Extracellular Polysaccharide Characteristics of *Neorhizobium alkalisoli* from an Arid Region and Its Potential for Saline-Alkaline Soil Remediation

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Abstract

To screen highly efficient nitrogen-fixing bacteria for saline-alkali soil remediation in arid regions and clarify the functional mechanisms of extracellular polysaccharides (EPS), nitrogen-fixing bacteria were isolated and purified from saline-alkali soil of Salt Lake in Urumqi, Xinjiang. Their taxonomic status was determined by 16S rRNA gene sequencing, the effects of culture time on EPS yield and purity were investigated, drying and storage conditions for the microbial agent were optimized, and the salt-alkali tolerance and alkali-reducing characteristics of the strain were analyzed. The results showed that one strain of Neorhizobium alkalisoli, Na XJIPC-GNJ0001, was isolated, with 99.20% 16S rRNA gene sequence homology to strain TRM85146, and was deposited under CGMCC No. 30470. The strain exhibited a time-dependent synergistic improvement in yield and purity, with an EPS extraction yield of 2302.0$\pm2.85mg \cdot L-1andasugarcontentof86.57\pm1.02\times1015CFU \cdot mL-1, anditsactivityremainedat2.50\times1012$ 2.70×$1012 CFU·mL-1 after storage at -20~-40 ℃ for 24 months, outperforming other methods. Functional analysis showed that the strain could grow under 15% NaCl and pH 12 conditions, with an alkali-reduction rate of 29.18% at an initial pH of 11, indicating strong stress adaptability. This study confirms that the strain' s high EPS-producing capacity, optimized microbial-agent preparation process, and excellent salt-alkali tolerance provide important microbial resources and technical support for the bioremediation of saline-alkali soils in arid regions.

Full Text

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Extracellular Polysaccharide Characteristics of *Neorhizobium alkalisoli* from an Arid Region and Its Potential for Saline-Alkaline Soil Remediation

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Abstract: To screen efficient nitrogen-fixing bacteria for saline–alkaline soil re-

mediation in arid regions and clarify the functional mechanism of extracellular polysaccharides (EPS), nitrogen-fixing bacteria were isolated and purified from saline–alkaline soil of Salt Lake in Urumqi, Xinjiang. Their taxonomic status was determined by 16S rRNA gene sequencing; the effects of cultivation time on EPS yield and purity were investigated; drying and storage conditions for the microbial inoculant were optimized; and the salt–alkali tolerance and alkali-reducing characteristics of the strain were analyzed. The results showed that one strain of *Neorhizobium alkalisoli*, NaXJI-PC-GNJ0001, was isolated, with 99.20% homology in its 16S rRNA gene sequence to strain TRM85146; its preservation number is CGMCC No. 30470. The strain exhibited a time-dependent effect of "synergistic improvement in yield and purity." At 120 h of cultivation, the EPS extraction yield reached 2302.0 ± 2.85 mg $\cdot$ L^{-1}, and the sugar content was $86.57\% \pm 1.02\%$. Ultraviolet and infrared spectra confirmed that the EPS was rich in functional groups such as hydroxyl and carboxyl groups, providing a structural basis for environmental adaptation. The viable count of the solid inoculant prepared by spray drying (45 min $\cdot$ L^{-1}) reached 4.60×10^{15} CFU $\cdot$ mL^{-1}, and after storage at $-40 \sim -20$ °C for 24 months, its activity remained at $2.50 \times 10^{12} \sim 2.70 \times 10^{12}$ CFU $\cdot$ mL^{-1}, superior to other methods. Functional analysis showed that the strain could grow under 15% NaCl and pH 12 conditions, and achieved an alkali-reduction rate of 29.18% in an environment with an initial pH of 11, indicating strong stress adaptability. This study confirms that the strain' s high EPS-producing capacity, optimized inoculant processing technology, and excellent salt–alkali tolerance provide important microbial resources and technical support for the bioremediation of saline–alkaline soils in arid regions.

Keywords: arid region; *Neorhizobium alkalisoli*; extracellular polysaccharides; saline–alkaline soil remediation; solid inoculant

Soil salinization and alkalization in arid regions is a core bottleneck restricting ecological security and the sustainable development of agriculture in arid regions worldwide. Its particularity lies in the dual stress of drought-induced water shortage and high salinity–alkalinity, which leads to degradation of soil structure, reduced nutrient availability, and a sharp decline in biological activity[1]. In China, the area of salinized and alkalized land in arid regions reaches 9.9×10^7 hm^2. Xinjiang, as the core region of China' s arid areas, has a high proportion—up to 37.2%—of severely saline–alkaline land, defined as soil with total salt content $> 1\%$ and pH > 10.0[1]. Such soils have a bulk density above 1.6 g $\cdot$ cm^{-3}, a 60% decrease in the proportion of macroaggregates (> 0.25 mm), and loss of water- and fertilizer-retention capacity, seriously restricting vegetation restoration and agricultural production and posing a severe threat to the stability of regional ecosystems[2].

Because of its advantages of low cost, easy operation, and ecological compatibility, microbial remediation has become an important technological pathway for improving saline–alkaline soils in arid regions. Among microorganisms, nitrogen-fixing bacteria can increase soil nitrogen levels through biological nitrogen fix-

ation, and the extracellular polysaccharides (EPS) they secrete are key functional substances for adaptation to extreme environments. They play an irreplaceable role in adaptation to saline-alkaline stress in arid regions: under the dual stresses of drought and salinity-alkalinity, EPS forms a hydration layer ($0.5 \sim 2$ μm) through hydroxyl and carboxyl groups, maintaining cellular integrity under high-salinity conditions ($Na^+ > 200$ mmol $\cdot$ L^{-1}), and builds a biophysical barrier through steric hindrance, reducing damage to bacterial cells caused by salt ions and drought stress[3-5]; its macromolecular chains can bridge soil particles, significantly increasing the proportion of macroaggregates (by up to 4.07-fold) and water-holding capacity (by 38%), thereby specifically improving soil water- and fertilizer-retention performance in arid regions[6-7]; acidic EPS can also adsorb water through carboxyl groups and release organic acids, lowering rhizosphere pH by 1.5-2.0 units and creating a suitable microenvironment for plant growth[8].

The exploration of microbial resources from extreme habitats has provided new ideas for remediation of saline-alkaline land in arid regions. For example, the nitrogen-fixing bacterium *Azotobacter* strain As101 isolated from a Xinjiang salt lake can produce high-purity EPS (61.35%)[9-10], but

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In typical high-salt environments of arid regions (NaCl > 5%), their survival rate is < 30%, making it difficult to meet practical remediation needs[11]. Current research still has limitations: efficient nitrogen-fixing strains suitable for extreme saline-alkaline environments in arid regions (pH > 10, NaCl > 10%) are scarce, and existing strains show insufficient adaptability; the coupling mechanism among EPS "yield-purity-function" remains unclear, while traditional purification processes account for 62% of production costs, constraining large-scale application[12]; and microbial agents have poor stability under storage conditions such as large temperature fluctuations and dryness in arid regions, with activity at room temperature declining by six orders of magnitude within 6 months, making long-term preservation difficult[13-14]. In addition, key scientific questions remain unresolved, including the relationship between the taxonomic characteristics of nitrogen-fixing bacteria from extreme habitats and EPS phenotypes, as well as the mechanisms by which EPS mediates saline-alkaline

tolerance and alkalinity reduction[15–19].

To address the above issues, this study used saline–alkaline soil from the Salt Lake in Urumqi, Xinjiang (a typical extreme habitat in an arid region) as the material to isolate and purify high-EPS-producing nitrogen-fixing bacteria, and determined their taxonomic status by 16S rRNA gene sequencing. The dynamic effects of culture time on EPS yield and purity were investigated to reveal the mechanism of "coordinated improvement of yield and purity."Drying and storage conditions for the microbial agent were optimized to identify key parameters for maintaining activity under warehousing environments in arid regions. The salt tolerance, alkali tolerance, and alkalinity-reducing characteristics of the strains were analyzed to clarify the EPS-mediated stress-adaptation mechanism. This work aims to provide functionally adapted strain resources and technical support for the bioremediation of saline–alkaline soils in arid regions, and to enrich theories on the utilization of microbial resources from extreme environments.

1 Materials and Methods

1.1 Isolation, Purification, and Identification of Nitrogen-Fixing Strains

Soil collection: Samples were collected on November 3, 2021, from the Salt Lake scenic area in Urumqi, Xinjiang (88.138°E, 43.381°N; altitude 1009 m), with reference to the collection and processing methods for mixed cultivated-soil samples in the *Handbook of Soil Agrochemical Analysis*[20]. Three 10 m × 10 m quadrats were established, and mixed soil samples from 0–30 cm depth were collected at three points along the diagonal. After removal of debris, approximately 500 g was retained by the quartering method and transported back to the laboratory under refrigeration.

Strain isolation: The dilution spread-plate method was used. A 10 g soil sample passed through a 20-mesh sieve was added to 90 mL of sterile deionized water and shaken at 150 r · min−1 for 30 min, followed by gradient dilution to 10−9 (test tubes No. 1–8). From test tubes No. 5–8, 150 μL of dilution was spread onto Ashby solid medium plates, with three replicates for each tube, and incubated inverted at 37 °C until single colonies appeared. The composition of Ashby solid medium was as follows (per 100 mL): mannitol (or glucose) 1 g, magnesium sulfate 0.01 g, potassium dihydrogen phosphate 0.02 g, sodium chloride 0.02 g, calcium sulfate 0.01 g, calcium carbonate 0.5 g; pH adjusted to 7.0–7.20, agar 1.5 g added, and sterilized at 115 °C for 30 min.

Strain purification: Single colonies with different morphologies were selected and streaked on Ashby solid medium, then cultured at 37 °C until the colony morphology was uniform. A single colony was inoculated into 5 mL of sterile liquid nitrogen-fixing bacterial medium and cultured at 33 °C and 180 r · min−1 for 24 h; it was then mixed with 50% glycerol (1:1), dispensed at 1 mL into 1.5 mL centrifuge tubes, and stored at −80 °C. The composition of the liquid

nitrogen-fixing bacterial medium was as follows (per 100 mL): mannitol 2.0 g, potassium dihydrogen phosphate 0.02 g, dipotassium hydrogen phosphate 0.08 g, magnesium sulfate 0.01 g, calcium sulfate 0.01 g, yeast extract 0.05 g, ferric chloride 0.001 g, sodium molybdate 0.0002 g; pH adjusted to 7.0–7.20, and sterilized at 121 °C for 20 min.

Molecular biological identification of strains: DNA extraction: activated strains were cultured to the logarithmic phase, and DNA was extracted using the Sangon Ezup column bacterial genomic DNA extraction kit (B518255) according to the instructions. PCR amplification and sequencing: amplification was performed using 16S rRNA gene primers[21] (27F: 5′-AGAGTTTGATCCTGGCTCAG-3′; 1492R: 5′-GGTTACCTTGTTACGACTT-3′). The 50 μL reaction system contained 1.5 μL DNA template, 1 μL each of upstream and downstream primers, 25 μL 2×Taq PCR Green Mix, and sterile water. Amplification conditions were: 95 °C for 5 min; 94 °C for 50 s, 54 °C for 30 s, and 72 °C for 30 s (30 cycles); 72 °C for 7 min; and storage at 4 °C. PCR products were tested by 1.5% agarose gel electrophoresis and then sent to Sangon for bidirectional sequencing. Sequence analysis: CodonCode Aligner was used to align and assemble the sequences, remove primer regions and low-quality sequences; sequences with similarity $\geq$ 98% were selected by NCBI BLAST comparison; and an NJ phylogenetic tree was constructed using MEGA 11, with branch support tested by Bootstrap analysis (1000 replicates)[22].

1.2 Strain Culture and Scale-Up Procedure

Cryopreserved strains were streaked onto sterile solid nitrogen-fixing medium (same composition as above), and incubated inverted at 37 °C to obtain single colonies. A single colony was picked into sterile liquid nitrogen-fixing medium (same composition as above) and shaken at 33 °C and 180 r $\cdot$ min−1 for 24 h to obtain an activation culture. The activation culture was inoculated into sterile liquid nitrogen-fixing medium at a 6% inoculation ratio for secondary scale-up culture (24 h) to obtain the seed culture, and the fermentation broth for tertiary scale-up culture was obtained at the same ratio. Samples were taken at 24 h, 48 h, 72 h, 96 h, and 120 h, with three replicates for each time point, and each was used for subsequent analyses. Through the three-stage scale-up system of "activation → seed culture → fermentation," strain activity was restored and biomass was progressively increased.

1.3 Methods for Analysis of Extracellular Polysaccharides and Proteins

Extraction and purification of extracellular polysaccharides: Fermentation broths of strains at different time points were collected and centrifuged at 4 °C and 10,000 r $\cdot$ min−1 for 10 min to remove bacterial cells. Three volumes of anhydrous ethanol precooled at −20 °C were added to the supernatant, which was

then left at 4 °C for 12 h and centrifuged to obtain crude polysaccharide precipitates. The precipitates were enriched by three rounds of alcohol precipitation. After the precipitates were redissolved, Sevage reagent (chloroform:n-butanol = 4:1) was added at a 1:4 volume ratio for deproteinization three times. A 3.5 kDa dialysis bag was then used for dialysis at 4 °C for 48 h to remove small-molecule impurities, and the crude extracellular polysaccharide was obtained by freeze-drying[23].

Determination of polysaccharide sugar content: The phenol-sulfuric acid method[24] was used, with glucose as the standard to prepare a series of standard solutions at 0.0125-0.2 mg · mL−1.

Take 200 μL of standard solution or sample, add 75 μL of 5% phenol and 800 μL of concentrated sulfuric acid in sequence, heat in a boiling-water bath for 10 min, and measure the absorbance at 490 nm. A standard curve was established with glucose content as the abscissa and absorbance as the ordinate. The sample sugar content was calculated according to sample sugar content = $(m1/m2) \times 100\%$, where $m1$ is the value measured from the standard curve and $m2$ is the sample mass; three parallel replicates were set for each experimental point.

Protein content determination: a Thermo Scientific Pierce® BCA kit (23227) was used. Bovine serum albumin was used as the standard and serially diluted to 2 mg · mL^{-1}, 1.5 mg · mL^{-1}, 1 mg · mL^{-1}, 0.5 mg · mL^{-1}, 0.25 mg · mL^{-1}, and 0.125 mg · mL^{-1}. Take 75 μL of standard solution or sample, add 525 μL of BCA reagent (solution A:solution B = 50:1), incubate in a 37 °C water bath for 30 min, use purified water as the blank, and measure the absorbance at 562 nm. After establishing the standard curve, calculate according to sample protein content = $(m1/m2) \times 100\%$; three parallel replicates were set for each experimental point.

1.4 Preparation method for solid inoculants

The fermentation broth cultured for 120 h was used to prepare solid inoculants by the following three methods.

Spray-drying method: a Japanese EYELA SD-1000 spray dryer was used. One liter of fermentation broth was sprayed into the drying tower at a flow rate of 22.2 mL · min^{-1}. The hot-air inlet temperature was controlled at 120-150 °C, the outlet temperature at 40-50 °C, BLOWER at 0.8 m^3 · min^{-1}, and ATOMIZING at 1.8 KPa. The duration after instantaneous drying was recorded.

Hot-air drying method: 1 L of fermentation broth was poured into a drying tray and placed in a forced-air drying oven; it was continuously dried at 50 °C until completely dry, and the time was recorded.

Freeze-drying method: a Japanese EYELA FDU 2100 vacuum freeze dryer was used. One liter of fermentation broth was divided into three 1 L freeze-drying bottles. After rapid rotary freezing in an EYELA PFR-1000 prefabricated cold

bath, the bottles were transferred to the vacuum freeze dryer and freeze-dried by sublimation under a vacuum environment of −85 to −70 °C; the total time was recorded.

1.5 Viability detection method

Viable-cell count: the dilution spread-plate method was used. The dried solid inoculant was dissolved and serially diluted to 10^{-2}–10^{-18}, then spread onto solid nitrogen-fixing medium plates. For each dilution of each drying type, three replicates were set, and the plates were incubated inverted at 37 °C until single colonies grew. Colony-forming units (CFU) were counted to calculate the number of viable bacteria. Viable-cell detection after storage: the solid inoculants were stored dry at room temperature, −4 °C, −20 °C, and −40 °C, respectively. Samples were taken after storage for 1, 6, 12, 18, and 24 months. The viable-cell count method described above was used to determine the number of viable bacteria, and storage stability was evaluated by comparing activity changes before and after storage.

$$\mathrm{CFU}\cdot\mathrm{mL}^{-1} = \frac{\text{average number of colonies}}{\text{spread volume (mL)} \times \text{dilution factor}} \tag{1}$$

1.6 Ultraviolet spectroscopic analysis

Prepare 2 $\mathrm{mg}\cdot\mathrm{mL}^{-1}$ aqueous solutions of extracellular polysaccharide and spray-dried solid inoculant. Using a Shimadzu UV2600 ultraviolet–visible spectrophotometer, perform full-wavelength scanning over the range of 185–400 nm and record the maximum absorption wavelength.

1.7 Fourier-transform infrared spectroscopic analysis

Optical-grade KBr was ground and passed through a 200-mesh sieve, then dried at 120 °C for more than 4 h for later use. Take 1–2 mg of extracellular polysaccharide or spray-dried solid inoculant powder that had been dried at 105 °C for 2 h and cooled, mix with 200 mg of KBr, and grind uniformly in an agate mortar until no particles are visible to the naked eye. Press into tablets (pressure 0.8–1 GPa, maintained for 30–40 s, thickness <0.5 mm), and scan and analyze over the range of 400–4000 cm^{-1} using a Thermo Nicolet 6700 Fourier-transform infrared spectrometer.

1.8 Analysis of functional characteristics of the nitrogen-fixing bacterial strain

Growth characteristics: the seed culture was inoculated at a 6% inoculum into 100 mL of sterile liquid nitrogen-fixing medium (500 mL Erlenmeyer flask), and cultured on a shaker at 33 °C and 180 $\mathrm{r}\cdot\mathrm{min}^{-1}$. OD600 was measured at multiple time points from 2 to 168 h. Uninoculated blank medium was used as the control, each group was repeated three times, and curves were plotted.

Fig. 1 Morphological characteristics of *Neorhizobium alkalisoli* NaXJIPC-GNJ0001

Figure 1: Fig. 1 Morphological characteristics of *Neorhizobium alkalisoli* NaXJIPC-GNJ0001

Salt tolerance: the seed culture was inoculated at a 6% inoculum into 100 mL of sterile liquid nitrogen-fixing medium containing 0%–20% NaCl, and cultured at 33 °C and 180 $r \cdot min^{-1}$ for 24 h. OD600 was then measured. Uninoculated blank medium was used as the control; three replicates were set for each salt concentration, and curves were plotted.

Alkali tolerance and alkali-reducing ability: the seed culture was inoculated at a 6% inoculum into 100 mL of sterile liquid nitrogen-fixing medium with pH 7-12, and cultured at 33 °C and 180 $r \cdot min^{-1}$ for 24 h. OD600 and pH were then measured. Uninoculated blank medium was used as the control; three replicates were set for each pH group, and alkali-reducing ability was calculated according to the formula.

Calculation formula for alkali-reducing ability of the strain:

$$\eta = \frac{pH_{before} - pH_{after}}{pH_{before}} \times 100\% \quad (2)$$

1.9 Data analysis

Experimental data are expressed as mean ± standard deviation (SD). IBM SPSS Statistics 27 software was used for statistical analysis. Multiple means were compared by one-way analysis of variance (One-Way ANOVA), with $P < 0.05$ as the criterion for significant differences. For the extracellular polysaccharide extraction amount and content indicators at different times, least significant difference testing and Tamhane' s T2 test were further performed. Origin 2018 software was used for plotting.

2 Results and Analysis

2.1 Isolation, purification, and identification of nitrogen-fixing bacterial strains

The collected soil was subjected to dilution spread-plate culture on Ashby medium, streak purification, and 16S rRNA gene molecular identification. Ultimately, 13 strains of nitrogen-fixing types were obtained. Colonies on the plates were selected···

growth, viscous colonies, stringiness, brownish color, and a viscous fermentation broth was selected for subsequent experiments. The morphology of the strain is shown in Fig. 1.

Fig. 2 Evolutionary analysis of *Neorhizobium alkalisoli* NaXJIPC-GNJ0001

Figure 2: Fig. 2 Evolutionary analysis of *Neorhizobium alkalisoli* NaXJIPC-GNJ0001

Fig. 1 Morphological characteristics of *Neorhizobium alkalisoli* NaXJIPC-GNJ0001

The selected strain was subjected to 16S rRNA gene amplification, electrophoretic detection, sequencing, and evolutionary analysis (Fig. 2). It was found to share 99.20% homology with the alkali-soil rhizobium *Neorhizobium alkalisoli* strain TRM85146. Therefore, this strain was designated as alkali-soil rhizobium (*Neorhizobium alkalisoli*), with the code NaXJIPC-GNJ0001. The strain was deposited on January 20, 2025, at the General Microbiology Center of the China Committee for Culture Collection of Microorganisms, with the deposit number CGMCC NO 30470 and the NCBI accession number PQ804431.

2.2 Effects of incubation time on extracellular polysaccharides and protein

As shown in Table 1, as the incubation time increased from 24 h to 120 h, the extraction amount of extracellular polysaccharides increased significantly from 261.67$\pm$1.98$mg \cdot L^{-}1(groupe)to2302.0\pm2.85mg \cdot L^{-}1(groupa).Thepolysaccharidecontentsimultaneouslyincreasedfrom66.20\pm0.68\pm\$1.02\%$ (group a), exhibiting a time-dependent "yield-purity synergistic improvement" effect, with significant differences among groups (P<0.05).

The change in protein content showed stage-specific characteristics: from 24-48 h, it increased from 7.86%$\pm$3.69$\pm$0.60$\pm$0.76$\pm$1.91$\pm$\$1.81%),

Fig. 2 Evolutionary analysis of *Neorhizobium alkalisoli* NaXJIPC-GNJ0001

Table 1 Effect of strain incubation time on exopolysaccharides extraction and content and protein

Treatment	Strain incubation time/h	Exopolysaccharide extraction amount/(mg · L^-1)	Polysaccharide content/%	Protein content/%
Strain culture broth	24	261.6667$\pm$1.9757e\|66.1988$\pm$0.6803e\|7.8643$\pm$3.6941b||*Strainculturebroth*|48|11		
		0.3925$\pm$1.9098\|\|*Strainculturebroth*\|120\|2302.0$\pm$2.8478a\|86.5671$\pm$1.0206a\|$-$		
		1.5702$\pm$1.8075\|\|*Spray-driedsolidbacterialagent*\|120\|\|11.23$\pm$0.33\|1.51$\pm$\$0.06		

Note: Different letters indicate significant differences among different incubation times (P<0.05). The same applies below.

This negative value does not represent an actual loss of protein; rather, it is caused by steric-hindrance interference from highly purified polysaccharides with the detection method (such as the Coomassie brilliant blue method), indirectly corroborating the improvement in polysaccharide purity.

The extraction amount and sugar content of extracellular polysaccharides showed an increasing trend with incubation time (Fig. 3), and both reached their maximum values at 120 h, indicating that this time point is the optimal fermentation duration for extracellular polysaccharide production. Compared with the 120 h spray-dried solid-state bacterial agent, its polysaccharide content ($11.23\% \pm 0.33\%$) and protein content ($1.51\% \pm 0.06\%$) were significantly lower than those of the extracellular polysaccharide extract, highlighting the enrichment effect of the separation and purification process on polysaccharides. These patterns reveal a "culture-time-driven product purification mechanism" and provide key parameter support for the large-scale preparation of extracellular polysaccharides from nitrogen-fixing bacteria.

Ultraviolet spectrophotometry (UV), as a commonly used method for chemical-component analysis, can be used in polysaccharide identification to analyze residual impurities such as proteins and nucleic acids through characteristic absorption peaks. UV scanning results for the 120 h extracellular polysaccharides and the 120 h spray-dried solid-state bacterial agent over the range of 185–400 nm (Fig. 4a) showed that both samples exhibited ultraviolet absorption, but the absorption intensity and peak positions differed. Both showed obvious absorption peaks at around 200 nm, corresponding to the characteristic absorption of carbohydrates; thereafter, the absorption intensity gradually decreased and became nearly flat around 280 nm. Among them, the 120 h spray-dried solid-state bacterial agent showed weak absorption peaks in the 260–280 nm region, suggesting that it may contain small amounts of protein or nucleic acid impurities.

Fourier-transform infrared spectroscopy (FT-IR) can be used to analyze glycosidic bonds, types of functional groups, and compound composition. FT-IR analysis of extracellular polysaccharides and the spray-dried solid-state agent prepared from fermentation broth over the range of 4000–600 cm^-1 (Fig. 4b) showed marked spectral differences: the spectrum of extracellular polysaccharides was smooth overall, whereas the spray-dried solid-state bacterial agent showed multiple absorption peaks in regions such as 1600–1700 cm^-1, 1400 cm^-1, and 800–900 cm^-1, indicating that it was an unpurified complex mixture. Analysis of the characteristic peaks showed that the strong absorption at 3400 cm^-1 corresponded to the stretching vibration of hydroxyl groups (–OH), and that at 2900 cm^-1 corresponded to aliphatic C–H bond vibration; the 1600–1700 cm^-1 region was associated with carbonyl groups (C=O), possibly indicating ester or carboxylic acid compounds; the peak at 1400 cm^-1 corresponded to C–H bending vibration, and the peaks at 800–900 cm^-1 corresponded to sugar-ring structures

Fig. 3 Effect of incubation time on the extraction amount and content of

exopolysaccharides

- **(a)** Extracellular polysaccharide extraction amount/(mg · L^-1) versus strain incubation time/h.
- **(b)** Extracellular polysaccharide content/% versus strain incubation time/h.
- Note: Different letters indicate significant differences among different incubation times ($P < 0.05$).

Fig. 4 UV full-wavelength scan and FT-IR spectra of extracellular polysaccharides and the spray-dried solid-state bacterial agent (120 h)

- **(a)** UV spectrum: absorbance versus wavelength/nm. Labels: extracellular polysaccharides; fermentation broth.
- **(b)** FT-IR spectrum: transmittance/% versus wavenumber/cm^-1. Labels: extracellular polysaccharides; spray-dried solid-state bacterial agent; -OH; C-H; C=O; C-O.

structures, the glycosidic bonds that support polysaccharide chains, and alkynyl characteristics; C-O vibrations in the 1000-1200 cm^{-1} region are associated with ether bonds or sugar-ring structures. The complexity of the spectrum of the spray-dried solid bacterial preparation may arise from coexisting free compounds such as polysaccharides, fatty acids, amines, or amides.

2.3 Effects of Drying Method and Storage Conditions on the Activity of Strains in Solid Bacterial Preparations

2.3.1 Effect of drying method on the activity of strains in solid bacterial preparations

As can be seen from Table 2, different drying methods showed significant differences in drying efficiency, appearance, and immediate activity. In terms of drying duration, spray drying required only about 45 min to process 1 L of fermentation broth, and its efficiency was significantly higher than that of hot-air drying (50 ℃, 360 min) and freeze drying (1440 min), giving it a clear advantage in time cost. In terms of appearance, the spray-dried sample was a uniform, dense dry powder, convenient for storage and use; the hot-air-dried sample tended to agglomerate into blocks and could be used only after grinding and pulverization, making operation cumbersome; the freeze-dried sample was flocculent, light, and easily dispersed, which was not conducive to precise application. The immediate activity assay showed that the viable count of the spray-dried solid bacterial preparation reached 4.60×10^{15} CFU · mL^{-1}, significantly higher than that of hot-air drying (2.67×10^{10} CFU · mL^{-1}) and freeze drying (1.75×10^{6} CFU · mL^{-1}), giving the best performance in retaining bacterial activity.

A comprehensive analysis of drying duration, appearance, immediate activity, and room-temperature storage time (Table 2) showed that different drying methods had significant effects on the physical form and biological activity of the solid bacterial preparation. Among them, spray drying performed best in terms of immediate activity and long-term retained activity, whereas freeze drying performed relatively poorly in terms of drying duration and immediate activity.

2.3.2 Effect of storage conditions on the activity of strains in spray-dried solid bacterial preparations

The effect of storage conditions on the activity of strains in spray-dried solid bacterial preparations was further investigated. The spray-dried solid bacterial preparations were stored under dry conditions at room temperature, −4 ℃, −20 ℃, and −40 ℃. Samples were taken after 1, 6, 12, 18, and 24 months of storage to determine the viable counts; the results are shown in Table 3.

As can be seen from Table 3, as storage time increased, strain activity decreased under all temperature conditions. Strain activity declined significantly under room-temperature conditions, whereas the decrease was smaller at lower temperatures—especially −20 ℃ and −40 ℃—indicating that low-temperature storage helped maintain strain activity. At room temperature, strain activity decreased from 3.50×10^{15} CFU · mL^{-1} at 1 month to 2.00×10^{7} CFU · mL^{-1} at 24 months. By contrast, at −40 ℃, strain activity decreased from 4.40×10^{15} CFU · mL^{-1} at 1 month to 2.70×10^{12} CFU · mL^{-1} at 24 months, a decline markedly smaller than under room-temperature conditions. This indicates that low-temperature storage plays an important role in maintaining the activity of strains in spray-dried solid bacterial preparations; moreover, the lower the storage temperature, the slower the decline in strain activity and the better the storage effect. These data are of great significance for understanding the effects of storage conditions on the activity of strains in spray-dried solid bacterial preparations, and provide a scientific basis for the long-term preservation and application of bacterial preparations.

2.4 Analysis of Functional Characteristics of the Nitrogen-Fixing Bacterial Strain

To investigate the growth characteristics, metabolic patterns, and environmental adaptability of the alkaliphilic soil *Neorhizobium* strain NaXJIPC-GNJ0001, its growth characteristics at different culture times (2–168 h) were analyzed. The results (Fig. 5) showed that the logarithmic growth phase of this strain was 8–26 h, and growth tended to stabilize by 36 h.

In the salt-tolerance test, observations in liquid nitrogen-fixing medium containing 1%–20% NaCl (Fig. 6a) showed that the alkaliphilic soil *Neorhizobium* could grow in environments with NaCl concentrations below 15%. According to the classification standard for halophilic bacteria[25] (extremely halophilic bacteria correspond to NaCl concentrations of 14.63%–30.40%), this bacterium

Table 2 Comparison of the characteristics of solid bacterial preparations obtained by three drying methods

Tab. 2 Characteristics of three drying methods for obtaining solid fungicides

Drying method	Drying time/(min · L^{-1})	Appearance	Immediate activity/(CFU · mL^{-1})	Activity after 6 months at room temperature/(CFU · mL^{-1})
Spray drying	45	Dense dry powder	$4.60 \times 10^{15} \pm 0.5752$a	$2.40 \times 10^{13} \pm 0.3656$a
Hot-air drying	360	Agglomerated into blocks; requires grinding and pulverization before use	$2.65 \times 10^{10} \pm 0.2654$b	$3.75 \times 10^{6} \pm 0.4195$b
Freeze drying	1440	Flocculent, light, and easily dispersed	$1.75 \times 10^{6} \pm 0.3916$c	$3.25 \times 10^{3} \pm 0.8612$c

Note: Data are means ($n = 3$). The same applies below.

Table 3 Effect of storage conditions on the activity of strains in spray-dried solid bacterial preparations

Tab. 3 Effect of storage conditions on the activity of spray-dried solid bacterial strains /(CFU · mL^{-1})

Storage condition	Spray-dried solid bacterial preparation storage time—1 month	Spray-dried solid bacterial preparation storage time—6 months	Spray-dried solid bacterial preparation storage time—12 months	Spray-dried solid bacterial preparation storage time—18 months	Spray-dried solid bacterial preparation storage time—24 months
Room temperature	$3.50 \times 10^{15} \pm 0.2648$a	$2.40 \times 10^{13} \pm 0.3656$b	$4.50 \times 10^{12} \pm 0.7604$c	$4.90 \times 10^{10} \pm 0.4329$d	$2.00 \times 10^{7} \pm 0.2058$e

Storage condition	Spray-dried solid bacterial preparation storage time—1 month	Spray-dried solid bacterial preparation storage time—6 months	Spray-dried solid bacterial preparation storage time—12 months	Spray-dried solid bacterial preparation storage time—18 months	Spray-dried solid bacterial preparation storage time—24 months
−4 ℃	$3.40 \times 10^{15} \pm$ 0.3604a	$5.00 \times 10^{15} \pm$ 1.0739a	$3.30 \times 10^{14} \pm$ 0.4347b	$1.50 \times 10^{12} \pm$ 0.7602c	$3.20 \times 10^{10} \pm$ 0.2947d
−20 ℃	$3.80 \times 10^{15} \pm$ 0.5642a	$3.70 \times 10^{15} \pm$ 0.2874a	$3.60 \times 10^{15} \pm$ 0.4583a	$3.30 \times 10^{15} \pm$ 0.5604a	$2.50 \times 10^{12} \pm$ 0.4702b
−40 ℃	$4.40 \times 10^{15} \pm$ 0.6647a	$4.30 \times 10^{15} \pm$ 0.4608a	$4.10 \times 10^{15} \pm$ 0.6306a	$3.90 \times 10^{15} \pm$ 0.3045a	$2.70 \times 10^{12} \pm$ 0.8305b

Fig. 5 Growth characterization curve of *Neorhizobium alkalisoli* NaXJIPC-GNJ0001

Fig. 5 Growth characterization curve of *Neorhizobium alkalisoli* NaXJIPC-GNJ0001

Fig. 6 Salt and alkali tolerance of *Neorhizobium alkalisoli* NaXJIPC-GNJ0001

Fig. 6 Salt and alkali tolerance of *Neorhizobium alkalisoli* NaXJIPC-GNJ0001

The strain belongs to the extreme halophiles and exhibits relatively strong salt tolerance.

The results of alkali tolerance and alkali-reducing ability analyses (Fig. 6b, Table 4) showed that *Neorhizobium alkalisoli* could grow in a strongly alkaline environment of pH 12, indicating significant alkali tolerance; meanwhile, its alkali-reducing capacity reached 29%. For strain Ba-chu49, the alkali-reducing rates at pH 8.0, 9.0, and 10.0 were 9.75%,

Table 4 Alkali-reducing properties of *Neorhizobium alkalisoli* NaXJIPC-GNJ0001

Tab. 4 Alkali-reducing properties of *Neorhizobium alkalisoli* NaXJIPC-GNJ0001

pH before inoculation	pH after inoculation	Alkali-reducing rate/%
7	6.74$±0.351|3.79±0.5017||8|7.21±0.163|9.94±0.2032||9|7.46±0.337|17.17±0.3747||1	

15.56% and 20.60%[26], respectively, whereas salt-tolerant *Citrobacter* showed the strongest alkali-reducing ability under pH 8.5, 9.0, and 9.5 conditions, with alkali-reducing rates of 14.5%, 13.2%, and 13.9%[27], respectively. This indicates that the present strain has excellent alkali-reducing capacity.

3 Discussion

In this study, a strain of the alkali-soil rhizobium *Neorhizobium alkalisoli* NaXJIPC-GNJ0001 with high production of extracellular polysaccharides (EPS) was isolated from soil in the Salt Lake scenic area of Urumqi, Xinjiang. The dynamic changes in EPS during its culture and fermentation process were analyzed; the role of EPS in the activity and stability of nitrogen-fixing bacterial agents was explored; and the EPS-mediated microbial functional mechanisms and their application potential for soil improvement in arid regions were revealed.

The taxonomic basis of the strain is linked to environmental adaptability in arid regions. Strain taxonomic identification is the prerequisite for functional studies. Through 16S rRNA gene sequence analysis, the target strain showed 99.20% homology with *Neorhizobium alkalisoli* TRM85146, and its viscous bacterial colony and brown fermentation broth phenotypes were consistent with the characteristics of EPS-producing strains in the genus *Rhizobium*[28–29]. Notably, this strain was isolated from the extreme saline-alkaline environment of a salt-lake scenic area (total soil salt content >1.5%, annual precipitation <200 mm). Its habitat is highly consistent with the "high salt–strong alkalinity–drought" combined stress characteristics of typical saline-alkaline soils in arid regions. Its systematic phylogenetic position suggests that the genus *Neorhizobium* may adapt to high-salt stress in arid regions through specialized metabolic pathways. This is consistent with the unique evolutionary branch characteristics of rhizobia in saline-alkaline habitats[17–19], and provides new clues for the development of nitrogen-fixing microbial resources for extreme environments in arid regions.

The synergistic effect between EPS yield and purity reflects an adaptation mechanism for arid regions. This study found a "time-dependent synergistic increase in yield and purity" phenomenon for EPS. During 24–120 h of cultivation, the EPS extraction amount increased nearly 9-fold ($261.67\$ \rightarrow 2302.0 mg \cdot L < sup > -1 < /sup >$), $and the polysaccharide content increased by 30.5 \rightarrow \$86.57\%$), while protein detection showed negative values after 96 h (Table 1). This effect arises from a dual mechanism: (1) biosynthetic kinetics: before 72 h, EPS and protein accumulated synchronously, reflecting metabolic activity during the logarithmic growth phase; in the later stage, the degree of EPS polymerization increased, and its three-dimensional network encapsulated protein impurities through hydrogen bonding, interfering with the Coomassie brilliant blue assay[30]. This pattern is consistent with the EPS synthesis mechanism of nitrogen-fixing bacteria such as *Burkholderia*, in which the carboxyl groups (−COOH) and hydroxyl groups (−OH) of repeating units

form steric barriers[31]. (2) Response to environmental adaptability in arid regions: to resist saline-alkaline stress in arid regions, the strain preferentially synthesizes acidic polysaccharides containing galactose–mannose, maintaining osmotic balance by adsorbing water molecules via carboxyl groups, while hydroxyl groups enhance intermolecular hydrogen bonding to reduce water loss[4]. In the FT-IR spectrum, the bands at 3400 cm−1 (−OH stretching vibration) and 1600 cm−1 (C=O)

confirmed this mechanism (Fig. 4b). EPS purity was further verified by ultraviolet (UV) and Fourier-transform infrared spectroscopy (FT-IR): UV showed that EPS had a typical carbohydrate absorption peak at 200 nm, whereas the weak peaks of the spray-dried solid inoculant at 260–280 nm indicated residual nucleic acids (Fig. 4a); in the FT-IR spectrum, the characteristic EPS peak (2900 cm^{-1}, C–H vibration) was clear, while the multiple peaks of the spray-dried solid inoculant at 1600 $\sim$ 1700 cm^{-1} reflected the complexity of the mixture (Fig. 4b). This result highlights the critical role of the separation and purification process in EPS enrichment; its high purity may reduce the inhibitory effect of drought stress on nitrogen-fixation activity by promoting biofilm formation.

Optimization of the compatibility of the solid inoculant process for applications in arid regions. The industrial application of solid inoculants depends on efficient preparation and stable storage technologies. A comparison of three drying methods showed that spray drying (45 $\min \cdot L^{-1}$) was significantly superior to hot-air drying and freeze drying in maintaining strain viability (4.60×10^{15} $CFU \cdot mL^{-1}$) and shortening treatment time, consistent with the mechanism that "rapid low-temperature atomization reduces damage to bacterial cells"[14–15]. Storage tests showed that inoculant viability declined most slowly under −20 °C and −40 °C; after 24 months it still remained at $2.50 \times 10^{12} \sim 2.70 \times 10^{12}$ $CFU \cdot mL^{-1}$, whereas viability during room-temperature storage decreased by 6 orders of magnitude. This was associated with low-temperature inhibition of enzymatic reactions and protection by the glassy structure of polysaccharides[13]. This result provides a theoretical basis for natural low-temperature storage of inoculants during cold seasons in arid regions, such as winter in northern Xinjiang.

EPS-mediated mechanisms for remediation of saline–alkaline soils in arid regions. Compared with traditional inoculants, high-purity EPS endows nitrogen-fixing bacteria with three stress-resistance capabilities: (1) Biofilm protection: UV and FT-IR analyses showed that purified EPS eliminated the nucleic-acid absorption peak at 260–280 nm (Fig. 4a), effectively isolating environmental toxins[3–5]. Similarly, the EPS of *Rhizobium leguminosarum* can assemble the biofilm matrix through the RapA protein to resist chemical stress[32]. In arid regions such as Xinjiang, the barrier function of EPS can resist osmotic damage to bacterial cells caused by high concentrations of Na^+, thereby prolonging the survival period of the inoculant in severely saline–alkaline soils with total salt $> 1\%$. (2) Soil structure improvement: the EPS of this strain contains 86.57% polysaccharides, and its high-molecular-weight chains can bridge soil particles. Studies have shown that microbial EPS increases soil macroaggregates (> 0.25 mm) by 4.07-fold and

significantly improves water retention[2,6], providing a new approach to solving the problems of "difficulty in retaining water and easy fertilizer loss" caused by soil compaction in arid regions (bulk density $> 1.6\ \mathrm{g \cdot cm^{-3}}$). (3) Enhancement of symbiotic nitrogen fixation: EPS-deficient mutants of *Mesorhizobium tianshanense* lose the ability to infect licorice[33], suggesting that the EPS of this strain may improve the efficiency of vegetation restoration in saline–alkaline land by strengthening symbiosis with salt-tolerant plants in arid regions, such as alfalfa and reed.

Demonstration of environmental adaptability and field-application potential in arid regions. Strain NaXJIPC-GNJ0001 could still grow under 15% NaCl and pH 12 conditions, and its alkalinity-reduction rate in an initial pH 11 environment reached 29.18%; its saline–alkaline tolerance was significantly superior to that of ordinary nitrogen-fixing bacteria[15]. This characteristic derives from: (1) the EPS hydration layer reducing osmotic-pressure damage to the cell membrane; and (2) the production of organic acids through metabolism to regulate microenvironmental pH[29]. This is highly compatible with the dynamic feature of saline–alkaline soils in arid regions, namely "alternating high salinity and high alkalinity," and can reduce the inhibition of inoculant function caused by seasonal surface accumulation of salts. Field trials showed that, after inoculation with a saline–alkaline-tolerant inoculant, the emergence rate of cotton in saline–alkaline land increased by 20%, seedling survival exceeded 90%, total soil salt decreased by 36.05%, and available nitrogen increased by 52.8%[34–35]; the growth-promoting effect of *Ensifer* sp. GMS14 in saline soil was also significant[36]. In addition, the combined application of saline–alkaline-tolerant strains with indigenous salt-tolerant microorganisms from arid regions, such as *Bacillus* HM-311, can synergistically enhance soil remediation, providing a reference for constructing region-specific remediation microbial consortia[37–38].

Although phased achievements have been obtained, the following limitations remain: (1) Fermentation-process cost: the 120 h fermentation period is too long, and EPS extraction energy consumption accounts for 62% of production cost[39–40]. It is recommended to use a gradient-altitude screening method to select and breed fast-growing strains from arid regions. (2) Soil adaptability: the pH gradient in arid regions is pronounced (8.5–10.2), and research on region-specific inoculant formulations is needed[1]. Future work should integrate transcriptomics to elucidate EPS biosynthesis pathways, use gene-editing technologies such as CRISPR to directionally modify strains, improve their nitrogen-fixation efficiency and colonization capacity in extreme environments[13,35], and verify through field trials the potential for scaled application of composite inoculants in the improvement of saline–alkaline land.

In summary, the high extracellular-polysaccharide yield, efficient inoculant-preparation process, and strong stress adaptability of the alkaline-soil neorhizobial strain NaXJIPC-GNJ0001 provide a new approach for the bioremediation of saline–alkaline soils in arid regions. Subsequent studies should further analyze the relationship between the chemical structure and function of extracellular

polysaccharides, develop a technical chain of "native strain bank–customized inoculant–ecological evaluation,"and verify through field trials its practical efficacy in increasing crop yield and improving soil.

4 Conclusion

In this study, one strain of alkaline-soil neorhizobia, *Neorhizobium alkalisoli* NaXJIPC-GNJ0001 (preservation number CGMCC No.30470), was isolated and identified; its 16S rRNA showed 99.20% homology with strain TRM85146. After 120 h of cultivation, the extracellular polysaccharide (EPS) extraction yield of this strain reached $2302.0 \pm 2.85\ \mathrm{mg \cdot L^{-1}}$, with a purity of $86.57\% \pm 1.02\%$, exhibiting a "yield–purity synergistic improvement" effect. Ultraviolet and infrared spectra confirmed that the EPS was rich in functional groups such as hydroxyl and carboxyl groups, laying a structural foundation for environmental adaptation. The viable count of the solid inoculant prepared by spray drying ($45\ \mathrm{min \cdot L^{-1}}$) reached $4.60 \times 10^{15}\ \mathrm{CFU \cdot mL^{-1}}$; after 24 months of storage at $-40 \sim -20\ ^{\circ}\mathrm{C}$, viability still remained at $2.50 \times 10^{12} \sim 2.70 \times 10^{12}\ \mathrm{CFU \cdot mL^{-1}}$, indicating significant process-optimization effects. Functional analysis showed that the strain could grow in 15% NaCl

and could grow in a pH 12 environment; in an initial pH 11 environment, the alkalinity-reduction rate reached 29.18%, indicating strong stress adaptability. In summary, the high EPS yield of this strain, the optimized inoculant preparation process, and its strong salt–alkali tolerance provide important support for the bioremediation of saline–alkali soils in arid regions.

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Characteristics of exopolysaccharides from *Neorhizobium alkalisoli* in arid region and their saline-alkali soil remediation potential

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Abstract: To screen for highly efficient nitrogen-fixing bacteria suitable for use in the bioremediation of saline-alkali soils in arid regions and to elucidate the functional effects of exopolysaccharides (EPS), nitrogen-fixing bacteria were isolated and purified from saline-alkali soils of the Salt Lake in Urumqi, Xinjiang. Taxonomic identification was conducted using 16S rRNA gene sequencing. The influence of culture duration on yield and purity of EPS was investigated, the drying and storage conditions of bacterial inoculants were optimized, and the salt-alkali tolerance and alkali-reducing properties of the strains were analyzed. A strain with 99.20% similarity in the 16S rRNA gene

sequence to strain TRM85146 was isolated and designated as *Neorhizobium alkalisoli* NaXJIPC-GNJ0001. This strain, deposited under the accession number CGMCC No. 30470, exhibited a time-dependent synergistic enhancement of yield and purity: after cultivation for 120 h, the yield of EPS reached 2302.0 ± 2.85 mg $\cdot$ L^{-1} and the polysaccharide purity was $86.57\% \pm 1.02\%$. UV-Vis and Fourier transform infrared (FT-IR) spectroscopy confirmed that EPS were enriched in functional groups such as hydroxyl and carboxyl groups, indicating a structural basis for the occurrence of environmental adaptation. Viable cell counts of solid inoculants prepared via spray drying at 45 min $\cdot$ L^{-1} reached 4.60×10^{15} CFU $\cdot$ mL^{-1}. After 24 months of storage at -40 ℃ to -20 ℃, the viability remained stable at 2.50×10^{12}–2.70×10^{12} CFU $\cdot$ mL^{-1}, a superior result to other drying methods. Functional assays revealed that the strain could grow in medium containing 15% NaCl and at pH 12, and the alkali reduction rate was 29.18% in medium with an initial pH of 11, indicating strong adaptability to adverse environments. The high EPS-producing capacity of the strain, optimized inoculant preparation process, and excellent salt-alkali tolerance provide valuable microbial resources and technical insights that can support the bioremediation of saline-alkali soils in arid regions.

Keywords: arid region; *Neorhizobium alkalisoli*; exopolysaccharides (EPS); saline-alkali soil remediation; solid-state bacterial

Note: Figure translations are in progress. See original paper for figures.