plant survival under saline-sodic conditions. The results also mean that these isolates may have a direct practical application in growth promotion through their PGP characteristics.

In this study, the plant growth parameters were measured 21 days after inoculation. It was clear that all three isolates tested could significantly promote rice growth in terms of increased plant height, leaf width, and fresh and dry weight in saline soil under pot experiments. Similar findings have been reported for different PGPB that showed positive effects with respect to plant growth promotion under salt stress. It was reported that inoculation with *Bacillus* species, such as *B. pumilus*, resulted in increased tolerance of rice plants to salt stress (Ali *et al*., 2022). The salt-tolerant *Bacillus amyloliquefaciens* strain SN13 also increases plant growth and salt tolerance in rice after inoculation onto rice plants under saline hydroponic or soil conditions (Nautiyal *et al*., 2013). A recent report showed that *Streptomyces* isolate C significantly enhanced the germination rate, germination percentage and uniformity, shoot length, and total plant dry weight of wheat compared to the control under saline soil conditions (Sadeghi *et al*., 2012). The results of current studies revealed that inoculation with cellulolytic *Streptomyces* strains had positive effects on plant parameters through combined inoculation or single inoculation of any strains under salt stress.

Conclusion

According to the findings of recent investigations, inoculation with cellulolytic Streptomyces strains under salt stress had a good impact on plant parameters by either combined inoculation or single inoculation of any strain. The combined inoculation consistently enhanced plant height, leaf width, and fresh and dry weight of rice to a level equal to that achieved by single inoculation, and far greater than that of the uninoculated control plants. Although the impact of root inoculation with *Streptomyces* on some plant growth parameters is being explored, this is the first report that cellulolytic *Streptomyces* strains from saline-sodic soil can be used to increase rice growth under saline-sodic conditions. Our results confirm the rice plant growth-promoting effects of cellulolytic *Streptomyces* strains isolated from natural saline-sodic soil under salinity stress. This appears to be an effective strategy for selecting promising strains for use in agriculture, especially on saline soils.

Acknowledgments

We thank International Science Editing (http://www.internationalscienceediting.com) for editing this manuscript.

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(Received for publication 16 November 2022)