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RESEARCH PAPER

Studies on Bacteriorhodopsin Gene and Sequence of 16S rRNA Encoding Genes of Halophilic Archaea of Xinjiang Aibi Lake, China

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Abstract: One hundred and forty-eight strains of halophilic archaea were isolated from 40 samples of soil, lake water, and silt. To study and analyze the species and bacteriorhodopsin (BR) protein resource, partial DNA fragments encoding BR protein from helix C to helix G and 16S rRNA encoding genes from 6 strains of halophilic archaea were amplified by polymerase chain (PCR), and their DNA sequences were determined. The results indicate that the reduced amino acid sequences of BR protein from helix C to helix G of ABDH11 is obviously different from those of other existing proteins. The results of homology analysis on BR gene and16S rRNA and phylogenetic analysis on 16S rRNA show that strains ABDH10 and ABDH40 are novel members of genus *Natronorubrum* and *Natrinema*, respectively; the sequence of ABDH40 was obtained from GenBank and the number of sequence is AY989910. The protein from helix C to helix G of ABDH11 is significantly different from that of other strains.

Key Words: halophilic archaea; bacteriorhodopsin; 16S rRNA; phylogenesis

Research on the genetic diversity of extremely halophilic bacteria has developed rapidly with the recent applications of molecular biology. New species and genera are constantly being reported. Extremely halophilic bacteria belong to *Halobacteriaceae* of *Halobacteriaceae,* which is part of Archaea domain. There are a total of 18 genera in *Halobacteriaceae,* according to the data of International Committee on Systematics of Prokaryotes (ICSP) up to September 2004. The 18 genera are *Halobacterium*, *Haloarcula*, *Haloferax*, *Halococcus*, *Halorubrum*, *Halogeometricum*, *Haloterrigenia*, *Halobaculum*, *Halorhabdus,* *Natrialba*, *Natrinema*, *Natronobacterium*, *Natrononococcus*, *Natronorubrum*, *Natronomonas*, *Halomicrobium*, *Halosimplex*, *Halobiforma*, *Halobaculum*, including members of *Halobacteriaceae* and more than 50 species.

Bacteriorhodopsin, BR, which is located in the plasma membrane[1] with the basic secondary structure of seven times α-helix, is the only protein in the cell membranes of halophilic bacteria. The BR protein isolated from *Halobacterium halobium* is composed of 248 amino acids. The molecular weight of BR is about 26 kD. The lysine at the 216th position of BR is linked by schiff base and molecular chromophores of retinoic acid[2]. In the natural state, every patch of purple membrane with two-dimensional hexagonal lattice is composed of trimers. Each trimer is formed of three identical BR monomers. The in-depth study of the membrane will help understand cell membrane receptor signal transduction

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pathway and the mechanism of the mode of transmission. BR is also the prototype membrane ion-channel protein. BR plays a guiding role in the study of protein structure and function of ion channels such as photovoltaic response in transmembrane proton mechanism and photochromic properties are employed in applications in solar batteries artificial retina, optical information storage, neural networks, and BR biochip. Therefore, the wild separated BR and artificial modification BR are used in the study of protein structure and function combining crystal study to clarify their response and proton-pump mechanism. BR protein research has become a hot topic for the nonce[3].

The ABDH10 strain and ABDH11 strain, which are halophilic archaea with the growth concentration of salt in the range of 20 %–50 %, have been purified from the Aibi Lake in the north of Xinjiang Province, China. Amino acid analysis of the amplified conservative fragment of the ABDH10 BR from the C-helical spiral to G-helical spiral was carried out, and the sequences were compared with the corresponding BR-protein sequences from other strains that have been reported.

1 Materials and methods

1.1 Strains

Both ABDH10 and ABDH11 strains are isolated from the Aibi Lake. The control strain is *Halobacterium halobium* R1M1, which is kindly donated by Professor Guo-qing Li at the College of Life Sciences, Fudan University, Shanghai, China.

1.2 Isolation and culture

Each strain is isolated and enriched, both using culture medium containing high salt concentration. The composition of the culture medium is as follows: 200 g NaCl, 20 g $MgSO_4 \cdot 7H_2O$, 3 g sodium citrate, 2 g KCl, 0.2 g $CaCl_2$, and 10 g of bacterial peptone (L37); pH 7.2. The volume of the culture medium is made up to 1 L using distilled water, and 20 g agar in solid medium is also added.

The high salt suspension medium is used to culture the soil samples, and then small amount of the suspension is added to the liquid suspension medium. A small amount of thawing ice-sample is directly added to the liquid medium, followed by the addition of a small amount of water to the liquid medium. The culture is performed at 37 °C in the presence of light and oscillations for 7 days Spread at each proper dilution, purify by repeatedly crossing until single colony.

1.3 Extraction of total genomic DNA

The extraction of total genomic DNA is carried out according to the extraction method described in the published reports[4].

1.4 PCR

The PCR is carried out based on the known 16S rRNA sequences that were obtained from the previous reports[5,6]. DNASTAR software is used for designing primers. The forward primer is 5′-ATTCCGGTTGA TCCTGC-3′. The reverse primer is 5′-AGGAGGTGATCCAGCCGCAG-3′. PCR conditions: 50 μL of reaction system, reaction cycles 30 times; modified: 94 °C, 50 °C, 45 s; annealing: 45 s; extension: 72 °C, 90 s.

One pair of degenerate primers was designed by Otomo[7]. The forward primer is 5′-CCGCTG (CT) TG (CT) TG (CT) T (AC) GACCTCG-3′. The reverse primer is 5′-AGGATGA (GA) (CG) CCGAA (CG) CCGACCTT-3′. The PCR conditions are as follows: 50 μL reaction system, reaction cycle 30 times; modified: 94 °C, 55 °C: 30 s; annealing: 30 s; extension: 72 °C, 30 s.

1.5 DNA sequencing and conversion

The PCR products were cloned using a direct method of T/A cloning. First, the fragments were purified. The purified fragments and plasmid pUCm-T were ligated, and the purified fragments were then transformed into *Escherichia coli* JM109, which was placed overnight in the growth plate with ammonia-p (AMP^+). Afterwards select leukoplakia by PCR and restriction enzyme test. Three positive one that are randomly selected were cloned and sequenced.

1.6 Construction of phylogenetic tree

The 16S rRNA sequences cloned from the AB1 strain is compared with the other strains from halophilic archaea obtained from the GenBank database. Clustalw1.8 order software is used for determining the matching alignment of multiple sequences. In the case of the existence of gaps, it is filled with the neutral elements. The DNAdist procedure from PHYLIP package was used to calculate the evolutionary distance. The matrix distance through sequence data is calculated according to ‘Kimura two-parameter’. The phylogenetic tree was then constructed using the Neighbor-joining method. The reproducibility of all the branches is analyzed using Seqboot and Consense program of the PHYLIP package with 1 000 repeats.

1.7 Accession number

The 16S rRNA sequences of AB1 strain and gene sequences of BR were obtained from the nucleotide sequence database of GenBank. The sequences of related strains obtained from the database are listed in Table 1.

2 Results

2.1 Halophilic archaea ABDH10 strain and phylogenetic analysis of 16S rRNA sequences

One pair of primers was used to clone ABDH10, and 1 473 bp were obtained. The electrophoresis of 16S rRNA sequences is shown in Fig. 1. The results of sequencing show that the size of the 16S rRNA sequences of ABDH10 is similar to that of the sequences from other strains that have been reported. However, the differences among nucleotide sequences are significant.

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