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Preliminary Isolation of two Halotolerant Bacteria from Saline-Alkali Soil of Tianjin, China

Jiang Wu*, Yingkai Tong and Litong Ban

Department of Agronomy, Tianjin Agricultural University, Tianjin 300384, China.

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Halophiles are organisms that can live in environments with salt. Tianjin has many saline-alkali soils, but the microbial composition have not yet been explored. In the present study, twelve bacterial strains with tolerance to salt were isolated from aline-alkali soil of Tianjin. The result showed that the two extreme halotolerants were gram-negative bacillus and could ferment using glucose and lactose. The result of methyl red test showed that these two strains were methyl-red-negative. This preliminary study showed that Tianjin saline-alkali soils are rich in halotolerant bacteria, which could be used in many industrial applications areas.

Key words: Halophile, Gram's staining, sugar fermentation assay, methyl red test.

There are abundant of extremophiles living in the extreme environment on the earth. To understand the mechanism, many researchers had studied these microorganisms[1-3]. Extremophiles living in the extreme conditions have its unique physiological mechanism. Revelation these mechanisms could make people to better understand the extremophiles survival behavior and to further explore its development value.

The largest part of the earth is saline, including the oceans and some lands[4, 5]. Microorganisms living in saline environment can be either halophilic or halotolerant. A slight halophile can live in < 3% NaCl, a moderate halophile in 3-15% and an extreme halophile in > 15%[5-7]. Now many biotechnological applications have been found from halophilic microorganisms, such as β-carotene production in fermented foods[8, 9].

* To whom all correspondence should be addressed.
Tel.: +86 22 23781298; Fax: +86 22 23781298;
E-mail: wujiangjack@tjau.edu.cn

Tianjin is renowned for its saline-alkali soil in China. In this study, we separated the halophilic bacteria from saline-alkali soil of Tianjin. To explore novel halophilic bacteria, the physiological and biochemical characteristic had been researched.

MATERIALS AND METHODS

Sampling

The bacteria were separated from saline-alkali soil in Tianjin. All samples were collected into sterile centrifuge tubes and stored at 4 °C for subsequent experiments.

Isolation and enrichment of bacteria

In order to recover the halotolerant bacteria, enrichment procedures were performed on NaCl gradient broth medium. The appropriately diluted sample was spread on the plate containing 2%-17% of NaCl and incubated at 30 °C. The separated strains were cultured several times with the same situation to obtain the pure cultures. The strains, which had been purified, were stored at -80 °C for subsequent experiments[10].

Morphological studies of isolated bacteria

The purified strains were characterized on the basis Gram's staining, sugar fermentation assay and methyl red test. Cell morphology was observed by using microscope.

RESULTS

Screening of salt resistant microorganisms

The bacterium suspension of 10-3, 10-4, 10-5 dilution ratio were spread on the plate with 2% NaCl. After incubation for 24 h at 30 °C, only the plate spread by the bacterium suspension of 10-3 dilution ratio grew some little clones, and others not (Fig. 1).

The obtained clones were spread on 5% - 20% NaCl to screen the extreme halotolerant bacteria. Fig. 2 showed the clones on the 5% NaCl plates. There were some visible clones. After screened by 5% NaCl, the clones was subject to a higher concentration of NaCl. Fig. 3 showed the clones on the 10% NaCl plate, and Figure 4 showed two clones on the 16% NaCl plate. As a result, two clones of halotolerantbacteria was screened successfully.

Table 1. The 12 clones screened from saline-alkali soil.

Categorie	NaClconcentration/%	Number of clones
Non-halophilic bacteria	1. 17	0 (0%)
Slightly halophilic bacteria	1. 17- 2. 93	3 (25%)
Moderately halophilic bacteria	2. 93-14. 63	7 (58%)
Extreme halophilebacteria	14. 63- 30. 4	2 (17%)
Total	1. 17-30. 45	12 (100%)

Fig. 1. 10^{-3} dilution ratio on the plate for 24 h at 30°C

Morphological studies of isolated bacteria

Fig. 5 showed that these two halotolerant bacteria had the same colony morphologies with flat and round clone and tidy margin.

Gram's staining

Fig. 6 showed that these two halotolerant bacteria were gram-negative bacillus.

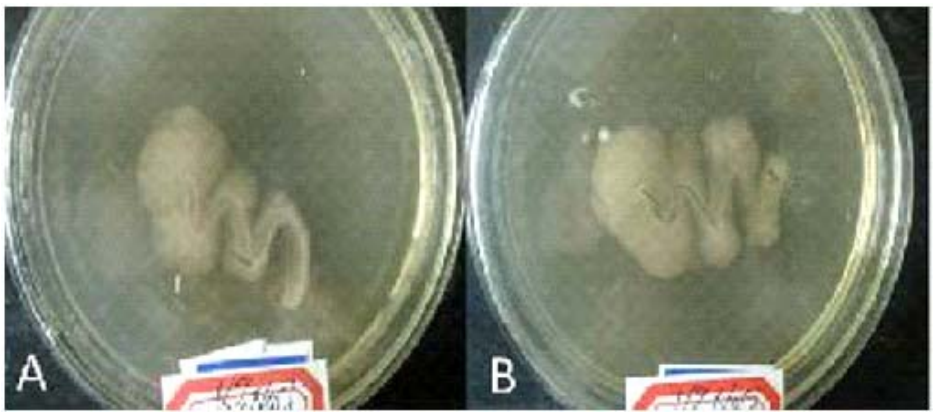

Fig. 2. The result of 5% NaCl screening. A, B and C were the clones on the 2% NaCl plate

Fig. 3. The result of 10% NaCl screening

Sugar fermentation assay.

Fig. 7 showed that purple medium turned yellow. So these two halotolerant bacteria could use the glucose and lactose to help them grow.

Methyl red test

Bland control was yellow (Fig. 8, C), but the others were orange (Fig. 8, A and B). So these two bacteria were methyl-red-negative.

After screened by NaCl concentration gradient, we separated 12 clones from the saline-alkali soil successfully (Table 1). In these clones, 2 clones were extreme halophile bacteria. So these two clones are the original strains for the subsequent experiments.

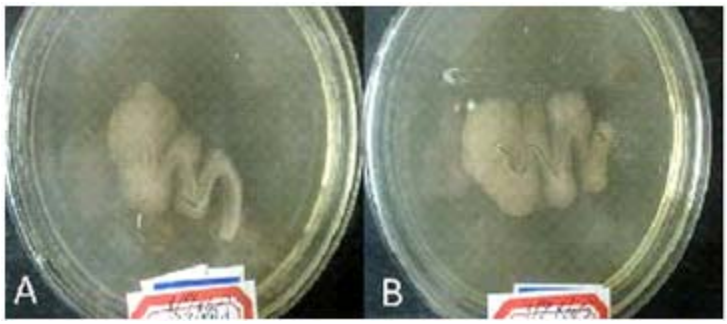

Fig. 4. The result of 16% NaCl screening. (A) was defined TN-H1 and (B) was defined TN-H2

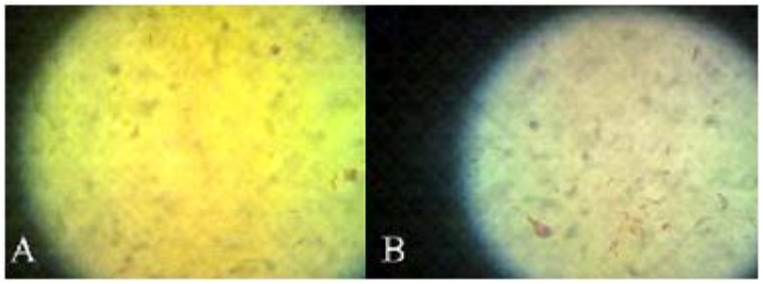

Fig. 5. The colony morphologies of TN-H1 (A) and TN-H2 (B)

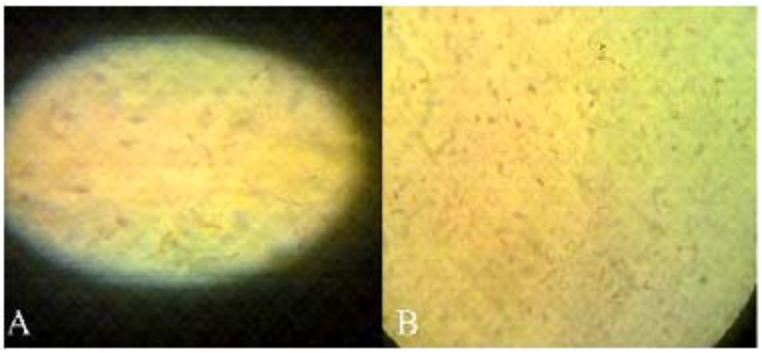

Fig. 6. Gram's staining results of TN-H1 (A) and TN-H2 (B)

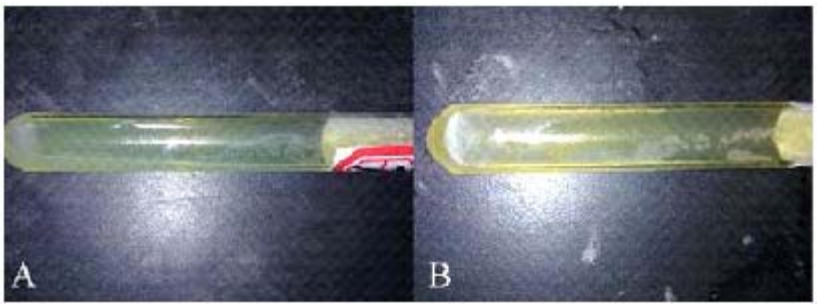

Fig. 7. Sugar fermentation assay result of glucose (A) and lactose (B)

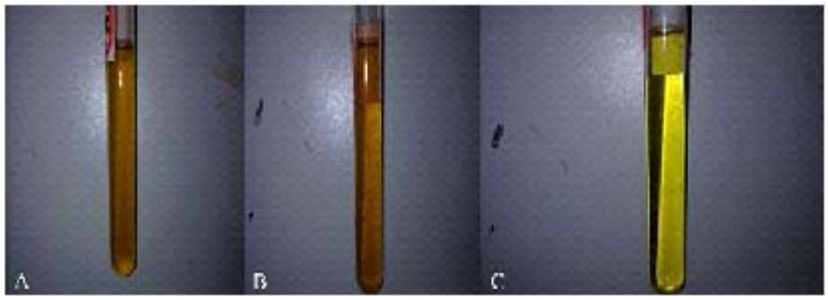

Fig. 8. Methyl red test result of TN-H1 (A), TN-H2 (B) and blank control (C)

DISCUSSION

Bacteria separated from the saline-alkali soil were found highly different group halotolerant and halophilic bacteria (Table 1). The reasonable explanation is standard separation method, because the bacteria growth depend on many conditions such as temperature, pH, medium component and also NaCl concentrations. Our results are n line with Fritze[11]. The halophilic bacteria isolated from saline-alkali soil were categorized on the basis of NaCl tolerance into Non-, slightly-, moderately-, and extremely-halophilic bacteria (Table 1). And the slightly- and moderately halophilic bacteria occupied the vast majority. This result was in line with Martinez-Canovas[12]. This is the first research on the bacterial diversity of Tianjin saline-alkali soil. In view of the salt tolerance of microbes, a source of genes can be isolated to increase the salt tolerance of crops species.

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