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Research Letter **Open Access**

Isolation and Characterization of An Alkaliphilic and Halotolerant *Nesterenkonia.sp* from An extreme Soda Saline-alkali Soil in the Northeastern of China

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Abstract A strain of alkaliphilic and halotolerant, rod-shaped bacterium, designated strain SA−3, was solated from an Extreme soda saline-alkali soil in Heilongjiang, northeastern China. Strain SA−3 was Gram-positive, catalase-positive, and oxidase-negative and formed Orange colonies, and starch is not hydrolyzed, optimal growth conditions were pH 9.0 and 0.5 mol/L NaCl. Strain SA−3 has strong resistance to Na_2CO_3, hight pH value, and NaCl. 16S rRNA a gene sequence alignment analysis show that the strain formed a separated line within a clade containing the genus *Nesterenkonia* in the phylum Actinobacteria and was related to the species *Nesterenkonia* lutea YIM 70081^T (sequence similarity 99.2%), *Nesterenkonia sandarakina* YIM 70009^T (99.2%), *Nesterenkonia jeotgali* JG−241^T (99.1%), *Nesterenkonia halotolerans* YIM 70084^T (99.0%), *Nesterenkonia aethiopica* DSM 17733^T (97.5%). This similarity, suggest that this salt-tolerant alkaliphilic bacterium belonged to genus *Nesterenkonia*. This strain is a great potential microbial resources for development.

Keywords *Nesterenkonia*; 16S rDNA sequence

Background

Nesterenkonia as a genus was put forward by Stackebrandt (Stackebrandt et al., 1995). Which be classified as which be classified as one genus of common bacterial form and hight G+C content Actinobacteria, Currently, the genus contai eleven species: *Nesterenkonia halobia*, *Nesterenkonia lacusekhoensis*, *Nesterenkonia halotolerans*, *Nesterenkonia xinjiangensis*, *Nesterenkonia lutea*, *Nesterenkonia sandarakina*, *Nesterenkonia aethiopica*, *Nesterenkonia jeotgali*, *Nesterenkonia halophila* and *Nesterenkonia flava*, *Nesterenkonia alba* (Luo et al., 2009). Most of this genus, were isolated from hypersaline or alkaline environments, maily are the actinomycete group with the ability of alkali resistance properties.

As enzymes and antibiotics resources, Alkaliphilic actinomycetesget more attention of scholars dedicated to research in this area, and find several new agents and antibiotics. In order to develop the more effective use of such extreme environmental actinomycetes, we surveyed the halophilic bacteria in the regions of no perennial plant covering and pH 10.5 of extreme soda saline-alkali soil lands in Heilongjiang, northeastern China. A sum of 52 aerobic halophile strains was isolated from there, including three actinomycetes, one was identified from in the growth of actinomycetes under the pH 11.0 conditions. According to morphological, phenotypic characteristics and 16S rDNA sequence analysis, we identified taxonomy and phylogenetic position of this strain.

1 Results and Analysis

1.1 Phylogenetic analysis

Strain SA−3 was Gram-positive, catalase-positive, and oxidase-negative, starch is not hydrolyzed, mean size 0.56 μm×0.73 μm (Figure 1A). Colonies were circular, convex, moist, opaque, 7.5 mm wide, smooth and Orange on medium I (Figure 1B).

1.2 Resistance characteristics of the analysis

E.coli JM109 as a control, the strain SA−3 was

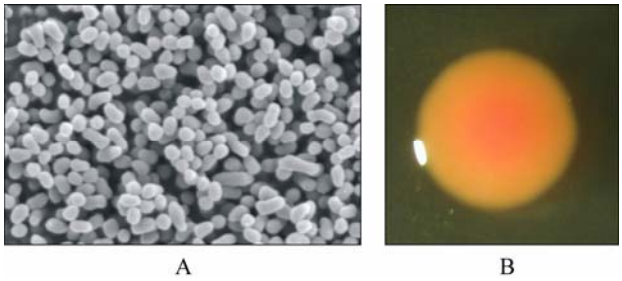

Figure 1 Scanning electron microscope (×10 000) and colony photos of strain SA−3

used in the analysis of resilience analysis showed (Figure 2): In the tough environment of Na_2CO_3, strain SA−3 growth occurred in media containing 0~250 mmol/L Na_2CO_3 and optimal growth were observed in media containing 30~100 mmol/L Na_2CO_3. On the condition of Na_2CO_3 not lower than 30 m we can't find *E.coli* JM109 grows; In the tough environment of NaCl, strain SA−3 growth occurred in media containing 0~2.0 mol/L NaCl and optimal growth was observed in media containing 0~0.5 mol/L. *E.coli* JM109 was grew in the media containing 0.0~0.5 mol/L NaCl, which is not grown in the media containing up to 0.5~2.0 mol/L NaCl; In the tough environment of pH value, strain SA−3 grew at pH 7.0~11.0, that showed optimal growth was pH 8.0~9.0, *E.coli* JM109 grew at pH 7~8, severely inhibited the growth of *E.coli* JM109 grew at pH 9~12. In short, in the tough environment of Na_2CO_3, pH value, NaCl, the strain SA−3 was better than *E.coli* JM109 on the salt and alkali resistances.

1.3 Phylogenetic analysis

Phylogenetic alignment analysis of the partial 16S rRNA gene sequence (GenBank databases under Accession Number JF937438) showed that the isolated SA−3 in a distinct lineage with *Nesterenkonia lutea* YIM 70081^T within the genus *Nesterenkonia* (Figure 3). These partial 16S rRNA gene sequences analysis suggested that strain SA−3 was most closely related to *Nesterenkonia lutea* YIM 70081^T (sequence consistency: 99.2%), *Nesterenkonia sandarakina* YIM 70009^T (99.2%), *Nesterenkonia jeotgali* JG-241^T (99.1%), *Nesterenkonia halotolerans* YIM 70084^T

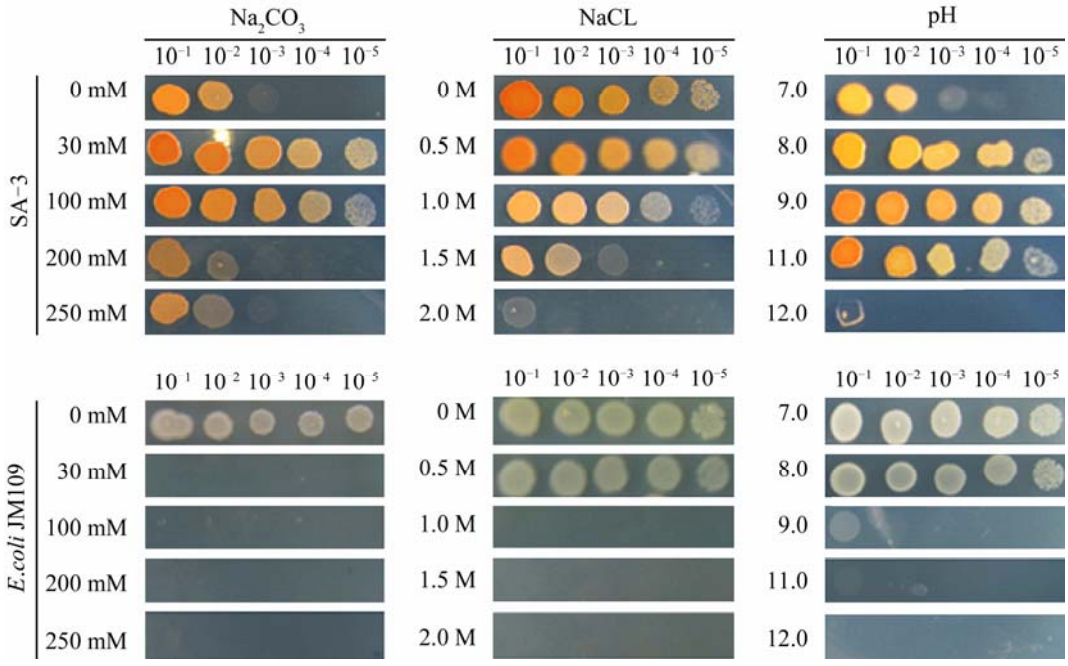

Figure 2 Resilience analysis of strain SA−3 and *E.coli* JM109

Note: The bacteria were grown in liquid medium until OD_{600}≈0.6, and then diluted 10^{-1}~10^{-5}. 3.5 microl liters of each dilution were inoculated to solid media Ⅱ and the LB medium supplemented with different concentrations of Na_2CO_3 (0~250 mmol/L)、NaCl (0~2.0 mmol/L) and varied pH (7~12). The bacteria were grown at 28℃ for 48 h with the monitor of the growth. In *E.coli* JM109 as a control, in addition to NaCl resistance experiments outside the medium pH adjustment 7.0, the remaining conditions are the same culture

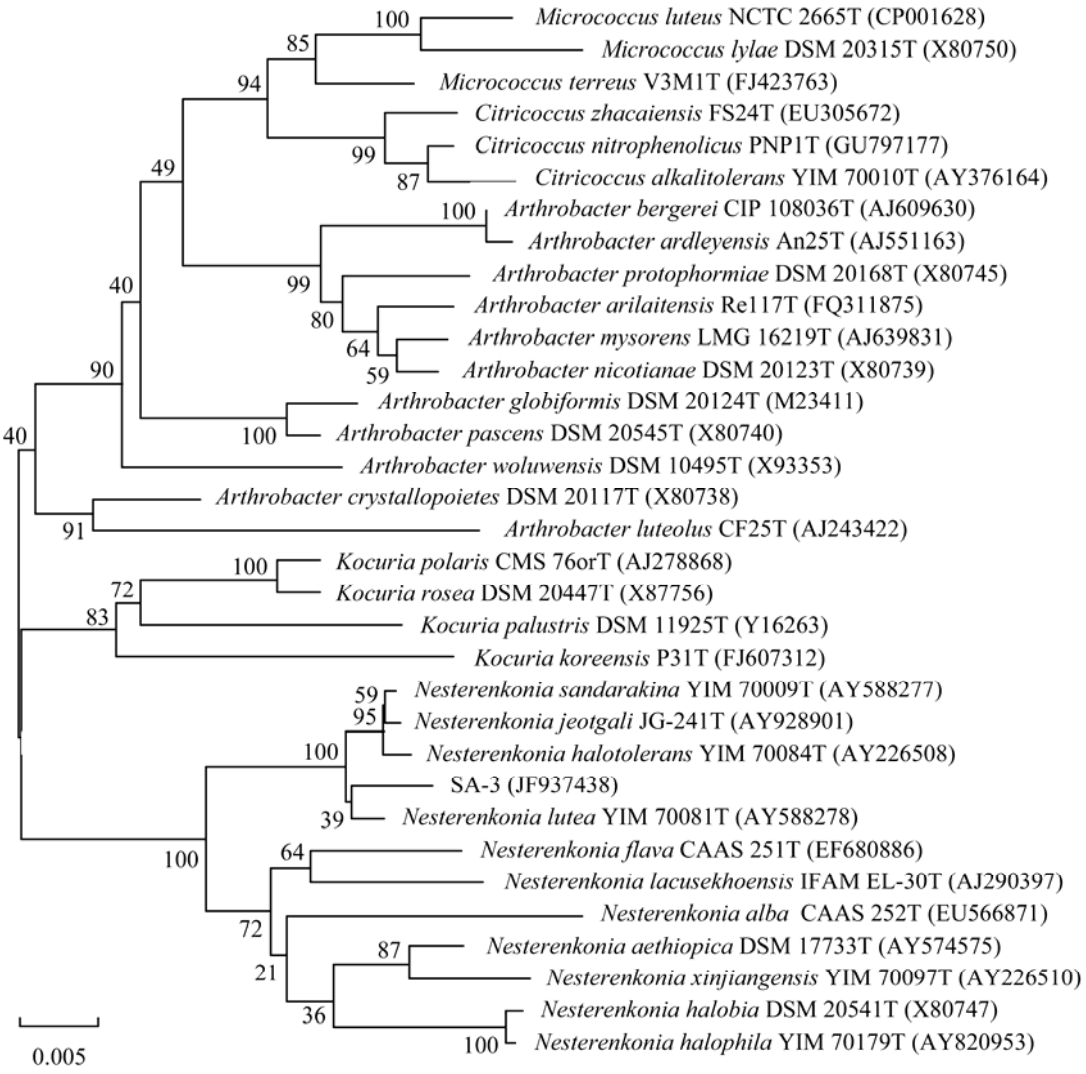

Figure 3 16S rRNA sequence phylogenetic tree

Note: Phylogenetic trees showing the relationships between strain SA−3 and related bacteria based on 16S rRNA gene sequences alignment analysis. Bootstrap values from 1 000 replicates are included, the scale bar corresponds to 0.005−estimated nucleotide substitution per sequence

(99.0%), *Nesterenkonia aethiopica* DSM 17733^T (97.5%), the highest similarity, higher than 97%. The results show that the strain SA−3 is belonged to genus *Nesterenkonia*.

Summary, the strain SA−3 was isolated from Extreme soda saline-alkali soil. It was Gram-positive, catalase-positive, and oxidase-negative and formed Orange colonies, and starch is not hydrolyzed, which is a member of the genus *Nesterenkonia*. In *E.coli* JM109 as a control, in the tough environment of Na_2CO_3, pH value, NaCl, the strain SA−3 was better than *E.coli* JM109 on the salt and alkali resistances. The strain SA−3 is salt-tolerant alkaliphilic actinomycetes. The salt-tolerant alkaliphilic actinomycetes is a particular taxa of actinomycete grow in extreme environment, which can produce alkaline proteases, amylases, cellulases and lipases that having unique properties under extreme conditions. These biocatalysts Widely used in industrial production. Wherefore the strain SA−3 have a great potentiality to develop.

2 Materials and Methods

2.1 Micro-organisms and culture conditions

In the couse of the survey of cultured bacteria of alkaline environments in heilongjiang, northeastern

China, strain H1 was isolated from a soil sample collected from the Extreme soda saline-alkali soil in Songnei Plain. The strain was isolated by the standard dilution-plating technique based on medium Ⅰ (KCl, 2.0 g; $MgSO_4 \times 7H_2O$, 1.0 g; NaCl, 30 g; Na_3-citrate, 3.0 g; yeast extract, 10.0 g; (1 mL/L of g/L) $MnCl_2 \times 4H_2O$, 0.36 g and $FeSO_4$, 50 g; agar, 15 g; distilled water, 1 000 mL, Media adjusted to the pH values 9.0) at 28℃. Strain H1 was subsequently maintained on medium Ⅰ slants and stored as 30% (v/v) glycerol suspensions at −80℃.

2.2 Phenotypic characteristics

Strain morphology was inspect by light microscopy (Olympus microscope BX41) and Scanning Electron Microscope (Hitachi model S-3400N). Determination of the Gram type using the standard Gram reaction and the KOH lysis test method (Oren et al., 1997), after incubation for 48 h on medium Ⅰ at 28℃. Degradation of gelatin, starch, and Catalase activity, oxidase activity et al were determined based on the protocols 'Research on soil microorganisms' (Institute of soil science, Chinese academy of sciences, and micro-organism lab, 1985, Science Press, pp 41-120).

2.3 Experimental bacterial resistance

The strain SA-3 was used in the analysis of resilience. The bacteria were grown in liquid medium until $OD_{600} \approx 0.6$, and then diluted $10^{-1} \sim 10^{-5}$. 3.5 microl liters of each dilution were inoculated to solid media Ⅱ (LB supplemented with 0.5 mol NaCl) and the LB medium supplemented with different concentrations of Na_2CO_3、NaCl and varied pH. The bacteria were grown at 28℃ for 48 h with the monitor of the growth. In *E.coli* JM109 as a control. In addition to NaCl resistance experiments outside the medium pH adjustment 7.0, The remaining conditions are the same as above.

2.4 Phylogenetic analysis of 16S rRNA gene sequences

Bacterium total genomic DNA was extracted and purified from bacteria according to the methods of Griffiths (2000). The 16S rRNA gene fragments were cloned as described by Shigematsu (Shigematsu et al., 2003). The sequence of forward universal primers used in PCR amplification was 27F (5`-AGAGTTTG ATCCTGGCTCAG-3`) and reverse primer was 1401R (5`-CGGTGTGTACAAGGCCC-3`). The reactions as followed: 95℃ for 2 min ; 35 cycles of 94℃ for 30 s, 54℃ for 40 s, and 72℃ for 2 min; 72℃ for 10 min. The 16S rRNA fragment was cloned into pMD18-Tvector (Takara, Biotechnology Co., Ltd) and transformed to competent cell JM109. The positive clones were identified by enzymatic digestion with *Hind*Ⅲ and *Bam*HⅠ and confirmed by PCR. The cloned 16S rRNA gene was sequenced. The 16S rRNA sequences were analysed by using BLAST program and Clustal X program (Thompson et al., 1997). 16S rRNA phylogenetic tree was created by using MEGA program version 3.1 (Saitou and Nei, 1987).

Authors' contributions

WS designed and conducted this experiment; TT participated the experiment design and data analysis; SKL is the person who takes charge of this project, including experiment design, data analysis, writing and modifying of the manuscript. All authors have read and approved the final manuscript.

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