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A Salt-Tolerant *Streptomyces paradoxus* D2-8 from Rhizosphere Soil of *Phragmites communis* Augments Soybean Tolerance to Soda Saline-Alkali Stress

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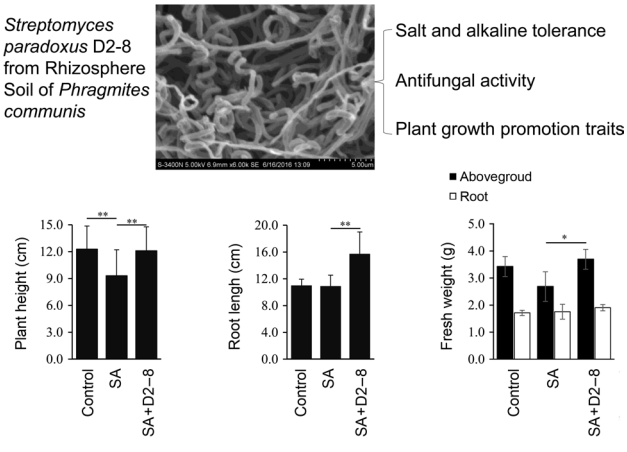
Abstract

Soil salinity and alkalization limit plant growth and agricultural productivity worldwide. The application of salt-tolerant plant growth-promoting rhizobacteria (PGPR) effectively improved plant tolerance to saline-alkali stress. To obtain the beneficial actinomycetes resources with salt tolerance, thirteen isolates were isolated from rhizosphere saline and alkaline soil of *Phragmites communis*. Among these isolates, D2-8 was moderately halophilic to NaCl and showed 120 mmol soda saline-alkali solution tolerance. Moreover, the plant growth-promoting test demonstrated that D2-8 produced siderophore, IAA, 1-aminocyclopropane-1-carboxylate deaminase (ACCD), and organic acids. D2-8 showed 99.4% homology with the type strain *Streptomyces paradoxus* NBRC 14887^T and shared the same branch, and, therefore, it was designated *S. paradoxus* D2-8. Its genome was sequenced to gain insight into the mechanism of growth-promoting and saline-alkali tolerance of D2-8. IAA and siderophore biosynthesis pathway, genes encoding ACC deaminase, together with six antibiotics biosynthesis gene clusters with antifungal or antibacterial activity, were identified. The compatible solute ectoine biosynthesis gene cluster, production, and uptake of choline and glycine betaine cluster in the D2-8 genome may contribute to the saline-alkali tolerance of the strain. Furthermore, D2-8 significantly promoted the seedling growth even under soda saline-alkali stress, and seed coating with D2-8 isolate increased by 5.88% of the soybean yield in the field. These results imply its significant potential to improve soybean soda saline-alkali tolerance and promote crop health in alkaline soil.



K e y w o r d s: Actinomycetes, *Phragmites communis*, rhizosphere, soda saline-alkali tolerance, plant growth promotion

Introduction

Salinization-alkalization of soil is a serious threat worldwide, and the total area of saline soils exceeds 8.3×10^8 hm^2 worldwide, including 53% alkaline soils and 47% saline soils. In China, it reaches 9.9×10^7 hm^2. The Songnen Plain, an important plantation base of soybean production, is one of China's five largest salt-affected soil regions and has 3.42×10^6 hm^2 salt soil (Huang et al. 2016). Salinity stress has detrimental effects on soybean growth and agronomy traits, such as nodulation, seed quality and quantity, and yield (Phang et al. 2008). Except for plant breeding and agricultural practices, applying a salt-tolerant bioagent may be an effective and sustainable means to enhance crop health and augment plant tolerance to salinity stress

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without environmental damage. To date, only several commercial bioagent products in the market have been applied in agriculture (Zeng et al. 2012; Panda et al. 2014). Moreover, the effect of the biological agent was influenced by variable conditions in the field (Chatterton and Punja 2010). Additionally, it is estimated that 50% of annual yield losses in major crops were caused by abiotic stress worldwide (Zörb et al. 2019). So, it is urgent to isolate more microorganisms with tolerance to various abiotic stress in the field, such as salt, alkaline, drought, etc., that could be applied as bioagents. Nowadays, some salt-tolerant plant growth-promoting rhizobacteria (PGPR) have shown great potential for alleviating salinity stress of many crops, such as *Bacillus*, *Rhizobium, Pseudomonas*, and others (Han et al. 2021). For soybean, *Bacillus*, *Pseudomonas*, and *Rhizobacteria* were reported to alleviate the harmful effects of salinity stress (Khan et al. 2019; Costa-Gutierrez et al. 2020; Abulfarajand Jalal 2021). To our best of knowledge, most PGPR were investigated under salt (NaCl) stress, and few actinobacteria were studied its potential to prime the alkaline or saline-alkali tolerance of soybean in soda alkaline soils. Alkaline or soda saline-alkali soils are composed primarily of $NaHCO_3/Na_2CO_3$ with excess Na^+, HCO_3^-/CO_3^{2-}, high-pH (>8.5), and poor soil structure imposes more damages on plant growth than saline soils. Hence, more microorganism resources with saline-alkali tolerance are essential to increase crop yields under soda saline-alkali stress.

Actinomycetes produce many bioactive compounds and have a great capacity to promote plant growth (Bhatti et al. 2017; Rehan et al. 2021). Nowadays, several commercial biocontrol agents are developed from actinobacteria, such as Actinovate based on *Streptomyces lydicus*, Mycostop based on *Streptomyces griseoviridis*, and Rhizovit based on *Streptomyces* sp. DSMZ 12424 (Lahdenperä et al. 1991; Berg et al. 2001; Minuto et al. 2006; Zeng et al. 2012). Actinobacteria can survive in various environments, such as saline or alkaline soils (Vasavada et al. 2006; Sadeghi et al. 2013). They are promising microorganisms to apply in abiotic stress environments for sustainable agriculture.

Phragmites communis (common reed), a tall perennial grass, is widely distributed in alkaline and saline soils of Songnen plain. The survival threshold of salt tolerance of the reed is 1%. It can grow normally in soil with a pH value of 6.5 ~ 9 and has strong salt and alkali resistance. Actinomycetes from rhizosphere soil of *Phragmites* were supposed to be salt and alkali tolerant and beneficial plant growth-promoting rhizobacteria. To test the above hypothesis and obtain soda saline-alkali tolerant PGPR to improve crop health in alkaline soils, the actinomyces were isolated from the rhizosphere soil of *Phragmites*, and antifungal activity of salt-tolerant strain D2-8 was screened. Soda saline-alkali tolerance was focused, and the survival ability of D2-8 in 0 ~ 120 mM mixed saline-alkali solution (NaCl, Na_2CO_3, $NaHCO_3$, and Na_2SO_4) was confirmed. Meanwhile, plant growth promotion characteristic of the strain was measured. Then, the polyphasic taxonomy analysis was carried out, and the genome was sequenced to reveal the antifungal, plant growth promotion, and saline-alkali tolerant mechanisms. Furthermore, pot and field experiments evaluated its soda saline-alkali tolerance improvement and growth promotion. This study provided the multifarious PGPR *Streptomyces* strain with soda saline-alkali tolerance, which has great potential to improve soybean productivity, and it is also an important source of new secondary metabolite substances.

Experimental

Materials and Methods

Isolation of actinomycete strains from rhizosphere soil of *P. communis*. Rhizosphere soil was collected from the rhizosphere of *P. communis* grown in the saline and alkaline land (34°290' N, 113°20' E), Daqing, China. The soil sample (pH 9.9 and salinity 20.89 g/kg) was air-dried at room temperature for 14 days, then ground into powder. The sample was suspended in sterile water and incubated at 28°C and 200 rpm on a rotary shaker for 30 min. A standard serial dilution technique was used to isolate actinomyces on the Gause No. 1 culture medium. Cycloheximide (50 mg/l) and nalidixic acid (20 mg/l) were added to the medium to inhibit the growth of bacteria and fungi. After seven days of aerobic incubation at 28°C, the colonies were picked out, continually purified, and finally maintained as glycerol suspensions (20%, v/v) at –80°C refrigerator.

Screening for antagonistic actinomycetes. The phytopathogenic strains and fungi used in this study were stored by the Heilongjiang Bayi Agricultural University (Daqing, China). Those fungi included *Gibberella zeae*, *Fusarium avenaceum*, and *Fusarium solani*. Antagonistic activity of isolates was evaluated with the culture plate assay. The isolates were firstly point-inoculated at the center of potato dextrose agar (PDA) plates. After three days of culture, four fresh mycelial PDA agars plugs of the fungus were put in the corresponding plate's margin and incubated for an additional seven days at 28°C. The inhibition activity of isolates against *Phytophthora sojae* was measured on CA plates following the same method. Each experiment was repeated three times. The inhibition rates were calculated according to the formula:

$$\text{inhibition rate (\%)} = Wi/W \times 100\%$$

Wi is the width of inhibition, and W is the width between the pathogen and actinomycetes.

NaCl and soda saline-alkali tolerance. NaCl tolerance (0–10% in 1% intervals, 12%, and 15%, w/v) of strain D2-8 was measured in Glucose-Yeast extract (GY) medium at 28°C for seven days on a rotary shaker. D2-8 was also tested for their tolerance to various concentrations (0, 10, 20, 40, 80, 120 160, and 200 mmol/l) of soda saline-alkali supplemented in GY medium. Soda saline-alkali solution contained NaCl, Na_2CO_3, $NaHCO_3$, and Na_2SO_4 (molar ratio 1:1:9:9). The strains were cultured at 28°C on a rotary shaker. After seven days, the cultures were visualized for the growth of the strains.

Morphological, physiological, and biochemical characteristics of strain D2-8. Gram staining was carried out by the standard Gram stain. After growing on ISP 3 agar at 28°C for four weeks, morphological characteristics of D2-8 were observed using scanning electron microscopy (Hitachi SU8010, Hitachi Co., Tokyo, Japan) according to Jin et al. (2019). The growth at different temperatures (10, 15, 20, 25, 28, 32, 35, 40, 45, and 50°C) was examined on ISP 3 medium after incubation for 14 days. The growth in the pH range (4.0 ~ 12.0 at intervals of 1.0 pH unit) was tested in GY (Glucose-Yeast extract) medium at 28°C for 14 days on a rotary shaker. Cultural characteristics were observed on the ISP 1 agar, ISP media 2 ~ 7, Czapek's, Bennett's, and Nutrient agar after14 days at 28°C. The utilization of sole carbon and nitrogen sources, decomposition of cellulose, hydrolysis of starch and aesculin, reduction of nitrate, coagulation and peptonization of milk, liquefaction of gelatin, and production of H_2S were measured.

Phylogenetic analysis of strain D2-8. For DNA extraction, strain D2-8 was cultured in GY medium for seven days. The biomass was harvested using centrifugation and stored at –80°C until use. Genomic DNA was extracted by the method of sodium dodecyl sulfate (SDS)-based DNA extraction. The 16S rRNA gene sequence was amplified using the universal bacterial primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACT-3'). The PCR product was sequenced by the HuaDa company (Beijing, China). The 16S rRNA gene sequence of strain was compared with type strains available at the EzBioCloud server (https://www.ezbiocloud.net/). Phylogenetic trees were constructed by Molecular Evolutionary Genetics Analysis (MEGA) software version 7.0. Neighbor-joining and maximum likelihood algorithms were used.

Evaluation of plant growth-promoting traits of strain D2-8. The strain D2-8 was inoculated into Gause No. 1 liquid medium supplemented with tryptophan (500 μg/ml) at 28°C, 180 rpm in the dark for seven days. Indole-3-acetic acid (IAA) content in the supernatant was measured according to the Salkowski colorimetric method (Gang et al. 2019). A standard curve of IAA solution (5, 15, 20, 30, 40, 50, and 60 μg/ml) was prepared to determine the IAA concentration of strain D2-8. To quantify siderophore production, D2-8 was placed on CAS (Chrome-Azurol S) plates and incubated at 28°C for seven days. The diameter of the orange loop around D2-8 was then measured. In 1-aminocyclopropane-1-carboxylic acid (ACC) degradation assays, D2-8 was streaked onto Dworkin and Foster (DF) minimal salts agar with 5 mmol/l ACC as a sole nitrogen source. Dworkin and Foster medium with 0.2% (wt/vol) $(NH_4)_2SO_4$ or no nitrogen source was inoculated as positive and negative controls. The plates were incubated at 28°C for 10 days before imaging. The growth of the strain was compared to that of the no-nitrogen medium. The supernatant (10 ml) of strain D2-8 was mixed with methyl red reagent (3–5 drops) to test organic acid production. The resulting color was recorded.

Draft genome sequencing and bioinformatic analysis of strain D2-8. For DNA extraction, strain D2-8 was cultured in tryptone soya broth (TSB). DNA library preparation and sequencing by Illumina MiSeq 2000 (Paired-end 2 × 250 bp) were performed at the Personalbio Genomics (Shanghai, China). The raw reads were adapter clipped, quality trimmed, and error corrected as standard procedure. All good-quality sequences were assembled into several scaffolds. The GeneMarkS was used to predict genes in the assembled genome. Genes were further annotated by BLAST in the Swiss-Prot, GO, eggNOG, and KEGG databases. The contig sequence was submitted to antibiotics and secondary metabolite analysis shell AntiSMASH, and the default options were selected (Weber et al. 2015). Genes associated with plant growth promotion and stress tolerance, such as IAA biosynthesis genes, ACC deaminase, ectoine biosynthesis gene cluster, production and uptake of choline and glycine betaine cluster genes, were analyzed.

Assessment of plant growth promotion of the strain in pot and field experiments. The pot experiment was carried out to measure the seedling growth promotion of strain D2-8 under normal condition. D2-8 spores were washed with ddH_2O from Gause No. 1 medium and diluted to the final concentration of 10^8, 10^7, and 10^6 CFU/ml. Seeds were soaked with the spores for 12 h, and seeds of control treatment were soaked with ddH_2O. Soybeans were planted in 10 cm pods. The soil: sand: vermiculite (1:1:1, pH 7.8) mixed substrate, which contained 54.19 mg/kg available nitrogen, 9.63 mg/kg available phosphorus, 128.45 mg/kg available potassium, 8.8 mg/g organic matter, was sterilized and used. The plants were cultured in the greenhouse with 16 h/8 h light and darkness for 14 d. The plant height, fresh weight of aboveground and root, dry weight of aboveground, and root were measured. Each treatment with five plants was repeated three times.

The effect of strain D2-8 on seedling growth under soda saline-alkali stress was further studied. Soybean

seeds were soaked with 10^7 CFU/ml of D2-8 spores for 12 h, and seeds of control treatment were soaked with sterile water. Soybeans were planted in 10 cm pods. The same soil composed of sand: vermiculite (1:1:1) mixed substrate was sterilized and watered with 80 mM saline-alkali solution containing NaCl, Na_2CO_3, $NaHCO_3$, and Na_2SO_4 (molar ratio 1:1:9:9) at first and 8th days, and sterile water was used in other days. The plants were cultured in greenhouse with 16 h/8 h light and darkness for 14 d. The plant height, root length, fresh weight of aboveground, and root were measured after 14 d. There were three treatments, including water soaking under normal condition (Control group), water soaking under saline-alkali stress condition (SA group), D2-8 soaking under saline-alkali stress condition (SA + D2-8 group), and each treatment was repeated five times.

The field experiment was carried out to measure the growth promotion of strain D2-8. Seeds were coated with 10^7 CFU/ml of D2-8 spores, and seeds of control treatment were coated without the strain. Soybeans were planted in the field on May 15th, 2020. The field soil contained 256.56 mg/kg available nitrogen, 65.49 mg/kg available phosphorus, 277.75 mg/kg available potassium, 52.9 mg/g organic matter. The soil pH was 7.1. There are four replicates in the field plot, and each plot has six lines with five meters. Soybeans were managed as routine. Soybeans were harvested on October 25th, 2020. The plant height, number of effective sections, pod number per plant, grain number per plant, 100 grains weight, the yield and theoretical yield were investigated.

Data analysis. Data analysis was performed using SPSS Statistics 22 software. All experiments were analyzed using unpaired *t*-tests (n = 15 plants).

Results

Isolation of salt and saline-alkali tolerant actinomyces strain D2-8 with antagonistic activity. To obtain the biocontrol actinomyces resources with soda saline-alkali tolerance, we isolated 13 strains from the rhizosphere soil of *P. communis* using the dilution plate method. The antagonistic activity of the 13 strains (D2-1 ~ D2-13) against *P. sojae* displayed significant variation (0 ~ 28 ± 0.77 mm). Specifically, strain D2-8 significantly inhibited the mycelia growth of *P. sojae* by 28 ± 0.77 mm (62%) compared to the control and other strains. In addition, it also had the antagonistic activity against *Fusarium* and *Gibberella* (Table SI). So, strain D2-8 was further identified and studied in detail. We measured the resistance of D2-8 to abiotic stresses. It could grow in the presence of 0 ~ 10% NaCl (w/v) with an optimal level of 4% (w/v) (Fig. 1a). Furthermore, it could grow well in 10 ~ 80 mmol soda saline-alkali solution (NaCl, Na_2CO_3, $NaHCO_3$, and Na_2SO_4, molar ratio 1:1:9:9) except for the weak growth in 120 mmol saline-alkali solutions (Fig. 1b).

Identification of strain D2-8. The morphological characteristics of strain D2-8 showed the typical characteristics of the genus *Streptomyces*. Strain D2-8 exhibited a white aerial spore mass and a grey substrate mycelium and formed chains of smooth spore. The spore (0.6 ~ 0.8 × 0.9 ~ 1.1 μm) was rhabditiform (Fig. 2a and 2b). The strain grew well and developed more abundant aerial mycelia on ISP 3 (oatmeal agar) and Czapek's agar (Table SII). The growth of strain D2-8 was observed at 15 ~ 40°C (optimum temperature, 28°C), and pH 5 ~ 9 (optimum pH 7). Detailed physiological characteristics are presented in Table I. Strain D2-8 could liquefy gelatin and produce H_2S and protease.

The 16S rRNA gene sequence (1,487 bp) of strain D2-8 was closely related to members of the genus *Streptomyces*. The strain shared a high 16S rRNA gene sequence similarity of 99.4% to *Streptomyces paradoxus* NBRC 14887T and *Streptomyces hawaiiensis* NBRC 12784T. The neighbor-joining tree showed that strain D2-8 formed a distinct phyletic line and occupied a subclade with *S. paradoxus* NBRC 14887T (Fig. 3). Based on morphological, physiological, and phylogenetic analysis, strain D2-8 was considered a species of the genus

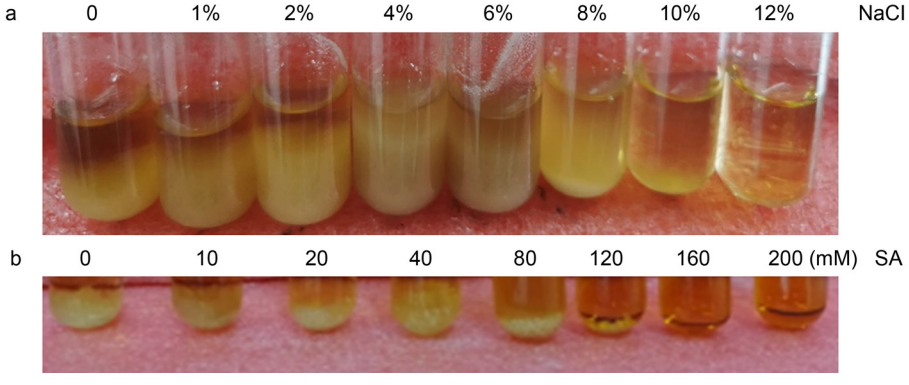

Fig. 1. a) Stress tolerance of strain D2-8 in different concentration of salt and b) saline-alkali solutions.
SA represents saline and alkaline solution, which contains NaCl, Na_2CO_3, $NaHCO_3$, and Na_2SO_4 (molar ratio 1:1:9:9).

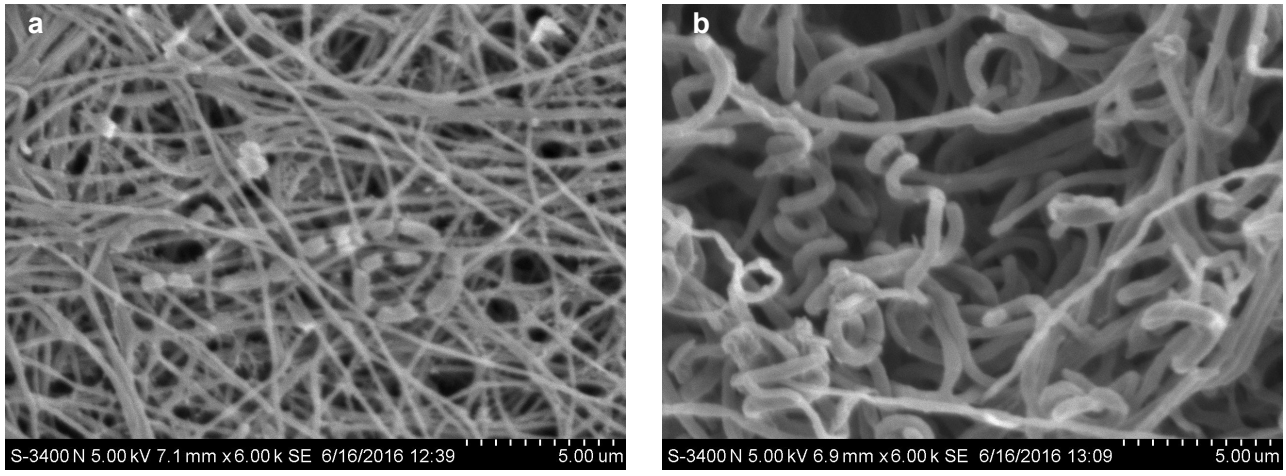

Fig. 2. Morphological characteristics of strain D2-8 observed by scanning electron microscopy.

Streptomyces. The name of *Streptomyces paradoxus* D2-8 was proposed. It was deposited in the China Center for Type Culture Collection (Wuhan) CCTCC M2019949.

Multiple plant growth promotion properties of strain D2-8. Stain D2-8 showed prominent properties for plant growth promotion. On the brilliant blue CAS plate, D2-8 could form an orange loop, which indicated the production of siderophore (Table II). It could produce major phytohormone indole acetic acid (IAA) in Gause No1 liquid medium with tryptophan (Table II), and the yield was 29.1 μg/ml. When testing for changes due to organic acids, the supernatant turned to yellow upon adding methyl red, which suggested D2-8 could produce organic acid (Table II). The ability of D2-8 to produce ACC deaminase was proved by the fact that it grew on the minimal salt medium supplemented with ACC (Table II). These results indicated that D2-8 produced IAA, organic acids, ACC deaminase, and siderophore, which likely contributed to the plant growth promotion by D2-8.

Table I
Physiological characteristics of strain D2-8.

Characteristic	D2-8	Characteristic	D2-8	Characteristic	D2-8
Carbon source utilization		Nitrogen source utilization		Melanin formation on peptone-iron agar	+
Lucose	+	L-threonine	+	Cellulose decomposition	–
Arabinose	+	L-tyrosine	+	Enzymatic activity	
Sucrose	–	L-asparagine	+	Protease	+
Xylose	+	Alanine	+	Liquefaction of gelatin	+
Inositol	+	Glycine	+	Production of H_2S	+
Mannitol	+	L-glutaminealanine	+		
Fructose	–	L-arginine	–		
Rhamnose	–	L-proline	+		
Raffinose	+				
Lactose	+				
D-ribose	+				
D-galactose	+				

+ – positive,
– – negative

Table II
Plant growth promotion properties of strain D2-8.

Characteristic of D2-8	Value or properties
IAA production	29.1 μg/ml
siderophore production	+
organic acids production	+
1-aminocyclopropane-1-carboxylate deaminase (ACCD) production	+

Identification of genes associated with plant growth promotion in the D2-8 genome. To reveal the molecular mechanism of antagonism and plant growth promotion, the D2-8 genome was sequenced. The assembled genome sequence of strain D2-8 was

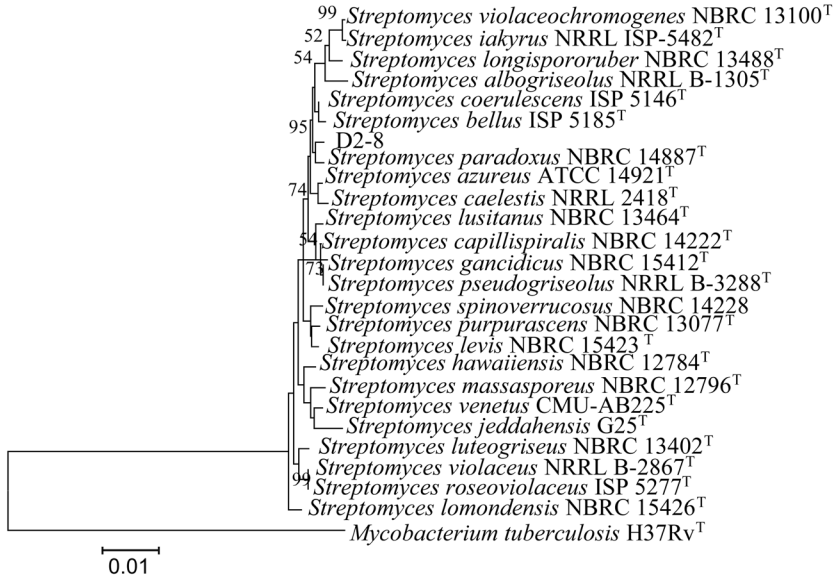

Fig. 3 Neighbor-joining tree of strain D2-8 and the related species of the genus *Streptomyces* based on the 16S rRNA gene sequences. The out-group used was *Mycobacterium tuberculosis* H37Rv^T. The stability of the topology of the phylogenetic tree was assessed by the bootstrap method with 1000 repetitions. Only bootstrap values above 50% (percentages of 1000 replications) are indicated. Scale bar represents 0.01 nucleotide substitutions per site.

8,732,707 bp long and composed of 72 contigs with N50 of 280,139 bp. The G+C content of the D2-8 genome was 71.15 mol %, and its coverage was 336×. The gene prediction resulted in 7,703 gene models. A total of 4,818 proteins were predicted (Table SIII). Annotation obtained via RAST assigned 18% sequences (1,504/8,377) to 24 subsystem categories and 82% sequences, not in subsystem. The high-ranking numbers in the subsystem categories were amino acids and derivatives, carbohydrates, protein metabolism, fatty acids, lipids, isoprenoids, respiration, nucleosides, and nucleotides (Fig. 4a). These subsystems belonged to the basic biological processes. Besides, the stress response and metabolism of aromatic compounds were relatively prominent.

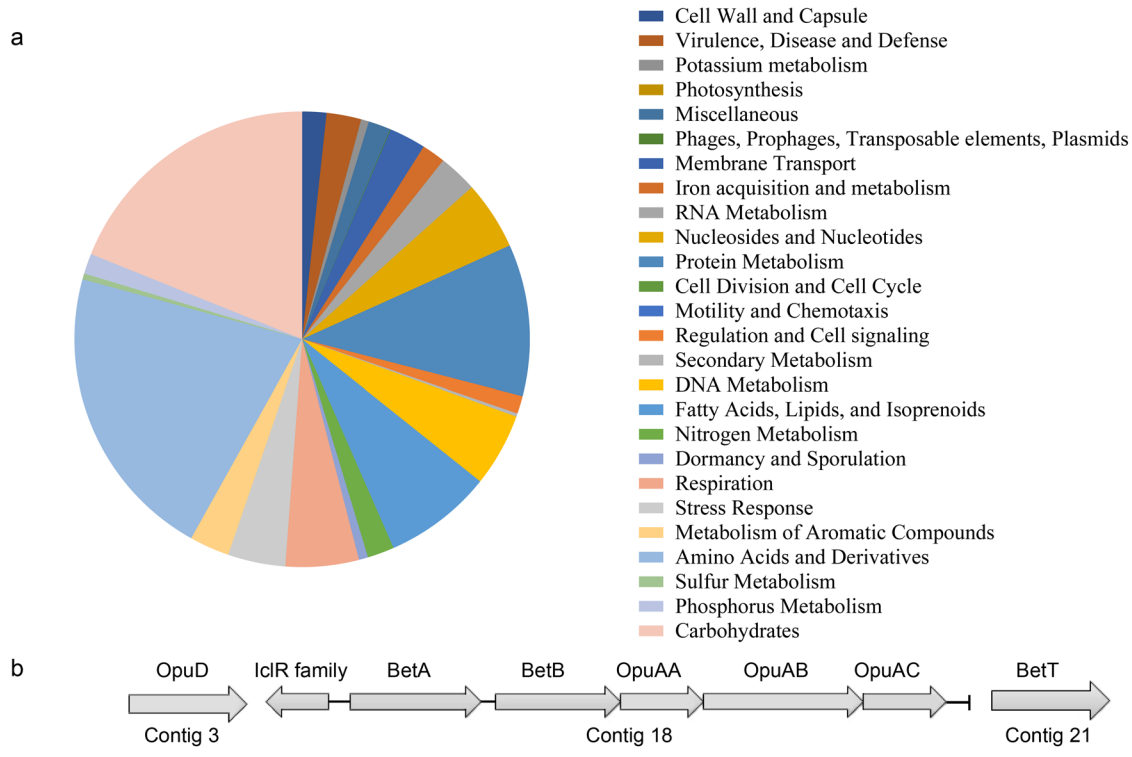

Fig. 4. a) The distribution of subsystems of the D2-8 genome annotated through the RAST webserver and b) stress response genes of D2-8 involved in the production and uptake of choline and glycine betaine.

According to the functional annotation, various genes related to plant growth-promoting traits were found in the genome. Strain D2-8 can produce IAA with a maximal yield of 29.1 μg/ml. Several genes related to IAA biosynthesis were found in the genome of D2-8, such as the genes encoding for indole-3-glycerol phosphate synthase (contig1_962), phosphoribosyl anthranilate isomerase (contig1_953), and anthranilate phosphoribosyl transferase (contig1_852). Tryptophan 2-monooxygenase (contig19_5733) and amidase (contig20_5854, contig3_1725, contig3_1776, contig13_4655, contig17_5213) in IAA biosynthesis IAM pathway were found in the genome. Aldehyde dehydrogenase (NAD^+) (contig7_3087, contig11_4153) in the IAA biosynthesis TAM pathway was also identified in the genome.

Additionally, genes of ammonia assimilation via both the GDH pathway using glutamate dehydrogenase (contig3_1507) and glutamine synthetase (GS)-glutamate synthase (GOGAT) pathway using glutamine synthetase (contig1_757, contig1_760, contig1_782, contig1_793, contig3_1528, contig3_1878) and glutamate synthase (contig1_209, contig1_975, contig1_976, contig1_1058) were identified. Furthermore, two genes encoding for 1-aminocyclopropane-1-carboxylate (ACC) deaminase (contig2_1144, contig8_3396) were found. These results were consistent with the potential ability of D2-8 to produce ACC deaminase.

Identification of genes responsible for the antifungal property in the D2-8 genome. Strain D2-8 had the potential to inhibit several plant fungi; therefore, the antiSMASH program and annotated genes sorting in the subsystem categories of RAST were performed to reveal the biologically active natural products. The antiSMASH analysis predicted that strain D2-8 contains 23 individual clusters which share homology to known gene clusters whose metabolic products are known and seven clusters that show no similarities to known clusters (Table III). These different secondary metabolite gene clusters included four siderophores, two melanins,

Table III
The secondary metabolism substances prediction results of antiSMASH.

Region	Type	From	To	Most similar known cluster	Similarity	
Region 1.1	siderophore	27,766	39,535	desferrioxamin B / desferrioxamine E	other	83%
Region 1.2	melanin, lanthipeptide	125,173	149,340	melanin	other	80%
Region 23.1	melanin	99,231	109,596	melanin	other	57%
Region 1.3	terpene, NRPS	542,592	599,827	SCO-2138	RiPP	85%
Region 8.1	terpene	65,776	90,277	isorenieratene	terpene	100%
Region 8.2	terpene	227,330	248,343	albaflavenone	terpene	100%
Region 3.1	terpene	232,223	258,904	hopene	terpene	92%
Region 9.2	terpene	221,324	243,489	geosmin	terpene	100%
Region 17.2	terpene	157,127	178,023	CC-1065	other	24%
Region 28.1	terpene	3,613	24,626	A23187 calcimycin	polyketide	10%
Region 31.1	terpene	11,837	32,925	rustmicin	polyketide: iterative type I	10%
Region 2.1	ectoine	76,359	86,757	ectoine	other	100%
Region 3.2	NRPS	364,532	415,460	coelichelin	NRP	100%
Region 36.1	NRPS	1	35,822	(2S,6R)-diamino-(5R,7)-dihydroxy-heptanoic acid	NRP	24%
Region 3.4	bacteriocin, lanthipeptide	786,397	813,467	informatipeptin	RiPP: lanthipeptide	100%
Region 5.1	PKS-like, butyrolactone	18,668	71,723	ulleungmycin	NRP	8%
Region 7.2	LAP, thiopeptide	331,828	365,008	diazepinomicin	terpene	7%
Region 3.5	T1PKS, T3PKS	874,824	939,794	alkylresorcinol	polyketide	100%
Region 9.4	T1PKS	400,230	434,396	mediomiycin A	polyketide	36%
Region 12.1	T1PKS	1	24,057	argimycin PI / argimycin PII / nigrifactin / argimycin PIV / argimycin PV / argimycin PVI / argimycin PIX	polyketide: modular type I	21%
Region 17.1	T2PKS	79,071	151,586	spore pigment	polyketide	83%
Region 39.1	T2PKS	1	25,935	collinomycin	polyketide	72%
Region 33.1	T3PKS	8,168	49,481	germicidin	other	100%

eight terpenes, one NRPS, two LAP, one ectoine, three bacteriocins, four T1PKS, two T2PKS, one T3PKS, one NRPS-like, and one PKS-like. Six clusters encoded the metabolites with antibacterial or antifungal activity, including collinomycin (72%) with antibacterial (enterococci), and no antifungal or antiviral activity, mediomiycin A (36%) with antifungal activity, calcimycin (10%) with inhibition of Gram-positive bacteria and some fungi, rustmicin (10%) with inhibition of elongation of germ tube of *Puccinia graminis*, ulleungmycin (8%) with antibacterial activity against *Staphylococcus aureus*, diazepinomicin (7%) with antibacterial, anticancer, and anti-inflammatory activity. Most such clusters displayed a low level of similarity to the known clusters. For antifungal metabolism, cluster 25 (Region 9.4) showed 36% similarity to the biosynthetic cluster of mediomiycin A in *Kitasatospora mediocidica* (Table III). It is a PKS gene cluster containing three PKS (contig 9_3944, contig 9_3945, contig 9_3946).

Cluster 15 (Region 3.2) and cluster 1 (Region 1) were predicted to produce siderophores (Table III). Cluster 15 showed 100% similarity to the biosynthetic cluster of coelichelin in *Streptomyces coelicolor* A3(2). Cluster 1 showed 83% similarity to the biosynthetic cluster of desferrioxamin B/desferrioxamine E in *S. coelicolor* A3(2). These genes further verified the ability of producing siderophores by strain D2-8.

Identification of genes responsible for salt stress adaptation of strain D2-8. For salt tolerance, several genes were found related to osmotic stress. Genes involved in production and uptake of choline and glycine betaine were found in D2-8, such as high-affinity choline uptake protein *BetT*, glycine betaine transporter *OpuD*, glycine betaine ABC transport system *OpuAA*, *OpuAB*, *OpuAC*, transcription regulator *IclR* family, choline dehydrogenase (*BetA*), and betaine aldehyde dehydrogenase (*BetB*) (Fig. 4b). These compounds could maintain membrane fluidity and enhance tolerance to salt (Guillot et al. 2000). D2-8 also contained an outer membrane protein A precursor gene. These genes were specific to D2-8, and absent from the genome of *Streptomyces avermitilis* MA-4680, *S. coelicolor* A3(2), and *Streptomyces griseus* subsp. *griseus* NBRC 13350.

Ectoine is a kind of compatible solute with protein-stabilizing properties. In addition, ectoine also stabilizes a higher-order nucleoprotein complex at the regulatory region of bacterial rRNA promoters (Pul et al. 2007). A group of microorganisms can synthesize the compatible solutes upon exposure to high salinity, including some *Streptomyces* species (Killham and Firestone 1984). In the strain D2-8 genome, there was an ectoine encoding gene cluster. Its ectoine biosynthetic gene cluster was highly like that of *Streptomyces* genus (Fig. S1). The compatible solutes ectoine and glycine betaine could influence (either as destabilizers or stabilizers) the melting temperature of the DNA helix (Rajendrakumar et al. 1997). These two substances both contributed to the salt tolerance of strain D2-8.

Plant growth-promoting activity of strain D2-8. D2-8 showed the potential to promote plant growth in the pot experiment, and the growth stage in the 10^7 CFU/ml D2-8-treatment was earlier than that of control. The height of soybean seedlings increased by 17.6% at 12 d in the 10^7 CFU-ml D2-8 treatment group compared to the control group (Fig. 5a). The treatment of strain D2-8 also affected the structure of the root. More lateral roots and longer main roots were observed. The root length increased significantly (21.3%) when the soybean seeds were soaked with 10^7 CFU/ml D2-8 spore's suspension (Fig. 5b). Fresh weight of root and aboveground were significantly improved by 65.6% and 27.4% in 10^7 CFU/ml D2-8 treatment ($p < 0.05$), respectively (Fig. 5c). These results implied that the concentration of 10^7 CFU/ml of D2-8 spores had the best effect on plant growth promotion. So, 10^7 CFU/ml of D2-8 spores were used further to investigate the plant growth under saline-alkali conditions. Compared with saline-alkali treatment (SA group), the D2-8 treatment increased the seedling's height of soybean under saline-alkali stress conditions (Fig. 5d and 5e). Additionally, the root length and fresh weight were improved by D2-8 treatment under saline-alkali stress conditions (Fig. 5f). The root length and fresh weight of aboveground increased by 44.1% and 37.5%, respectively. These results indicated D2-8 had the potential of alleviating the saline-alkali stress to promote soybean growth.

In the field experiment, seeds coating of D2-8 also showed significant plant promotion. Soybean yield increased by 5.88% (Table IV). Above all, D2-8 has not only the potential to promote soybean growth and increase its production, but also this strain can augment the soybean tolerance to saline-alkali stress.

Table IV
Effect of *Streptomyces* D2-8 on the soybean production in the field experiment.

Treatment	Density (Ten thousand/ha)	Plant height (cm)	Number of effective sections	Pod number per plant	Grain number per plant	100 grains weight (g)	Yield (kg/ha)
D2-8	33.85 ± 0.76*	92.30 ± 4.02	12.40 ± 0.37	25.45 ± 1.75	55.95 ± 1.68	19.51 ± 0.49*	2463.85 ± 56.36**
Control	31.54 ± 1.28	93.95 ± 2.42	11.90 ± 0.35	24.45 ± 0.54	57.60 ± 2.76	18.73 ± 0.29	2327.85 ± 42.46

The symbols * and ** indicate *p* values 0.05 and 0.01.

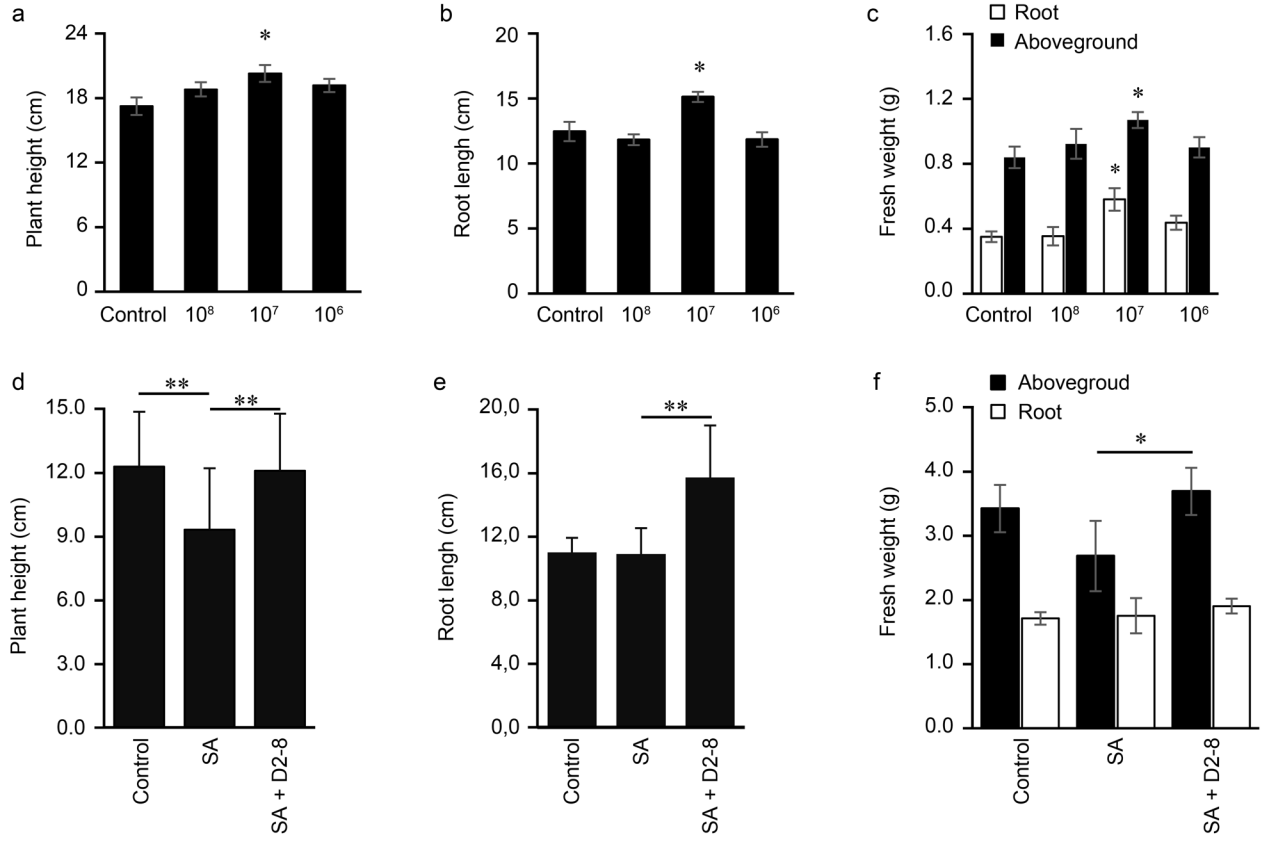

Fig. 5. The growth promotion effect of D2-8 under normal conditions (a, b, c) and saline-alkali stress (d, e, f) on soybean. For a), b), c), control group: water treatment, 10^8 D2-8: the D2-8 spore concentration of 10^8 CFU/ml; 10^7 D2-8: the D2-8 spore concentration of 10^7 CFU/ml; 10^6 D2-8: the D2-8 spore concentration of 10^6 CFU/ml. For d), e), f), control group: normal condition, SA group: saline-alkali stress treatment, SA+D2-8: D2-8 treatment under saline-alkali stress condition; Plants height, roots length, and fresh weight of roots and aboveground were analyzed using unpaired *t*-tests (n = 15 plants). The symbols * and ** indicate *p* values 0.05 and 0.01.

Discussion

A diverse range of microorganism is dispersed in the rhizosphere of plant, and rhizosphere microbial communities play a crucial role in ensuring the stability and productivity of the agricultural ecosystem, including nutrition, disease suppression, and resistance to both biotic and abiotic stresses (Newitt et al. 2019). Lots of plants growth-promoting rhizobacteria have been isolated, and some were used as microbial agents and biofertilizers in agricultural production. Many actinomyces, especially the genus *Streptomyces*, were isolated from the plant rhizosphere and were known to enhance plant growth and protect plant health (Liu et al. 2019). Three *Streptomyces* strains have been developed into commercial biocontrol agents called Actinovate, Rhizovit, and Mycostop. *P. australis* is one of the most extensively distributed plant species used for phytoremediation of different types of wastewater, soil, and sediments. As a halophyte, the rhizosphere of *P. australis* harbored some new and vital microbial groups, which are of great importance in the vegetation restoration and ecological reconstruction of salinized soil. Although the bacterial community of rhizosphere of *P. australis* was revealed and some rhizobacteria were isolated (He et al. 2019), few *Streptomyces* with alkaline tolerance from the rhizosphere of *P. australis* were isolated (Pereira et al. 2015). In this study, *Streptomyces* strain D2-8 was isolated from the rhizosphere of *Phragmites*, which grows in the saline and alkaline land. It exhibited a broad antifungal activity against the pathogen of soybean rot diseases, such as *P. sojae* and *Fusarium*. Moreover, strain D2-8 showed moderate saline and alkaline stress tolerance. It can grow in the 10% NaCl and 120 mM soda saline and alkaline solution. Some *Streptomyces* strains also showed salt tolerance, such as *Streptomyces sparsus* sp. nov. (0–15% NaCl, pH 6–10.0) (Jiang et al. 2011), and *Streptomyces clavuligerus* strain Mit-1 (up to15% NaCl, pH 11.0) (Thumar and Singh 2009). However, their tolerance to soda alkaline solution was not measured. Although D2-8 was not a halophilic bacterium, its tolerance to saline and alkaline and antifungal activity make it a promising microorganism to apply in alkaline land to protect crops against disease and improve plant production.

The beneficial microorganism can promote plant growth through nitrogen fixation, potassium solubilization, and phosphorus solubilization; meanwhile, it produces siderophores, IAA, and ACC deaminase to

enhance plant stress tolerance. In genus *Streptomyces*, two antagonistic *Streptomyces* WZS1-1 and WZS2-1 produced IAA, siderophores, solubilized phosphorus and potassium, harbored nitrogenase activity, and can promote wheat growth (Han et al. 2018). *Streptomyces* sp. NEAU-S7GS2 also produces IAA, siderophores, ACC deaminase, and inoculation of NEAU-S7GS2 showed the potential of growth promotion in soybean (Liu et al. 2019). In the current study, D2-8 was confirmed to produce IAA, siderophore, and organic acid. Its ability of growth promotion was further measured in soybean. D2-8 spores can promote the root length under normal conditions and the saline and alkaline condition in pod experiments. Moreover, seeds coated with D2-8 can increase soybean production in field experiments. The plant growth promotion of strain D2-8 under saline and alkaline condition would be valuable for agricultural production.

The genome analysis of D2-8 gave insight into its plant growth promotion mechanism. Multiple siderophores biosynthetic and uptake systems identified in this strain suggested functional duplication conferring an advantage for the bacterium as it colonizes different ecological niches. In this study, IAA biosynthesis genes were also found in D2-8, and IAA production of D2-8 consisted with the analysis results *in silico*. The plant intake of IAA released by *Streptomyces* was one of the reasons for soybean growth promotion. Streptomyces' saprotrophic and spore-forming lifestyle is beneficial to survive well under unfavorable conditions. The detailed mechanism of plant growth promotion of *Streptomyces* D2-8 under saline and alkaline stress needs further research.

Streptomyces spp. have a high performance in producing various bioactive secondary metabolism and enzymes (Zhang et al. 2017). The genomic analysis of D2-8 identified many PKS and NRPS gene clusters. Six clusters encoded the metabolites with antibacterial or antifungal activity, but the similarity of gene cluster was low, which indicate that the strain may produce some new metabolisms. Although three PKS enzymes were found, whether D2-8 produced the mediomycin or its similar antibiotics still need more experiments. Moreover, RAST analysis results revealed that production and uptake of choline and glycine betaine genes in the strain genome is uniquely present in D2-8 but absent from the related Streptomyces complete genome of *S. avermitilis* MA-4680, *S. coelicolor* A3(2), and *S. griseus* subsp. *griseus* NBRC 13350. We proposed that ectoine, choline, and glycine betaine are particularly important for adaptation to saline and alkaline stress. Altogether, the presented data demonstrate that strain D2-8 is a moderate saline and alkaline stress tolerance streptomyces with the potential of plant growth promotion and antifungal activity and streptomyces to find novel bioactive compounds.

Conclusion

Saline and alkaline tolerant actinomyces, designated as *S. paradoxus* D2-8, was isolated from rhizosphere soil of *Phragmites*, which grown in the saline and alkaline land. Except for the antifungal activity, the strain has significant saline-alkali tolerance and plant growth promotion. Moreover, genome analysis showed that IAA and siderophore biosynthesis pathway, genes encoding ACC deaminase, and secondary metabolite gene clusters were identified in the D2-8 genome. Soybean growth promotion experiments further confirmed that the strain improved plant tolerance to saline and alkaline stress and promoted host health. The yield increase of soybean after D2-8 seed's coating treatment in the field suggested that strain D2-8 could be a potential candidate for the development of bioagent with abiotic stress tolerance used in sustainable agriculture in the future.

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Availability of data and materials

All data generated or analyzed in this study are presented within this manuscript. All materials used in this study, including raw data, shall be available upon reasonable request. The 16S rRNA nucleotide sequences for strain D2-8 and its genome sequence were deposited in GenBank (NCBI) under the accession numbers MT027002 and PRJNA605321.

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Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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