Scholars Journal of Engineering and Technology

OPEN ACCESS

Abbreviated Key Title: Sch J Eng Tech
ISSN 2347-9523 (Print) | ISSN 2321-435X (Online)
Journal homepage: http://saspublisher.com/sjet/

Isolation and Preliminary Identification of Resistant (Salophilic) Microorganisms in Daqing

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DOI: 10.36347/sjet.2020.v08i01.003 | **Received:** 08.01.2020 | **Accepted:** 15.01.2020 | **Published:** 18.01.2020

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Abstract **Original Research Article**

Halophilic bacteria have special metabolic mechanism and physiological structure, which can change soil properties to achieve economic benefits, and have great research value and development potential. The development of new salt-tolerant strains can tap the strain potential and contribute genotypes of new strains to the database. The ultimate goal is to improve the crop yield, enrich the variety of planted crops and increase the utilization rate of saline-alkali land. So as to improve the soil conditions to increase food production, protect the environment and green saline land and other goals. Targeted enrichment method and liquid dilution coated plate method were used to isolate and screen bacteria with high salt tolerance, and then phylogenetic analysis method based on 16S rDNA gene sequence was used to establish the bacterial gene tree.

Keywords: Separation, Appraisal, Salinity tolerance, Phylogenetic analysis, PCR.

INTRODUCTION

Now the land salinization is serious, in order to improve the soil environment, we need to use chemical, physical, biological and other ways to carry out experiments. Biology as the common method to improving soil salinization is the joint action of plants and salt-tolerant microorganisms. Nowadays, due to the pursuit of efficiency, people generally only choose efficient chemical reagents, which leads to the aggravation of land consolidation and is one of the causes of land salinization. Studies have shown that nitrogen-fixing bacteria, rhizobia and other microbial fertilizers have stronger affinity to soil and almost no negative effect on soil. Therefore, if a new salt-tolerant microorganism is found, it can effectively provide value for improving land salinization. At the same time, it can also provide new gene fragments for transgenic plants, which provides a reference for constructing efficient transgenic saline-tolerant plants. As a new microbial resource with great development value, salt-environment microorganisms have their unique biological groups, special physiological structure, metabolic mechanism and genetic genes. Because there are excellent characters, so the development potential is great, suitable for development and utilization.

MATERIALS AND METHODS

Bacterial Culture Medium

Salinity of the S - G medium: ryptone, 10g/L, yeast extract, 5g/L, casein, 5g/L, KCl, 2g/L, sodium citrate, 3g/L, $MgSO_4.7H_2O$, 20g/L, distilled water, 1000 mL, 15g/L agar was added to prepare solid screening medium. For the enrichment, isolation and screening of salt-tolerant bacteria [1], NaCl was selected in accordance with the required concentration requirements. When the solid medium is configured, KOH is used to regulate the medium, because its positive ions are K^+ and will not affect the concentration of Na^+.

Classification of salt-tolerant and salt-loving microorganisms

There are five types of microbial tolerance to salt [2], the optimum growth salinity (NaCl concentration) of non-halophilic bacteria was less than 2%. The optimum growth salinity was 2% ~ 5%. Moderate halophilic bacteria, the optimum growth salinity is 5% ~ 20%. Extremely halophilic bacteria, optimum growth salinity 15% ~ 30%. Salt-tolerant bacteria, the optimal growth salinity is more than 25%, among which some extreme halophilic bacteria are halophilic archaea [3]. In recent years, through 16S rRNA gene research and analysis [4], many scholars

have found a large number of salt-tolerant and salt-loving fungi groups growing in the ocean, salt lake, salt field and saline-alkali land. Such as *Hanaerobiales* mesh (Firmicutes door) and *Halomonadaceae* family (Gammaproteo bacteria classes) are all halophilic [5].

Enrichment and Isolation of Halophilic Bacteria

Using the liquid medium directional enrichment method. The samples were placed in a sterilized 250 mL conical flask containing 5% (w/v) NaCl, 160 r/min, and shock cultured at 28℃. The bacterial enrichment process was ended when the bacterial solution became turbidized and the bacterial concentration reached the point where it could be screened. By using liquid dilution coated tablet, will complete the bacteria suspension of enrichment in 0.9% (w/v) sterile saline classification dilution. Then 0.1mL of bacterial suspensions of various concentrations were absorbed and coated on the modified S-G medium containing 5%, 10%, 15% and 20% (w/v) NaCl series of plates [6, 7], and the single colony removed was transferred to a clean liquid medium and cultured to a suitable concentration.

Extraction of Genomic DNA from Bacteria

A bacterial genome kit was used to extract the bacterial genome of the target species. By centrifugation cracking bacterial genes, through split-phase, filter, combination and elution steps, will do well the genome of the gel electrophoresis, gel, spot, run 10 min, observations. PCR products with suitable amplified fragment length, no obvious heterozygosity and high purity were selected and sent to bgi for product sequencing. Seqman was used to splix the upper and lower fragments of sequencing results, and the primer fragments were manually deleted. The wrong bases were modified with stable bases of wave peaks through find conflict. The splaining sequence was saved in seq format, opened in test format, and the sequence was modified in fasta format, and the sequence name was named.

Based on 16S rDNA gene sequence established bacteria phylogenetic tree

DNAMAN 6.0 software was used to compare the 16S rDNA gene sequence returned to us. Bacteria whose sequence similarity was ≥99.9% were discarded as the same strain temporarily. If the similarity is less than 99%, it is temporarily regarded as a new strain, and a sequence with less homology is selected as the representative sequence. Submit the representative sequence to GenBank database, analyze and find the serial number. Using blast search online analysis, the genomes with high similarity and homology of representative sequences were searched in NCBI database. With MEGA 5 application CLUSTAL W for sequence alignment, the Neighbor - Joining method, using the bootstrap is found that the proposed new bacteria genetic tree is established. Genetic tree analysis, preliminary determine the strains of bacteria.

Halophilic bacteria - determination of resistance to alkalinity

Mainly based on the determination of alkali resistance under different concentration, the growth of bacteria, according to the results of the determination of salt resistance, the ratio of salt concentration and had the same growth conditions, pH value was adjusted to 6.0, 7.0, 8.0, 9.0, 10.0, 11.0, 12.0, the salinity of the S - G medium plate, will select representative bacteria inoculation on the tablet, 28 ℃ inversion training after 3 d to observe the growth of bacteria.

RESULTS AND ANALYSIS

After the enrichment and preliminary screening of the bacterial liquid, it could be seen that the bacterial liquid in the conical flask became cloudy and had a relatively atmospheric taste after the first colony enrichment, which basically indicated that there were bacteria in it for large-scale reproduction. After 7 days of culture, the color of the bacterial liquid was yellow with obvious turbidity (Figure-1).

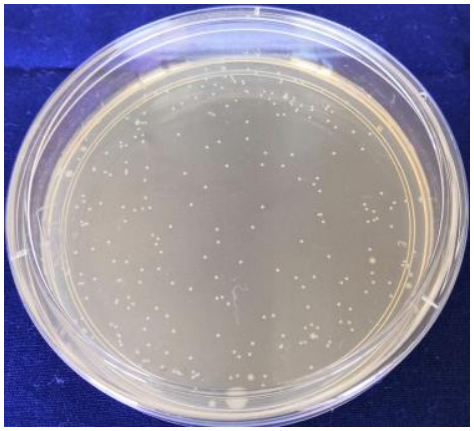

Fig-1: Bacteria growing at salt concentration of 1 mol/L

The bacteria were initially screened with medium with different salinity concentrations, and were coated with solid medium with salt concentrations of 5%, 10%, 15% and 20%, respectively, and cultured at 28℃ to observe the colony growth. The suitable individual colonies of high-salinity tolerant bacteria were selected and transferred to the liquid medium for reproduction. After dilution to the appropriate concentration, the molar concentration was selected for plate screening to determine the range of salt tolerance. Strains were selected and labeled for liquid culture, and tubes with good growth conditions were selected for bacterial genome extraction, and then PCR was used to obtain the amplified DNA fragments for examination (Figure-2).

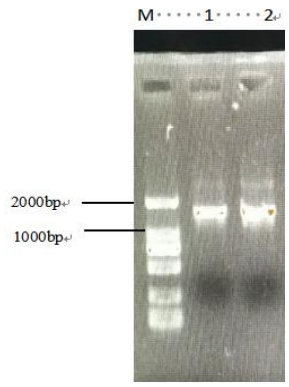

Fig-2: Agarose gel electrophoresis of 16SrDNA

In salt under the same conditions of use for the cultivation of the pH, observe its growth conditions. The alkali resistance was determined to be between 7 and 10, and the growth was not good beyond this range, so the alkali resistance was determined and sequenced, the bacteria with low similarity were selected as the representative bacteria. The sequence of the detected strain was compared in GenBank database of NCBI to construct the bacterial gene tree and understand its biological morphology and traits. It was found that CGM20190520 belongs to the genus bacillus, and its gene sequence is the gene tree as shown in the Figure-3.

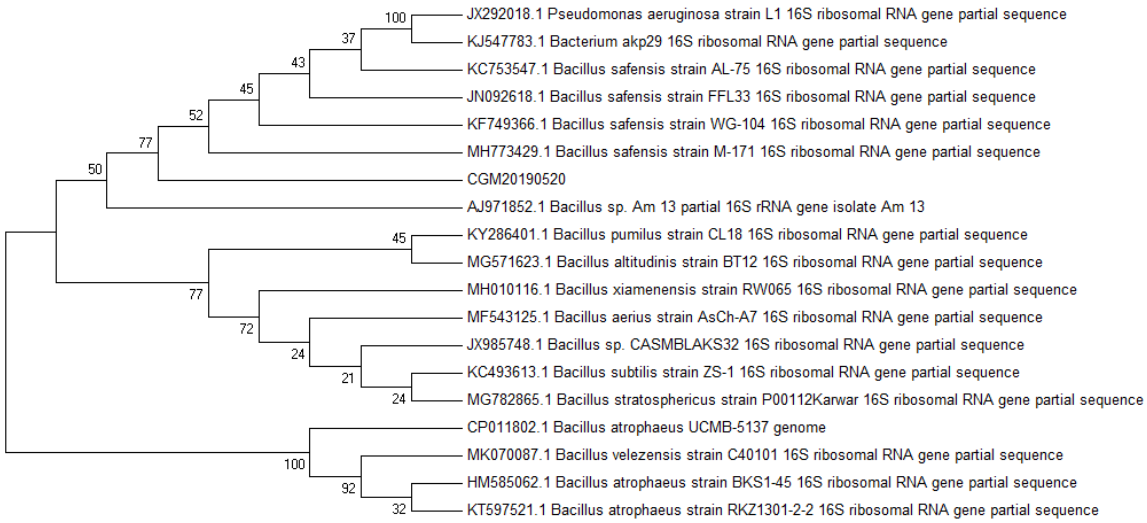

Fig-3: Establishment of bacterial genome tree based on 16S rDNA gene sequence (CGM20190520 is the proposed discovery of new bacteria)

According to the test results, among the 10 strains tested, 5 strains had the tolerance of 5% (w/v) NaCl, 4 strains had the tolerance of 10% (w/v) NaCl, 2 strains had the tolerance of 15% (w/v) NaCl, and no strain had the tolerance of 20% (w/v) NaCl, indicating that the salt tolerance of the strains was mainly between 5% and 10%. In the determination of alkali resistance, the alkali resistance was determined to be between pH 8 and 10. Na_2CO_3 and $NaHCO_3$ were the main soil types in saline-alkali land, and their pH values were roughly between 8 and 10.

CONCLUSION

Experiments were carried out from the separation of salt-tolerant and halophilic bacteria, salt-tolerant S-G culture medium was used to provide salt-tolerant environment, and single colony was selected to conduct aseptic isolation of single bacteria. By using the database to carry out the bacterial gene comparison, the halophilic microorganisms in saline soil were isolated and recultured, and the single colony of bacteria was obtained. After PCR amplification technology, through a database of 16 S rDNA gene sequence similarity comparison and phylogenetic analysis of (heavy) salt resistant bacteria for genotype analysis, to determine whether its for strain has been found, according to the experimental results, the following conclusion: the experiment with directional enrichment method and liquid dilution coated tablet method for screening and identification of salt-tolerant bacteria found in soil bacteria. They found that the genotypes of two samples of the bacteria were significantly different from those of the known bacteria in the gene pool. For the proposed discovery of new strains, among the four submitted strains, the genotypes of the two strains and the 16S rDNA gene sequences of the known strains in the NCBI database were found to be between 97.0%-99.2%. Both strains were subspecies of the genus bacillus, which may be newly discovered strains. The results of salt-alkali tolerance test showed that the salt-tolerant strains were all pH 7-10. It is similar to the soil environment in which it grows.

ACKNOWLEDGEMENTS

This work was supported by Innovation and Entrepreneurship Program for College Students in Heilongjiang Province (201910223085) and

Heilongjiang Natural Science Foundation (LH2019C050).

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