

KONG Fanjing and CHEN Susu. 2014. Cloning of Na^+/H^+ Antiport Gene from *Dunaliella salina*. *Acta Geologica Sinica* (English Edition), 88(supp. 1): 78-79.

Cloning of Na^+/H^+ Antiport Gene from *Dunaliella salina*

KONG Fanjing and CHEN Susu

MLR Key lab of saline lake and resources, Institute of Mineral resources, Chinese Academy of Geological Sciences, Beijing 100037, China

1 Introduction

Dunaliella Salina, which taxi *Dunaliella*, Volvocales, Chlorophyceae Chlorophyta, is unicell algae with double flagllum at top, and cup shaped chloroplast without cell wall. *Dunaliella Salina* is the most salt tolerance eucaryotes. It can grow at the range of salt concentration from 0.05 to 5.5 molL-1NaCl. The *Dunaliella Salina* is the unique primary produce and plays important role in hyper saline lake ecosystem. It has been proposed that Na^+/H^+ antiport provides an efficient mechanism to avert the deleterious effects of Na^+ in the cytolol and maintains osmotic balace by using Na^+ accumulated in the vacuole to drive water into the cells. This Na^+/H^+ antiport transport Na^+ into the vacuole by using the electrochemical gradient of protons generated by the vacuolar H^+ translocating enzymes, H^+ adenosine triphosphatase and H^+ inorganic pyrophosphase. In early 1949, it's firstly proposed Na^+/H^+ antiport activity in the eucaryotes. Na^+/H^+ antiport was reported first time from mouse kidney cell in 1976. Research on Na^+/H^+ antiport from animal, plants, yeast

and algae has been reported in detail. However, Na^+/H^+ antiport form *Dunaliella salina* has not been reported. In this paper, we report the cloning of Na^+/H^+ antiport from *Dunaliella salina*.

2 Materian and methods:

Dunaliella. Salina was stored at our lab. The 5ml mother *Dunaliella Salina* algae liquid were transferred into flask with 200ml new made media. The algae grew at $28 \pm 2^\circ\text{C}$ with light(light: dark= 14h: 10h), shaking the flask three times a day. The Total RNA of *Dunaliella. Salina* was extract using the TRNzol. The RT-PCR of cDNA was carried out according to instruction of QIAGEN One Step RT-PCR Kit. The product of PCR was cloned to pGEM-T vector using pGEM-T vector kit according to instruction of protocol (Promega cor.). The sequencing of cDNA were submitted to GenBank and get the accession number. BLAST was done on NCBI website on line. The phulogenetic tree was built using N-J method.

3 Results and discussion:

TheTotal RNA was extracted from algae using RNA Extaction Kit ()according to manufacure's instruction. The degenerate primers were designed according to the consensus sequences of Na^+/H^+ gene sequences from saltbrush, Suaeda salsa, Arabidopsis thaliana. primer upsteam: 5'-ATTGG (T/A) GCAAT (A/C) TT (C/T) GCTGC-3'; primer downstream: 5'-TCTCAAT (A/G) TCCA (T/G) (T/G/A) GCATCC-3'. RT-PCR was performed using total RNA as template, and RT-PCR Kit. A specific DNA fragment was generated. The sequencing was carried out and was submitted to GenBank, got the accession nuber: EF654513.

In this study, the cloned gene DsNH1 from *Dunaliella. Salina* has close relative with Arabidopsis

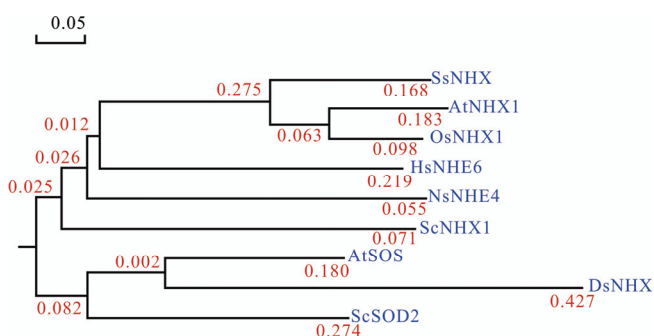


Fig. 1. phylogenetic tree of Na^+/H^+ of antiport gene. SsNHX: Suaeda salsa; AtNHX1: Arabidopsis thaliana (vacuole membrane); OsNHX1: rice (vacuole plasma membrane); HsNHE6: Human; NsNHE4: Norway mice; ScNHX1: Saccaromyces cerevisiae; AtSOS: Arabidopsis thaliana (plasma membrane); DsNHX: *Dunaliella. Salina* (plasma membrane); ScSOD2: zygosaccharomyces

* Corresponding author. E-mail: kfjb2002@aliyun.com

thaliana SOS1 gene in the evolution tree. SOS1 coded a polypeptide of 1146 amino acid residues. The molecular weight of the polypeptide is 127kDa, and SOS1 has high hydrophobic nature at N – end and 12 transmembrane regions. The transmembrane regions of SOS1 were much similar with Na⁺ / H⁺ antiporters from animals and bacteria. For example, NHE1 of SOS1 from Chinese hamster has homology of 26% and similarity of 45%. SOS1 has homology of 31% and similarity of 48%. With Nhap from *P. aeruginosa*. The hydrophilic C-end of SOS1 keep residue in plasma and the long hydrophilic C –end become the largest Na⁺ / H⁺ antiport factor as known. The BLAST result showed that the DsNHX in this study has no high homology sequences, so it's new gene fragment. In the future work, the full long gene will be amplified by RACE technique and study its characters.

Dunaliella. Salina is one of the most salt tolerance algae, but the genetic background was not known so far. The genes has not screened from Genomic library successfully. The degenerate primers are mixtures of oligonucleotides which are different in one or few nucleotide. The known gene family can be detected by the PCR using degenerate primers, or the homology comparing. The degenerate primers were designed through the consensus sequences between species. It may the simple method to screen the gene application of PCR with degenerate primers. In this study, we designed the degenerate primer according to the sequences of Na⁺/H⁺ antiport from degenerate primers and the Na⁺/H⁺ antiport gene successfully generated by

PCR. The results indicated the method was efficient. The BLAST analyses results showed this is a new gene fragment since no high homology sequences has been searched in GenBank.

Key words: *Dunaliella. Salina*; Na⁺/H⁺ antiport gene; gene cloning; degenerate primers; RT-PCR.

Acknowledgements

This study was supported by National Natural Science Foundation of China (40772065) and the Open project from the key lab of saline lake resources and environments, MLR (2010-SYS-06) .

Reference

- Xue Z Y, Zhi D Y, Xue G P, et al Enhanced salt tolerance of transgenic wheat (*Triticum aestivum* L.) expressing a vacuolar Na⁺ / H⁺ antiporter gene with improved grain yields in saline soils in the field and a reduced level of leaf Na⁺ . *Plant Sci*, 2004, 167:849~859.
- Hamada A, Hibina T, Nakamura T, et al Na⁺ / H⁺ antiporter from *Synechocystis* Species PCC 6803, Homologous to SOS1, contains an aspartic residue and long C-Terminal tail Important for the carrier activity. *Plant Physiol*, 2001, 125(1): 437~446.
- Shi H , et al . The *Arabidopsis thaliana* salt tolerance gene SOS1 encodes a putative Na⁺ / H⁺ antiporter. *Proc Natl Acad Sci* , 2000 ,97 :6896~6901.
- Apse M P , et al . Salt tolerance conferred by overexpression of a vacuolar Na⁺ / H⁺ antiport in *Arabidopsis*. *Science* ,1999 ,285 : 1256~1258.