



# Salt Tolerance Mechanism and Species Identification of the Plant Rhizosphere Bacterium JYZ-SD2

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## Abstract

A salt-tolerant microbe strain JYZ-SD2 was investigated to develop biological soil amendments to stimulate salix growth and acclimation in costal salt-affected soils. The salt tolerance mechanism of strain JYZ-SD2 was investigated by detecting the salt-tolerant growth characteristics, biofilm formation, ion distribution, secondary metabolites, and zymogram profiling. The strain was identified by physiological and biochemical characteristics (Biolog), 16S rDNA sequencing, and *cry1/7/9* gene expressing. With increasing of NaCl concentration, strain JYZ-SD2 adapted to the increased osmotic pressure by prolonging the retardation period, slowing down the growth rate of the logarithmic phase, increasing *spo0A* gene expression, increasing biofilm formation, reducing Na<sup>+</sup> uptake, and changing the expression of metabolites and intracellular soluble proteins. The results showed that strain JYZ-SD2 could be assigned to *Bacillus cereus*.

**Keywords** Plant rhizosphere bacterium · Salt tolerance · 16srDNA · Biofilm · *spo0A*

## Introduction

Salinity affects 33% of the world's potential arable land, with 950 million hectares of salt-affected land in arid and semi-arid regions [1]. Increasing salt reduces soil organic matter and causes soil degradation [2], resulting in physiological drought and the death of plants. Due to the shortage of land resources, developing and utilizing saline–alkali land is becoming a major research area. Investigating plant growth promoting rhizobacterium (PGPR) has become one of the hot topics in soil microbiology and microbial ecology. PGPR can colonize plant roots through biofilms, stimulate root growth, provide nutrients, and enhance plant tolerance to salt stress [3]. *Bacillus* sp. have a strong resistance to stress due to the generation of spores. Ahmad et al. [4] found

that PGPR can reduce the toxicity of saline soils to plants through biofilm creation. PGPR is also widely distributed in nature and can be isolated from many saline and alkaline soils, so they have broad application prospects.

At present, halophiles can be divided into non-halophiles (are able to grow in NaCl < 1.2%), mild halophiles (1.2–3%), moderate halophiles (3–15%), and extreme halophiles (> 15%) [5]. Extreme halophiles are known to tolerate salts in a "salt-soluble" manner by maintaining high concentrations of Na<sup>+</sup> or K<sup>+</sup> in the cytoplasm. Relative to the extremely halophilic bacteria, research of the salt tolerance mechanisms of moderate halophilic bacteria is lacking. To avoid dehydration and maintain their normal physiological functions in hypertonic environments, small molecules such as sugars, amino acids, betaine, and four hydrogen pyrimidine accumulate in high amounts in extremely halophilic microbial cells to provide osmotic regulation [6]. In the current research focused on moderate halophilic bacteria, Ivey et al. [7] found that *E.coli* with *NhaA* transporter showed high Na<sup>+</sup> tolerance. Na<sup>+</sup>/K<sup>+</sup> and Na<sup>+</sup>/H<sup>+</sup> reverse transporters are widely found in prokaryotes and eukaryotes, where they are not only involved in the output of Na<sup>+</sup> and the maintenance of intracellular homeostasis, but also participated in physiological activities such as cell size regulation, nutrient absorption, and cell movement. Na<sup>+</sup>/H<sup>+</sup> reverse transporter proteins are ubiquitous in bacteria, which can

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catalyze intracellular cation outflow to exchange for external protons, reduce cationic toxicity in cytoplasm, and maintain cell homeostasis [8]. As an important  $\text{Na}^+/\text{H}^+$  reverse transport protein in *Enterobacterium*, *NhaA* determines whether the strain can tolerate high sodium concentrations [9]. Few studies have investigated the salt tolerance mechanisms of gram-positive bacteria. Therefore, work on the adaptation mechanisms of moderate halophiles to salt stress is of great significance to the future development and utilization of saline–alkali soil.

*Bacillus* is a moderately salt-tolerant halophile. Compared to bacteria without biofilms, *Bacillus subtilis* and *Pseudomonas putida* have a stronger adsorption capacity for metal cations [10]. Most *Bacillus* can produce biofilms and form colonies, which can help plants retain more water molecules [11] to resist higher salt stress [12]. While PGPR helps plants resist stress, they also face changes in osmotic pressure. However, little is known about the formation of biofilms and their own salt tolerance mechanisms. Samples of the *Bacillus* sp. strain JYZ-SD2 that were isolated from rhizosphere of poplar trees were obtained in our lab. The poplar inoculation test demonstrated that these bacteria can improve the salt resistance and promote the growth of poplar trees, but the salt-tolerant characteristics of the bacteria are not clear. In this investigation, the microbial classification and salt tolerance of the JYZ-SD2 strain were studied.

## Materials and Methods

### The Tested Strain and Its Culture Medium

#### The Tested Strain, JYZ-SD2

The tested strain, JYZ-SD2, was isolated from the rhizosphere soil of a 2-year-old poplar grove at the Chenwei forest farm in Sihong County, Jiangsu, China. The strain was stored at the storage center of typical culture in China (CCTCC) with the number M2018468.

#### Medium Preparation

LB solid medium (peptone 10 g, yeast powder 5 g, NaCl 10 g, agar 15–20 g, pH 7.0) and LB liquid medium (peptone 10 g, yeast powder 5 g, NaCl 10 g, pH 7.0) were prepared.

#### Saline Medium

A NaCl concentration gradient for the above test medium was set as 0, 1% (0.17 mmol/L), 3% (0.51 mmol/L), and 6% (1.02 mmol/L). The strain was cultured to activate two times. The activated strain was shaken at 200 r/min and grown in the saline medium at 28 °C for 48 h.

### Determination of Salt Tolerance of the Strain

Strains were inoculated on plates with different salt concentrations and the growth of the strains on the plates was observed 24–48 h later. 50  $\mu\text{L}$  of the activated bacterial solution was inoculated into test tubes containing 4950  $\mu\text{L}$  of the different salt concentrations, shaken, and then the culture broth was pipetted into each well in a 96-well plate and cultured in a growth curve automatic analyzer (Bioscreen C, FP-110-C, Finland). The growth curve was measured at 28 °C and automatically measured every two hours for 48 h.

The activated strains were cultured in different concentrations of NB medium with 2% inoculation, and the cells were collected after 24, 48, and 72 h and dried by centrifugation to constant weight to obtain dry weight.

### Biofilm Change and Ion Distribution Determination of the Strain Treated with Salt

The observation of biofilms with scanning electron microscopy (SEM) was completed as described by Castelijm et al. [13]. The biofilm observed by scanning electronic microscopy (QUANTA200, FEI, USA) and the surface and internal ions of the strain were analyzed by energy-dispersive spectrometer (EDS).

### Preparation and Quantitative RT-PCR of Total RNA

The strain was incubated in LB medium for 36 h using a TIANGEN RNAprep pure DP430 RNA extraction kit (China) according to the manufacturer's instructions. The cDNA samples were prepared using HiScript II Q Select RT Supermix for qPCR (China). The expression of *spo0A* was determined by qRT-PCR using ABI 7500 (Applied Biosystems, USA) and 16S rRNA as an internal reference. The primer sequences selected in this study were based on previous studies, such as *spo0A* [14] and 16S rRNA [15]. The RT-PCR experiment consisted of three independent experiments with three to five replicates per experiment.

### Determination of Secondary Metabolites of the Strain Treated with Salt

The culture medium was prepared as above and the activated cells were inoculated with 2% seed. The cells were removed for 48 h (12,000 r/min, 15 min, 4 °C), and 5 mL of the fermentation broth was extracted with dichloromethane (chromatographically pure). The organic phase was collected and filtered through a 0.22- $\mu\text{m}$  filter and the filtrate was loaded directly. The metabolites were determined by gas chromatography–mass spectrometry (GC–MS). The relative content

of each component was determined by the area normalization method. The structure of the compound corresponding to the chromatographic peak was identified based on the mass spectrometry database.

Gas chromatographic (GC) conditions were as follows: column: DB-5MS (30 m × 0.25 mm × 0.25 μm); carrier gas: high purity helium; column flow rate: 1.0 mL/min; column initial temperature: 50 °C for 3 min, increasing 10 °C to the final temperature of 250 °C which was held for 20 min; injection volume: 1 μL; injection method: split. Mass spectrometry (MS) conditions were as follows: ionization mode: EI; ion source temperature: 250 °C; interface temperature: 250 °C; data acquisition rate: 0.2 s/time.

### Determination of Zymogram Profiling of the Strain Treated with Salt

The culture medium was prepared as above. The activated cells were inoculated with 2% seed and cultured until stable phase when they were centrifuged (12,000 r/min, 10 min, 4 °C) to collect the cells. The cells were washed twice with phosphate buffered to pH 7.4. 4 mL of lysate (30 mmol/Tris-HCl, 0.3% SDS, 1.0% DTT, pH 8.0) was added, then mixed, and sonicated on ice for 20 min (power 80 W). Samples were sonicated for 9 s at an interval of 1 s, cooled on ice at 4 °C, centrifuged at 11,000 r/min for 10 min, and the supernatant was collected as the total protein in the cells. The cell lysate and loading buffer were mixed in a boiling water bath at a ratio of 1:4 for 5 min for spotting. The samples were subjected to polyacrylamide gel electrophoresis (SDS-PAGE) using 5% concentrated gel and 12% separating gel and colorized using Coomassie Brilliant Blue and decolorized by acetic acid-methanol.

A standard curve was drawn and bovine serum albumin was used as the standard protein to determine protein concentration. Standard protein solution, distilled water, and Coomassie G-250 solution were added, mixed, and dropped for 5 min at room temperature, and the OD595 value was measured at 595 nm with a spectrophotometer. 5 mL of Coomassie G-250 solution was added to 50 μL of the cell lysate, shaken, and mixed for 10 min, and the OD595nm value was measured.

### Identification of the Strain

The morphology and physio- and biochemical reactions used to identify the strain are given in Bergey's Bacterial Identification Manual [16]. The use of strains for different carbon sources was determined by Biolog identification plates [17]. The similarity index was read after 16, 24, 36, and 48 h, respectively. According to the instructions of the DNA extract kit, the strain was cultured for 24 h and extracted using the NANOFAST bacterial genome DNA extraction

kit (China). The extracted bacterial DNA was used as a template, and a pair of universal primers of bacterial 16S rDNA was used [18] (27F, 1492R) to amplify the bacterial genome. The amplified PCR product was ligated into Amp-resistant pClone007 Simple Vector, transformed into competent *E. coli* Trelief™ 5α, uniformly coated onto an Amp-containing medium, and allowed to stand at 37 °C overnight. The grown transformants were selected and sent out to sequence (Nanjing Kingsray Biotechnology Co., Ltd).

The 16S rDNA sequences obtained were compared to the NCBI database, and some representative 16S rDNA sequences were selected for further analysis. These sequences were analyzed by MEGA7.0 software to construct a phylogenetic tree. By using the prepared strain's genomic DNA, the *cry1*, 7, and 9 genes were amplified according to the primers described by Kuo and Chak [19].

## Results

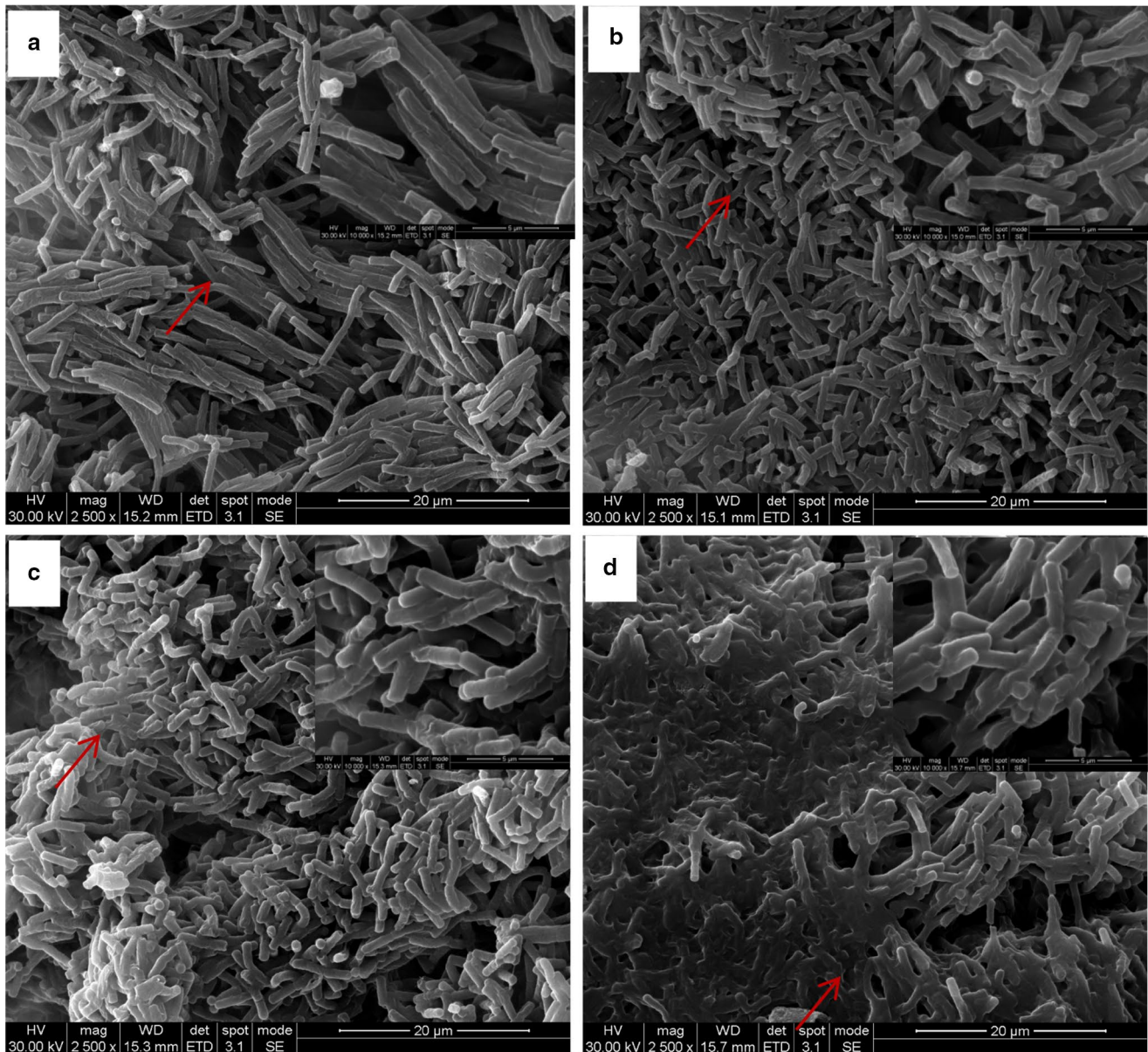
### Salt-Tolerant Growth Characteristics of the Strain JYZ-SD2

The strain JYZ-SD2 was able to grow on the plate containing 0–6% NaCl. It grew slowly and had a small colony (Table S1) indicating it was a mild halophile.

The strain had the shortest retardation period when the salt content was 1%. When the salt content was 6%, the retardation period reached 14 h and the growth rate of the strain was slowest in the logarithmic growth phase (Fig. S1a). The biomass dry weight of the strain decreased gradually with time (24, 48, 72 h) at a salt concentration of 1–3%. When the salt concentration was 6%, the dry weight of the strain increased first and then decreased depending on time; the dry weight at 72 h was slightly higher than that at salt concentrations of 0 and 1% at 72 h (Fig. S1b).

### Biofilm Formation and Expression of *spo0A* Gene of the Strain Treated with Salt

SEM results showed that when the NaCl concentration was 0 the strain had a slender rod shape with many visible shrinkage locations on the surface covered and a lower exopolysaccharide (EPS). The biofilm formed was the simplest single-layer cell arrangement (Fig. 1a). The length of the strain became smaller and the diameter increased depending on the NaCl concentration. The surface of the strain gradually formed a complex biofilm structure, and the arrangement structure became complicated with more cell clusters. When the NaCl concentration was 1–6%, the EPS became thicker and the cells were tightly bound to each other (Fig. 1b, c, d). According to the relative expression of the *spo0A* gene



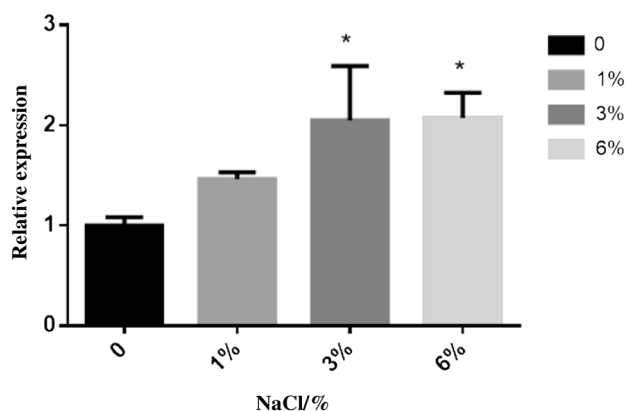
**Fig. 1** Effect of different salt concentrations on biofilm of JYZ-SD2 strain (the arrow indicated the aggregation of biofilm). Scanning electron microscopic images of strain JYZ-SD2 with NaCl content of 0, 1%, 3%, and 6% in **a–d**, respectively, with a scale of 20/5 micron

(Fig. 2), the expression level of the *spo0A* gene of the strain increased gradually with increasing of NaCl concentration.

### Changes in Elemental Content in the Strain Treated with Salts

The cells were observed by X-ray energy spectroscopy and a large amount of Na was found in the intracellular/extracellular phase of the strain (Fig. S2). In the intracellular strain (Table 1a), the content of Na increased at first, then decreased, and finally increased again as the NaCl concentration increased. The intracellular Na was about 69.86% at a

NaCl concentration of 3% and the intracellular Na reached 85.99% at 6% salt concentration. The relative content of each element in the strain (Table 1b) had a similar tendency to the intracellular trend. Regardless of intracellular or extracellular, the relative contents of  $\text{Na}^+$  and  $\text{Cl}^-$  were lowest at a NaCl concentration of 3% and the relative contents of  $\text{Mg}^{2+}$ ,  $\text{Fe}^{3+}$ , and  $\text{Zn}^{2+}$  increased.



**Fig. 2** The relative expression of JYZ-SD2 *spo0A* gene treated with salt

### Differences in Metabolites Between the Strain at Different Salt Concentrations

After removing the silicon oxide compound, and the LB medium, the salt tolerance-related metabolites were displayed based on the GC–MS analysis. The metabolites of the strain were different depending on salt concentrations (Table S2).

### Analysis of Zymogram Difference of the Strain Treated with Salts

The total intracellular protein content of the strain decreased at first at all NaCl concentrations and then decreased depending on the NaCl concentration (Fig. S3a). The total protein content reached a maximum of 72.60  $\mu\text{g}/\text{mL}$  at a NaCl concentration of 3%. The total protein was 11.32  $\mu\text{g}/\text{mL}$  and 22.75  $\mu\text{g}/\text{mL}$  at NaCl concentrations of 1% and 6%, respectively. Based on SDS-polyacrylamide gel electrophoresis (Fig. S3b), a total of 18, 14, 33, and 31 protein

bands appeared at NaCl concentrations of 0, 1%, 3%, and 6%, respectively.

### Identification of the Strain JYZ-SD2

The strain colony was round and slightly bulged, pale milky white, with a dull surface, opaque, rough, waxy, and the edges were irregular and fine. The cells were rod-shaped with peripheral flagella, Gram stain positive, with spores, no parasporal crystal, and the size was 1.0–1.1  $\mu\text{m} \times 2.6$ –5.0  $\mu\text{m}$ . The spores were round (Table S3). Based on the results of the physio-biochemical analysis and Biolog system, the strain JYZ-SD2 was identified as *Bacillus cereus*/*Bacillus thuringiensis*, with a similarity index (SIM) of 0.674. The result was relatively reliable when the similarity index was  $\geq 0.5$  due to bacterial identification in the Biolog system. According to Zeng et al.'s [20] article, the result is available. It was finally identified as *Bacillus cereus* due to the absence of parasporal crystals. The result of 16S rDNA of the strain confirmed the strain was *Bacillus cereus* (Fig. 3) because no products of *cry1*, 7, and 9 belonging to *Bacillus thuringiensis* were amplified by PCR.

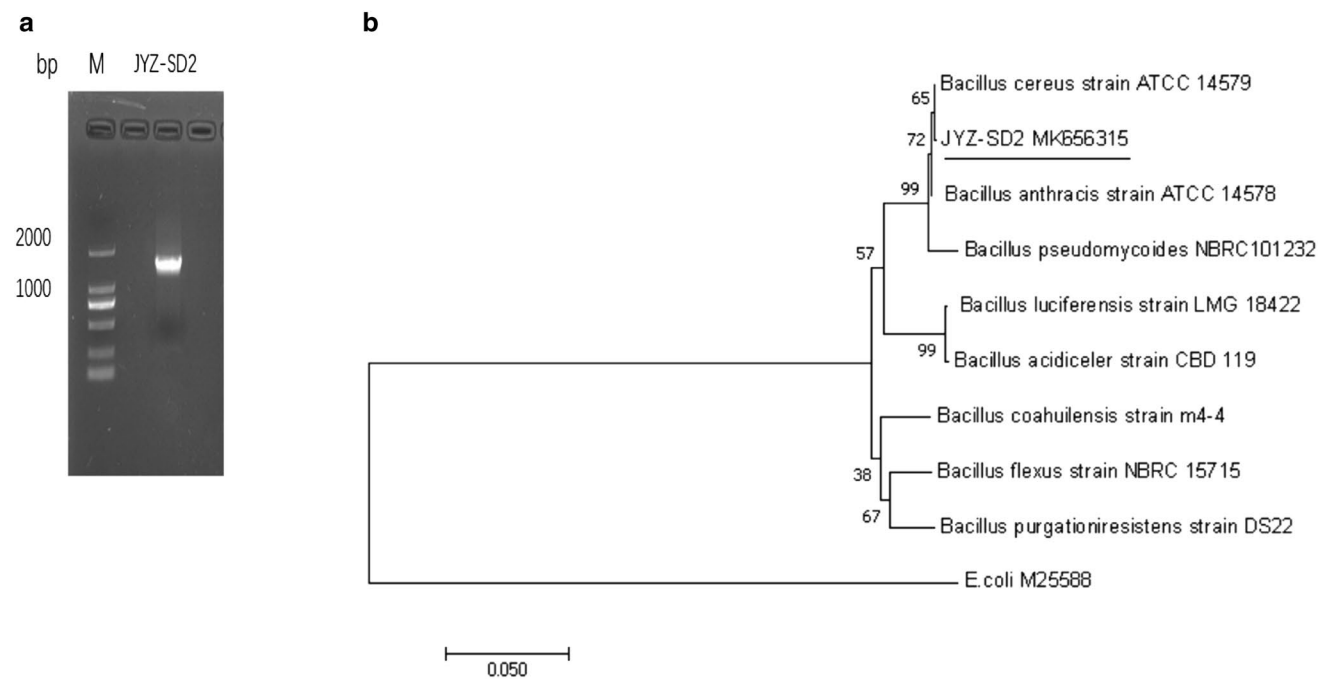
### Discussion

When cells adapt to a new osmotic environment and exceed their optimal salt concentration for growth, they may change the expression level of related genes. The strain JYZ-SD2 could tolerate 1–6% salt concentrations and the most suitable NaCl concentration was 3%. In order to adapt to the balance of intracellular osmotic pressure, some osmotic regulators and protective substances needed to be synthesized and accumulated in, for example, tetrahydropyrimidine [21] and glycine betaine [22], alleviating the effects of salt stress.

Bacterial biofilm is mainly formed by extracellular polysaccharides (EPS), proteins, peptidoglycans, lipids, phospholipids, DNA, RNA, and so on [23]. It is self-wrapped

**Table 1** Effects of different salt concentrations on the relative content of elements in JYZ-SD2 strain

Element NaCl (%)	Na	Cl	K	Ca	Mg	Fe	Zn
a. Relative content of intracellular elements							
0	73.9	7.96	–	2.48	4.57	3.18	5.41
1	82.71	6.73	0.61	0.71	1.12	0.71	5.52
3	69.86	–	0.722	–	6.99	2.12	13.81
6	85.99	2.88	0.49	–	6.24	1.62	1.49
b. Relative content of extracellular elements							
0	81.68	7.09	–	2.29	–	3.72	5.23
1	84.19	6.96	1.48	3.08	–	4.28	–
3	70.65	3.38	3.68	3.91	–	4.6	13.79
6	95.3	2.34	0.63	0.69	–	0.04	1



**Fig. 3** JYZ-SD2 strain was identified based on 16SrDNA. **a** Electrophoresis of the PCR product of JYZ-SD2 strain, **b** Neighbor-joining trees showing the phylogenetic position of strain JYZ-SD2 next to its phylogenetic neighbors

to form a large bacterial population, such as multicellular aggregates that are membranously bound to liquid or solid surfaces [24]. As the environment changes, the expression of given genes will be inhibited or enhanced [25]. Park et al. [26] found that the up-regulated expression of the *spo0A* gene helps to enhance biofilm formation, while the gene *spo0A* was inhibited by high salinity [27]. In the present work, the biofilm on the surface of the strain became thicker gradually and the bacteria became tubbiness depending on the salt concentration. The *spo0A* gene was a positive regulator of biofilm formation. Under high salt concentrations, the expression of the *spo0A* gene by the strain was significantly increased, and the strain could prevent the separation of the cytoplasmic wall and cell rupture and maintain the morphological structure of the organism to ensure it could cope with the osmotic pressure. Waheed and Nasim [28] found that the salt-tolerant strains *Halomonas variabilis* (HT1) and *Planococcus rifietoensis* (RT4) can accumulate extracellular polysaccharides to form biofilms under salt stress. Li et al. [29] reported that the strain *Paenibacillus edaphicus* NUST16 has a high salt tolerance. Kaluzhnaya et al. [30] found that the five obligate methane oxidizing bacteria they isolated can accumulate a large amount of EPS on the cell walls and form biofilms at high  $\text{Na}^+$  concentrations. Under high salt stress, the bacteria was like a sugarcoat that was closely bound together. Salt increased the accumulation of EPS spines, which promoted the formation of the biofilm barrier. The barrier reduced the amount of Na moving into

the cell. This indicated that under high osmotic pressure environments, bacteria will aggregate in a complex arrangement as an adaptation to survive [31].

In prokaryotes, the  $\text{Na}^+/\text{H}^+$  antiporter plays an important role in the salt tolerance process. Southworth et al. [32] found that GerN is a strong  $\text{Na}^+/\text{H}^+-\text{K}^+$  reverse transporter protein found in *Bacillus cereus* that can catalyze the efflux of  $\text{Na}^+$  and intake of  $\text{K}^+$ . This study found that under different salt concentrations, the total amount of Na detected in the cells of the strain showed an upward trend. When the salt concentration was 3%, Na in the cell accounted for only 69.86% of the detected elements. The relative contents of K, Mg, and Zn in the cell were 7.22%, 6.99%, and 13.81% respectively. In order to reduce the continuous intake of  $\text{Na}^+$ , the cells must ingest other metal cations to achieve charge balance and maintain the membrane resting potential. When the salt concentration reached 6%, the intracellular Na content of the cells was the highest of all treatments. The strain formed a biofilm and the main component of the biofilm was glycoprotein, which may contain acid amino acid residues such as aspartic acid and glutamic acid. Excessive acidic amino acid residues would form a negative electric region [33].

Salt stress can cause microbial hyperbaric osmosis and nutritional stress, inducing new microbial metabolic pathways to activate silent gene expression. Based on the differences in the strain metabolites under different salt concentrations, the small molecular metabolic pathways

of bacteria changed, and almost all of them were low polar small molecular substances. The lower the polarity of the substances, the easier the water miscibility. With an increase in NaCl concentration, the content of acetamide decreased gradually. Acetamide is a substance that is easily miscible with water and may participate in the formation of biofilms as a negative regulator. Chen et al. [34] found that amide was one of the reasons for the biosorption of *Bacillus cereus*. In addition, a decrease in acetamide may increase the surface hydrophobicity of the strain, which may increase the rate of biofilm formation [35]. In hypertonic environments (NaCl 3% and 6%), the content of 3-methyl butyric acid was much higher than that in low osmotic environments. In addition, when the NaCl concentration was 3% and 6%, the appearance of pyrrole [1,2-a] pyrazine-1,4-dione (a derivative of proline) nitrogen-containing substances might be related to the salt tolerance of the strain. In Gram-positive bacteria, information exchange between bacteria is usually carried out through amino acids, polypeptides, and other substances [34].

When bacteria are exposed to a hypertonic environment, gene expression will be affected. Some genes may be silenced. Others may activate the expression, regulate the translation of corresponding proteins, and synthesize some carriers, proteins, or enzymes that can adapt to a high salt environment, such as the Na/H–K anti-transporter, Na/H anti-transporter, betaine transporter (BetH), and so on. Fang et al. [36] found that the strain ID05-A0528<sup>T</sup> could synthesize univalent cation/proton reverse transporter proteins, thus tolerating high NaCl concentrations. When osmotic pressure changed in the environment, the intracellular receptors could detect and accept this signal, and then downward transmit the signal to the transport system, proteins and enzymes, so that the cells could react quickly to the activity of their own proteins and maintain normal life metabolism to meet their needs for survival. In this work, the intracellular soluble protein changed significantly depending on NaCl concentration. Many proteins were not expressed or underexpressed significantly at the highest NaCl concentration. It could be speculated that such proteins would be involved in the accumulation and transport of compatible substances. A special band appeared at a molecular weight of 56 kDa, possibly a glycine betaine transporter (BetH). Lu et al. [37] predicted that the betaine transporter of *Halobacillus* is 55.2 kDa. The protein and BetH have a high similarity with *Bacillus subtilis* OpuD [38].

In summary, this work analyzed the physiological and biochemical traits of salt tolerance of the JYZ-SD2 strain, which indicated the strain was *Bacillus cereus*. The strain could tolerate very high concentrations of NaCl by forming biofilms, secreting small organic molecules, changing morphology, and so on to adapt to environmental stresses.

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## Compliance with Ethical Standards

**Conflict of interest** The authors hereby declare that they have no conflict of interest.

## References

- Munns R, Tester M (2008) Mechanisms of salinity tolerance. *Annu Rev Plant Biol* 59(1):651–681
- Nusrat N, Shahbaz M, Perveen S (2014) Modulation in growth, photosynthetic efficiency, activity of antioxidants and mineral ions by foliar application of glycine betaine on pea (*Pisum sativum* L.) under salt stress. *Acta Physiol Plant* 36(11):2985–2998
- Mishra S, Upadhyay S, Shukla RK (2017) The role of strigolactones and their potential cross-talk under hostile ecological conditions in plants. *Front Physiol* 7:691
- Ahmad AF, Iqbal A (2018) Plant growth promoting attributes and alleviation of salinity stress to wheat by biofilm forming, *Brevibacterium* sp. FAB3 isolated from rhizospheric soil. *Saudi J Biol Sci*. <https://doi.org/10.1016/j.sjbs.2018.08.003>
- Vaidya S, Dev K, Sourirajan A (2018) Distinct osmoadaptation strategies in the strict halophilic and halotolerant bacteria isolated from lunsu salt water body of north west Himalayas. *Curr Microbiol* 75(7):888–895
- Saum SH, Volker M (2010) Growth phase-dependent switch in osmolyte strategy in a moderate halophile: ectoine is a minor osmolyte but major stationary phase solute in *Halobacillus halophilus*. *Environ Microbiol* 10(3):716–726
- Ivey DM, Guffanti AA, Bossewitsch JS, Padan E, Krulwich TA (1991) Molecular cloning and sequencing of a gene from alkaliphilic *Bacillus firmus* OF4 that functionally complements an *Escherichia coli* strain carrying a deletion in the *nhaA* Na<sup>+</sup>/H<sup>+</sup> antiporter gene. *J Biol Chem* 266(34):23483
- Jiang J, Wang L, Zou Y, Lu W, Zhao B, Zhang B et al (2013) Identification of important charged residues for alkali cation exchange or pH regulation of NhaH, a Na<sup>+</sup>/H<sup>+</sup> antiporter of *Halobacillus dabanensis*. *BBA-Biomembranes* 3:997–1003
- Ritika K, Gupta MK, Kumar N, Kanwar S (2017) Analysis of *nhaA* gene from salt tolerant and plant growth promoting *Enterobacter ludwigii*. *Rhizosphere*. <https://doi.org/10.1016/j.rhisph.2017.07.002>
- Wei X, Fang L, Cai P, Huang Q, Chen H, Liang W et al (2011) Influence of extracellular polymeric substances (EPS) on Cd adsorption by bacteria. *Environ Pollut* 159(5):1369–1374
- Kasim WA, Gaafar RM, Abou-Ali RM, Omar MN, Hewait HM (2016) Effect of biofilm forming plant growth promoting rhizobacteria on salinity tolerance in barley. *J Agric Sci Cambridge* 61:217–227
- Dimkpa C, Weinand T, Asch F (2010) Plant–rhizobacteria interactions alleviate abiotic stress conditions. *Plant Cell Environ* 32(12):1682–1694
- Castelijn GAA, Stijn VDV, Zwietering MH, Moezelaar R, Abbe T (2012) Diversity in biofilm formation and production of curli fimbriae and cellulose of *Salmonella*, *typhimurium* strains of different origin in high and low nutrient medium. *Biofouling* 28(1):51–63

14. Gao T, Foulston L, Chai Y, Wang Q, Losick R (2015) Alternative modes of biofilm formation by plant-associated *Bacillus cereus*. *Microbiology Open* 4(3):452–464
15. Lucking G, Dommel MK, Scherer S, Fouet A, Ehling-Schulz M (2009) Cereulide synthesis in emetic *Bacillus cereus* is controlled by the transition state regulator *abrB*, but not by the virulence regulator *plcR*. *Microbiology* 155(3):922–931
16. Buchanan RE, Gibbons NE (1974) *Bergey's manual of determinative bacteriology*, 8th edn. The William & Wilkins, Baltimore, pp 729–759
17. Li GE, Wu XQ, Ye J-R (2013) Isolation and identification of phytate-degrading rhizobacteria with activity of improving growth of poplar and masson pine. *World J Microb Biotechnol* 29(11):2181–2193
18. Frank JA, Reich CI, Sharma S, Weisbaum JS, Wilson BA, Olsen GJ (2008) Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes. *Appl Environ Microbiol* 74(8):2461–2470
19. Kuo WS, Chak KF (1996) Identification of novel cry-type genes from *Bacillus thuringiensis* strains on the basis of restriction fragment length polymorphism of the PCR-amplified DNA. *Appl Environ Microbiol* 62(4):1369–1377
20. Zeng Q, Wu X, Wen X (2016) Identification and characterization of the rhizosphere phosphate-solubilizing bacterium *Pseudomonas frederiksbergensis* JW-SD2, and its plant growth-promoting effects on poplar seedlings. *Ann Microbiol* 66(4):1343–1354
21. Sadeghi A, Soltani BM, Nekouei MK, Jouzani GS, Mirzaei HH, Sadeghizadeh M (2014) Diversity of the ectoine biosynthesis genes in the salt tolerant streptomycetes and evidence for inductive effect of ectoines on their accumulation. *Microbiol Res* 169(9–10):699–708
22. Shivanand P, Mugeraya G (2011) Halophilic bacteria and their compatible solutes—osmoregulation and potential applications. *Curr Sci India* 100(10):25–2011
23. Dow JM, Ryan P (2008) Diffusible signals and interspecies communication in bacteria. *Microbiology* 154(7):1845
24. Morikawa M (2006) Beneficial biofilm formation by industrial bacteria *Bacillus subtilis* and related species. *J Biosci Bioeng* 101(1):1–8
25. Zeng Q, Wu X, Wen X (2016) Effects of soluble phosphate on phosphate-solubilizing characteristics and expression of *gcd* gene in *Pseudomonas frederiksbergensis* JW-SD2. *Curr Microbiol* 72(2):198–206
26. Park EJ, Hussain MS, Wei S, Kwon M (2019) Genotypic and phenotypic characteristics of biofilm formation of emetic toxin producing *Bacillus cereus* strains. *Food Control* 96:527–534
27. Widderich N, Rodrigues C, Commichau F, Fischer K, Ramirez-Guadiana FH, Rudner DZ et al (2016) Salt-sensitivity of  $\sigma^H$  and Spo0A prevents sporulation of *Bacillus subtilis* at high osmolarity avoiding death during cellular differentiation. *Mol Microbiol* 100(1):108–124
28. Waheed QA, Nasim SA (2012) Bacterial exopolysaccharide and biofilm formation stimulate chickpea growth and soil aggregation under salt stress. *Braz J Microbiol* 43(3):1183–1191
29. Li J, Xu H, Chen X, Xu L, Cheng R, Zhang J et al (2017) Characterization of an exopolysaccharide with distinct rheological properties from, *Paenibacillus edaphicus* nstr16. *Int J Biol Macromol* 105(1):1–8
30. Kaluzhnaya M, Khmelena V, Eshinimaev B, Suzina N, Nikitin D, Solonin A et al (2001) Taxonomic characterization of new alkaliphilic and alkalitolerant methanotrophs from soda lakes of the southeastern transbaikalian region and description of *Methylomicrobium buryatense* sp nov. *Syst Appl Microbiol* 24(2):166–176
31. Taketo K, Soichi F, Naoki N, Chisato H et al (2009) Biofilm formation by *Escherichia coli* in hypertonic sucrose media. *J Biosci Bioeng* 107(6):630–635
32. Southworth TW, Guffanti AA, Moir A, Krulwich TA (2001) Gern, an endospore germination protein of *Bacillus cereus*, is a Na<sup>+</sup>/H<sup>+</sup>-K<sup>+</sup> antiporter. *J Bacteriol* 183(20):5896–5903
33. Roeßler M, Müller V (1998) Quantitative and physiological analyses of chloride dependence of growth of *Halobacillus halophilus*. *Appl Environ Microbiol* 64(10):3813
34. Chen Z, Pan X, Chen H, Guan X, Lin Z (2015) Biomineralization of pb(ii) into pb-hydroxyapatite induced by *Bacillus cereus* 12–2 isolated from lead-zinc mine tailings. *J Hazardous Mater* 301:531–537
35. Karunakaran E, Biggs CA (2011) Mechanisms of *Bacillus cereus* biofilm formation: an investigation of the physicochemical characteristics of cell surfaces and extracellular proteins. *Appl Microbiol Biotechnol* 89(4):1161–1175
36. Fang H, Hu B, Nie Y, Tang YQ, Wu XL (2017) The complete genome of *Dietzia timorensis* id05-a0528(t) revealed the genetic basis for its saline-alkali tolerance. *J Biotechnol* 241:11–13
37. Lu W, Zhang B, Zhao B et al (2007) Cloning and characterization of the genes encoding a glycine betaine ABC-type transporter in *Halobacillus trueperi* DSM10404<sup>T</sup>. *Curr Microbiol* 54(2):124–130
38. Li FY, Ju QJ, Bai SZ, Bo Z (2006) A Na<sup>+</sup>/H<sup>+</sup> antiporter gene of the moderately halophilic bacterium *Halobacillus dabanensis* D-8<sup>T</sup>. *FEMS Microbiol Lett* 255(1):89–95

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