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# Cloning and Characterization of a $Na^+/H^+$ Antiporter Gene of the Moderately Halophilic Bacterium *Halobacillus aidingensis* AD-6$^T$

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**A gene encoding a $Na^+/H^+$ antiporter was obtained from the genome of *Halobacillus aidingensis* AD-6$^T$, which was sequenced and designated as *nhaH*. The deduced amino acid sequence of the gene was 91% identical to the NhaH of *H. dabanensis*, and shared 54% identity with the NhaG of *Bacillus subtilis*. The cloned gene enable the *Escherichia coli* KNabc cell, which lack all of the major $Na^+/H^+$ antiporters, to grow in medium containing 0.2 M NaCl or 10 mM LiCl. The *nhaH* gene was predicted to encode a 43.5 kDa protein (403 amino acid residues) with 11 putative transmembrane regions. Everted membrane vesicles prepared from *E. coli* KNabc cells carrying NhaH exhibited $Na^+/H^+$ as well as $Li^+/H^+$ antiporter activity, which was pH-dependent with the highest activity at pH 8.0, and no $K^+/H^+$ antiporter activity was detected. The deletion of hydrophilic C-terminal amino acid residues showed that the short C-terminal tail was vital for $Na^+/H^+$ antiporter activity.**

***Keywords*:** *Halobacillus aidingensis*, $Na^+/H^+$ antiporter, *nhaH*, Inverse PCR

Sodium/proton antiporter is a secondary transporter, which is an integral ubiquitous membrane protein playing an important role in maintaining inwardly directed $Na^+$ gradient and constant intracellular pH, and widely found in cytoplasmic and organelle membranes of cells from prokaryotes to eukaryotes. In bacteria cells, the $Na^+/H^+$ antiporter serves a variety of physiological functions, such as exclusion of $Na^+$ and $Li^+$ from cells for survival in presence of these ions at high concentrations (Padan *et al*., 1989; Inoue *et al.*, 2001), regulation of cytoplasmic pH and cell volume under alkaline conditions (Krulwich *et al*., 1983; Padan *et al*., 1994), signal transduction, establishment of an electrochemical potential of $Na^+$ across the cytoplasmic membrane which is the driving force for $Na^+$-dependent flagellar motor, $Na^+$-dependent multidrug efflux pump, as well as $Na^+$ symporters for alanine, glutamate, proline, serine and inorganic phosphate (Tsuchiya *et al.*, 1977). Sodium proton antiporter has been extensively identified and classified into two groups including a single gene superfamily and a gene cluster superfamily. Single gene encoding $Na^+/H^+$ antiporter include *nhaA* (Karpel *et al.*, 1988), *nhaB* (Pinner *et al.*, 1992), *chaA* (Ohyama *et al.*, 1994), and *mdfA* (Lewinson *et al.*, 2004) from *Escherichia coli*, *nhaA*, *nhaB*, and *nhaD* from *V. cholerae* (Herz *et al.*, 2003), *nhaC* from *Bacillus firmus* (Ito *et al.*, 1997), *napA* from *Enterococcus hirae* (Waser *et al.*, 1992), *nhaK* and *nhaG* from *B. subtilis* (Gouda *et al*., 2001; Fujisawa *et al*., 2005), *nhaH* from *H. dabanensis* (Yang *et al*., 2006) and *nhaP* from *Pseudomonas aeruginosa* (Utsugi *et al.*, 1998). In contrast, another group of genes encoding $Na^+/H^+$ antiporter includes *mrp* from *B. subtilis* (Ito *et al*., 1999), *mnh* from *Staphylococcus aureus* (Hiramatsu *et al.*, 1998), *pha* from *Sinorhizobium fredii* RT19 (Jiang *et al*., 2004), *sha* from alkaliphilic *Bacillus* sp. C-125 (Kosono *et al.*, 1999) and *sno* from *Staphylococcus aureus* (Bayer *et al.*, 2006).

Moderately halophilic bacteria contain a heterogeneous physiological group of microorganisms belonging to different genera, which grow optimally between 0.5 and 2.5 M salt concentration, for successful salt adaptation at high salt conditions, they actively extrude $Na^+$, and maintain internal ion concentration through $Na^+/H^+$ antiporters (Ventosa *et al.*, 1998). But the $Na^+/H^+$ antiporter gene in moderately halophilic bacteria has not been fully investigated. Most physiological experiments of Gram-negative moderate halophiles, such as *Salinivibrio costicola*, have clearly revealed the existence of $Na^+/H^+$ antiporters. However, the situation in Gram-positive moderately halophilic bacteria, especially in spore-forming ones, is not as well undertaken (Ventosa *et al.*, 1998). To date, only a primary $Na^+/H^+$ antiporter and a secondary $Na^+/H^+$ antiporter gene from Gram-positive moderately halophilic bacterium *H. dabanensis* have been identified (Yang *et al*., 2006). In order to study $Na^+/H^+$ antiporters in depth, we searched for more $Na^+/H^+$ antiporters from other moderately halophilic bacteria. *Halobacillus aidingensis* AD-6$^T$ is an endospore-forming, Gram-positive, moderately halophilic bacterium isolated from the Aidin salt lake in Xinjiang, China, which can grow under a broad range of salinitiy from 0.5% to 20% (w/v) NaCl with optimal growth occurring at 10% (w/v) NaCl (Liu *et al.*, 2005). It is

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most likely that *H. aidingensis* AD-6$^T$ possesses high $Na^+/H^+$ antiporter activity. Here, we reported identification of a $Na^+/H^+$ antiporter gene from *H. aidingensis* AD-6$^T$, and the deletion of the C-terminal tail in it.

## Materials and Methods

### Bacteria and growth

*H. aidingensis* was grown in Gibbson medium under aerobic condition at 35°C (Liu *et al.*, 2005). The *Escherichia coli* KNabc, which lacks three major $Na^+/H^+$ antiporters (NhaA, NhaB, and ChaA), were grown in a modified Luria-Bertani medium (LBK medium) (Goldberg *et al.*, 1987), to which NaCl or LiCl was added at indicated concentrations when necessary. The *E. coli* DH5α used for cloning experiments was maintained on LB agar and grown at 37°C. Plasmid pUC18 and pGEM-T easy vectors (Promega, USA) were used in cloning and sequencing experiments.

### Probe construction and labeling

To obtain a fragment of the $Na^+/H^+$ antiporter gene from *H. aidingensis*, forward primer HP-F; 5'-CTKATGACYGCK ACAGAYCC-3', and reverse primer HP-R; 5'-CATSCCGAT YYTBGCRCC-3' were designed for PCR with the chromosomal DNA of *H. aidingensis* as a template. The reaction was conducted using the following conditions: 94°C for 30 sec, 52°C for 1 min and then 72°C for 1 min, with a total of 30 cycles. The resulting product was cloned into the pGEM-T easy vector and sequenced. The recombinant plasmid was digested completely with *Eco*RI; then the DNA fragments of about 800 bp were purified and labeled as a probe to detect the entire $Na^+/H^+$ antiporter gene in *H. aidingensis*. Labeling of the fragments was performed with a Dig High Prime DNA Labeling and Detection Starter Kit I (Roche Diagnostic GmbH, Germany) as recommended by the manufacturer.

### Southern blot hybridization

Total DNA of *H. aidingensis* AD-6$^T$ was digested wholly with *Eco*RI, *Hin*dIII, and *Kpn*I, respectively. The resulting fragments were separated by electrophoresis in 0.8% agarose gel and transferred to a positive-charged nylon membrane (Roche Molecular Biochemicals, Germany), and then hybridized with the achieved probe. Southern hybridization and detection were performed according to standard techniques (Sambrook *et al.*, 1989), using the Dig High Prime DNA Labeling and Detection Starter Kit I as described above.

### Inverse PCR (IPCR)

IPCR was performed essentially as described by Ochman *et al.* (1988). PCR was carried out with chromosomal DNA of *H. aidingensis* as a template. A pair of primers was used in IPCR as follows: HIP-up; 5'-GAACAAGTACTGCTGCTCA CC-3' and HIP-down; 5'-ACCGAAGACGAATCCACC-3'. The DNA fragment generated by IPCR was cloned into the pGEM-T easy vector and sequenced.

### Preparation of membrane vesicles and assay of $Na^+/H^+$ antiporter activity

Everted membrane vesicles were prepared from cells of KNabc carrying the hybrid plasmids or pUC18 by the French press method essentially as described by Rosen (1986). The protein content in everted membrane vesicles was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as a standard. The $Na^+/H^+$ antiporter activity in the everted membrane vesicles was detected based on its ability to collapse a transmembrane pH gradient, and acridine orange fluorescence was monitored to estimate $\triangle$pH as previously described (Goldberg *et al.*, 1987) using Hitachi F-4500 fluorescence spectrophotometer (Hitachi Ltd, Japan). The excitation light was 495 nm, and emission light was measured at 530 nm. The measurements of antiporter activities at various pH values were performed in Tris titrated with Mes.

### DNA manipulations and sequence analysis

Nucleic acid manipulations and general cloning procedures were performed with standard methods (Sambrook *et al.*, 1989). The PCR products were purified with the EZ-10 Spin Column DNA Gel Extraction Kit (Bio Basic, Canada). Restriction enzymes, DNA Blunting Kit, T4 DNA ligase and TaKaRa ExTaq$^{TM}$ for PCR amplification were purchased from TaKaRa (China). Synthesis of oligonucleotides and DNA sequencing were conducted at Sangon Biotech (China). Data bank searches were performed through the National Center for Biotechnology Information (NCBI) using the BLAST program. The possible TMs of the protein sequence were deduced the TopPredII computer program.

### Complementation of antiporter deficient KNabc

The forward primer; 5'-GGAATTCCATGCACGGATTTCA TGATGT-3' containing the *Eco*RI restriction site and the reverse primer; 5'-CGGGATCCTTCGTGACTCCTTTCAA CCT-3' containing *Bam*HI site, were used for PCR with chromosomal DNA of *H. aidingensis* as a template to obtain the full *nhaH* open reading frame. The amplified DNA was purified and digested with *Bam*HI and *Eco*RI, then ligated into pUC18, which had also been digested with *Bam*HI and *Eco*RI. The ligation products were transformed into the *E. coli* KNabc, and the transformants were grown on the LBK medium containing 0.2 M NaCl. Recombinant transformants were selected by conventional techniques. The presence of the insert was confirmed, and the gene was sequenced.

### Construction of mutant with C-terminal tail deletion

The C-terminal tail-deleted mutant was constructed using the PCR method with the forward primer; 5'-GGAATTCC ATGCACGGATTTCATGATGT-3' containing the *Eco*RI restriction site, and the reverse primer; 5'-CGGGATCCTTA AGGCTTTAATGTCAGCCC-3' containing the *Bam*HI restriction site. The amplified DNA was purified and digested with *Bam*HI and *Eco*RI, then ligated into pUC18 also digested with *Bam*HI and *Eco*RI. The ligation products were transformed into the *E. coli* KNabc, and the resulting plasmid did not contain the last 8 amino acid residues.

### Nucleotide sequence accession number

The new nucleotide sequence data were deposited in GenBank with accession number EU159451.

### Statistics

Data were analyzed using one-way analysis of variance, and matched-pair comparisons were further made by Student's *t-test.*

## Results and Discussion

### Cloning of the $Na^+/H^+$ antiporter gene from *H. aidingensis* by IPCR

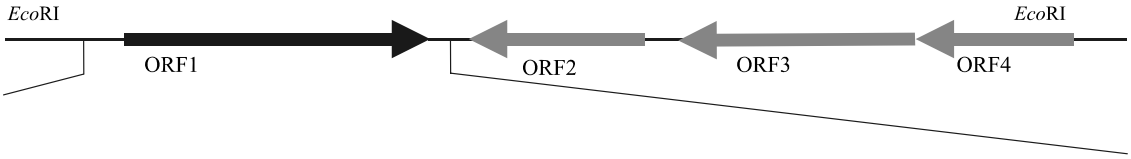
To isolate a fragment of $Na^+/H^+$ antiporter gene from *H. aidingensis*, the degenerate primers designated HP-F and HP-R were designed based on well-conserved polypeptide regions of several $Na^+/H^+$ antiporters, including NhaH of *H. dabanensis* (accession number GI 74418678), Nhe2 of *Bacillus* sp. NRRL B-14911(accession number GI 89096813) and NhaG of *Bacillus subtilis* (accession number GI 6714545). By PCR amplification using the total genomic DNA of *H. aidingensis* as the template, a DNA fragment with expected size (786 bp) was obtained and sequenced. The deduced amino-acid sequence of the DNA fragment showed great similarity to NhaH of *H. dabanensis* (91% identity), so the above fragment might be a part of the *nhaH* gene sequence from *H. aidingensis*. In order to obtain the entire *nhaH* gene, Southern hybridization experiments were carried out, and the *nhaH* gene was detected on a 3.7 kb *Eco*RI fragment. A set of primers derived from the 786-bp PCR product were used to amplify the 3.7 kb fragment including the entire ORF of *nhaH* by IPCR.

### Sequence analysis of the 3.7 kb fragment

As shown in Fig. 1, the protein sequence analysis of the 3.7 kb *Eco*RI fragment indicated the presence of four open reading frames (ORFs), and were not oriented in the same direction. ORF1 (1,212 bp) showed high level sequence similarity with other $Na^+/H^+$ antiporter: NhaH of *H. dabanensis* (91% identity), Nhe2 of *Bacillus* sp. NRRL B-14911 (69% identity), NhaG of *Bacillus subtilis* (54% identity), and NhaP of *Aphanothece halophytica* (37% identity), so we designated ORF1 as NhaH. Alignment of amino acid sequences was performed with CLUSTAL W, and the results are shown in Fig. 2. In addition, ORF2 (723 bp) was predicted to encode NAD-dependent deacetylase showing a high level of amino-acid sequence homology from *Geobacillus kaustophilus* HTA426 (45% identity). ORF3 (738 bp) was predicted to encode a membrane protein showing a high level of amino acid sequence homology to NADPH-flavin oxidoreductase from *Oceanobacillus iheyensis* HTE831 (55% identity) with unknown function. ORF4 (651 bp) showed similarity to a protein with unknown function of *O. iheyensis* HTE831 (22% identity).

### Features of the *nhaH*-encoded gene product

The *nhaH* gene encoded a 403-residue protein with a calculated molecular mass of 43,523 Da and a pI of 5.44. A hydrophobicity plot of NhaH revealed an alteration of hydrophobic and hydrophilic segments characteristic for integral membrane proteins, and suggested the presence of 11 trans-

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181   CGATGGTATTTAATCACTTCTTGTCGGAAGATGTGGATGAGATCGAAGACCAATTGGCCA
        R  W  Y  L  I  T  S  C  R  K  M  W  M  R  S  K  T  N  W  P      61

241   TCATACTTTCAGATCATCAGCTGATAGTTAAAGAATAACGGCAGCGCGGACGTTTTTTTA
        S  Y  F  Q  I  I  S  *  *  L  K  N  N  G  S  A  D  V  F  L      81

301   CGTCCGTTTTTTTATGGGTAAGGGGCGAGCAAGAAGGGAAAGACTAATTATAGCGCATTC
        R  P  F  F  F  Y  G  *     -35     K  K  G  K  T  N     -10     A  F     101

361   ATACTGGGAGGGTTTCTCATGCACGGATTTCATGATGTTTTCATTCAAATTCTACTGTTA
                 SD             M  H  G  F  H  D  V  F  I  Q  I  L  L  L
        I  L  G  G  F  L  M  .  .  .  .  .  .  .  .  .  .  .  .  .  .     121

1551  CCTGTCATCAAAAAACTCGGCTTAGCCTAACATCCATATTCTGGTGTACCAGGTCATACA
        P  V  I  K  K  L  G  L  A  *  H  P  Y  S  G  V  P  G  H  T      521

1621  GATAATGGAAAAAGCCTCCTTCATCGAAGGAGGCTTTTTTACATCTTTTCTTTTATTTTT
        D  N  G  K  S  L  L  H  R  R  R  L  F  Y  I  F  S  F  I  F      541

1681  GCTATGGCTTTTGACAAATCCTCGTGCACGACATAGTCAAATAGACGGTCCTTATCTGTT
        A  M  A  F  D  K  S  S  C  T  T  *  S  N  R  R  S  L  S  V      561
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**Fig. 1.** Physical map of the 3.7 kb *Eco*RI fragment and *Halobacillus aidingensis nhaH* sequence. Part of the DNA sequence of *H. aidingensis nhaH* and regions upstream and downstream of the coding region: Bases are numbered on the left (numbering according to full sequence as found in NCBI nucleotide sequence database: EU159451), the deduced amino acid sequence is below the codons and numbered on the right. -35, -10 putative promoter, SD putative Shine-Dalgarno sequence after putative promoter and putative start codons ATG, the putative termination TAA, and inverted repeat following the ORF are underlined.

membrane-spanning segments (Fig. 2). In the sequence 29 residues are basic, 26 are acidic and another 81 are uncharged polar, yielding a total of 136 (33.7%) hydrophilic and 267 (66.3%) hydrophobic residues. This complies with the overall composition of integral membrane proteins. The most abundant residue of this protein was Leu (65 residues out of 403), followed by Val (38 residues), Gly (38 residues) and Glu (37 residues). The least abundant residue was Cys (zero residue). The importance of negatively charged amion-acid residue Asp in the transmembrane domains has been well documented in several $Na^+/H^+$ antiporters, such as the Asp-133, Asp-163 or Asp-164 conserved in the *nhaA* from *E. coli*, the three conserved amion-acid residue Asp are located in 3rd and 4th putative transmembrane helices, and play important roles in cation binding and transport (Inoue *et al*., 1995). The Asp-138 in SynnhaP from *Synechocystis* sp. which is essential for $Na^+/H^+$ antiporter activity, has been demonstrated (Hamada *et al*., 2001). In NhaH, alignment

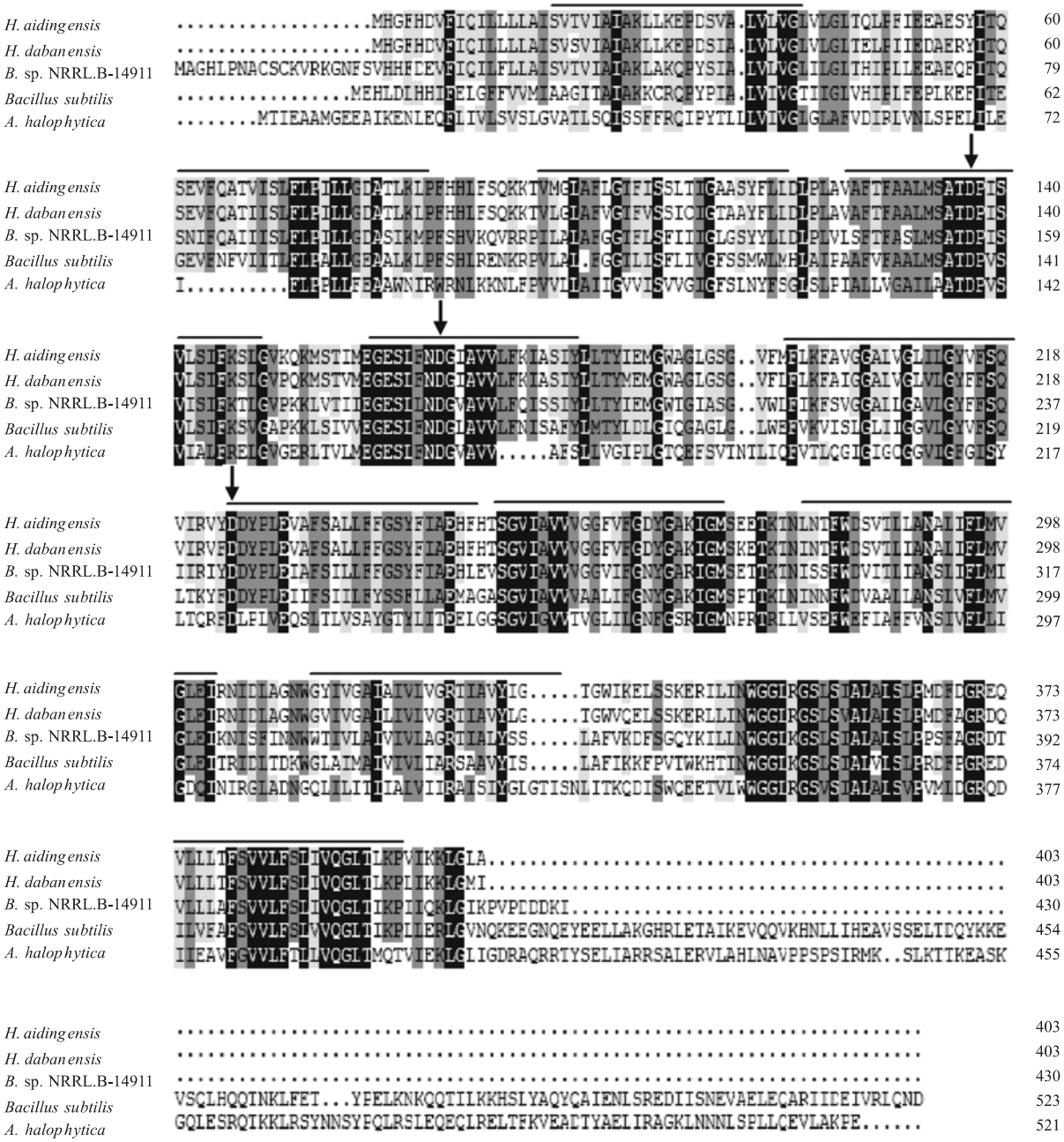


**Fig. 2.** Alignment of the predicted amino sequence of NhaH from *H. aidingensis* with NhaH from *Halobacillus dabanensis* D-8$^T$, Nhe2 from *Bacillus* sp. NRRL B-14911, NhaG from *Bacillus subtilis* and NhaP from *Aphanothece halophytica*. The amino acid residues conserved in all sequences are shown in black, and conservative substitutions are shown in grey, Three conserved Asp residues are indicated by downward arrows. The predicted 11 transmembrane domains are marked with bold lines above the alignment.

of NhaH with $Na^+/H^+$ antiporters of five microorganisms showed that three aspartates, Asp-137, Asp-166, and Asp-224, which are in the hydrophobic TMS (Trans Membrane Segments) were conserved in NhaH (Fig. 2). Three conserved Asp residues may be important for antiporter function. Therefore, the role of these Asp residues is worth studying in the future.

## Functional complementation of *E. coli* KNabc

The function of NhaH protein from *H. aidingensis* was assessed by an *in vivo* complementation assay using an antiporter-deficient *E. coli* KNabc, since the strain lacks the major $Na^+$ transport systems, which is unabled to grow in LBK medium containing 0.2 M NaCl. An expression vector from pUC18 where the lactamase is replaced *in-frame* by *nhaH* was constructed, named pUCADNhaH, and the plasmid pUC18 (as a negative control) were both transformed into *E. coli* KNabc. Colonies harboring pUCADNhaH appeared on the LBK medium containing 0.2 M NaCl after 24 h at 37°C, but *E. coli* KNabc harboring pUC18 did not grow on the medium. The *E. coli* KNabc harboring pUCADNhaH which can grow could be considered due to the *nhaH* fragment on it. Given that the cloned gene was expressed, it is assumed that NhaH was expressed under the control of the pUC18 *lac* promoter.

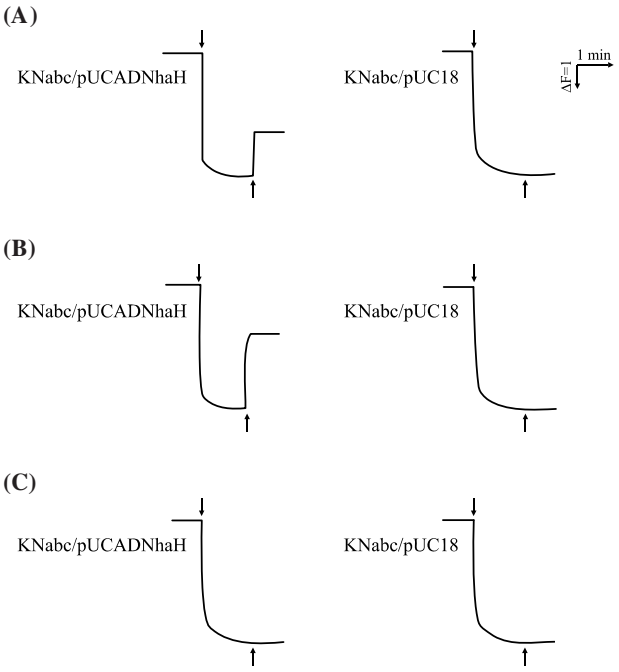


**Fig. 3.** Assays of $Na^+/H^+$ antiporter and $Li^+/H^+$ antiporter activities in membrane vesicles at pH 8.5. The activity measurements for $Na^+/H^+$ antiporter (A), $Li^+/H^+$ antiporter (B), and $K^+/H^+$ antiporter (C) were performed in everted membrane vesicles prepared from cells of *E. coli* KNabc/pUC18 or KNabc/pUCADNhaH by the French press method. The potassium lactate (final concentration of 5 mM) was added to the assay mixture to initiate fluorescence quenching at the time points indicated by downward arrows. NaCl (final concentration of 5 mM) or LiCl (final concentration of 5 mM) was added to the assay mixture at the time point indicated by upward arrows. Fluorescence quenching is shown in arbitrary units.

## The $Na^+/H^+$ antiporter activity in everted membrane vesicles

Everted membrane vesicles were prepared from cells of *E. coli* strains KNabc/pUC18 and KNabc/pUCADNhaH, and the $Na^+/H^+$ antiporter activity was determined by measuring the fluorescence dequenching of acridine orange. As shown in Fig. 3, both $Na^+/H^+$ and $Li^+/H^+$ antiporter activities were measured in membrane vesicles from KNabc/pUCADNhaH, while no $Na^+/H^+$ or $Li^+/H^+$ antiporter activity was determined in those from KNabc/pUC18. These results are consistent with the growth of KNabc/pUCADNhaH cells in the presence of $Na^+$ or $Li^+$. No $K^+/H^+$ antiporter activity was detected in everted membrane vesicles prepared from KNabc/pUCADNhaH or KNabc/pUC18.

The effect of pH on the activity of $Na^+/H^+$ as well as $Li^+/H^+$ antiporter was also measured. NhaH exhibited $Na^+/H^+$ and $Li^+/H^+$ antiporter activity at a wide range of pH between 6.5 and 9.5. The optimal pH for the $Na^+/H^+$ and $Li^+/H^+$ antiport activity was 8.0 (Fig. 4). Meanwhile NhaH in the *H. dabanensis* showed $Na^+/H^+$ antiporter activity in the pH range of 6.5 to 9.5 and $Li^+/H^+$ antiporter activity in the pH range of 7.5 to 9.5, with the optimal pH for the $Na^+/H^+$ and $Li^+/H^+$ antiport activity was 8.5~9.0 and 8.5, respectively. The pH profile for the NhaH of *H. aidingensis* was wider than that of *H. dabanensis*. Furthermore, the $Li^+/H^+$ antiporter activity was higher than the $Na^+/H^+$ antiporter activity, which suggested that $Li^+$ had more affinity than $Na^+$ for the antiporter. However, in the *H. dabanensis*, $Na^+$ was more affinity than $Li^+$ for the the NhaH antiporter (Yang *et al*., 2006). When we compared $Na^+/H^+$ and $Li^+/H^+$ antiporter activity between *H. aidingensis* and *H. dabanensis* directly by quinacrine fluorescence quenching method. The results showed that the activity in vesicles possessing *H. aidingensis* NhaH is higher than with vesicles possessing *H. dabanensis* NhaH when measured (Fig. 5). Hence, NhaH

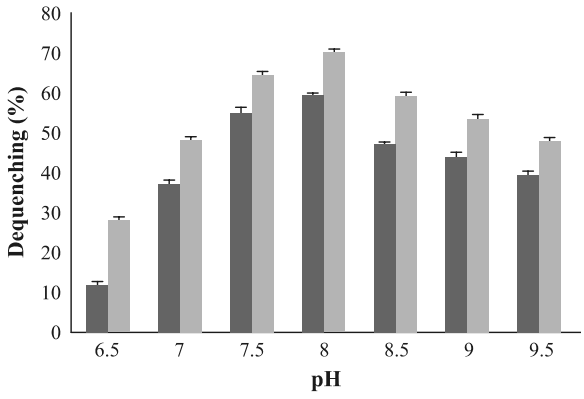


**Fig. 4.** pH profile of the $Na^+/H^+$ and $Li^+/H^+$ antiporter activities of NhaH. The antiporter activities were measured by the fluorescence quenching method. $Na^+/H^+$ antiporter activity (black column) and $Li^+/H^+$ antiporter activity (grey column) were measured at the indicated pH. The wave length of excitation light was 495 nm and fluorescence was monitored at 530 nm. Each value represents the average of three independent determinations, the level of significance was taken at $P<0.05$.

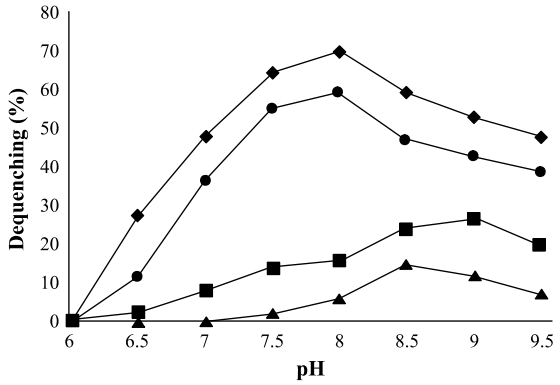


**Fig. 5.** Comparison of the $Na^+/H^+$ and $Li^+/H^+$ antiporter activities of NhaH from *H. aidingensis* and *H. dabanensis*. The antiporter activities were measured by the fluorescence quenching method. $Na^+/H^+$ antiporter activity from *H. aidingensis* (●), $Na^+/H^+$ antiporter activity from *H. dabanensis* (■), $Li^+/H^+$ antiporter activity from *H. aidingensis* (◆), and $Li^+/H^+$ antiporter activity from *H. dabanensis* (▲) were measured at the indicated pH. The wave length of excitation light was 495 nm and fluorescence was monitored at 530 nm. Each value represents the average of three independent determinations.

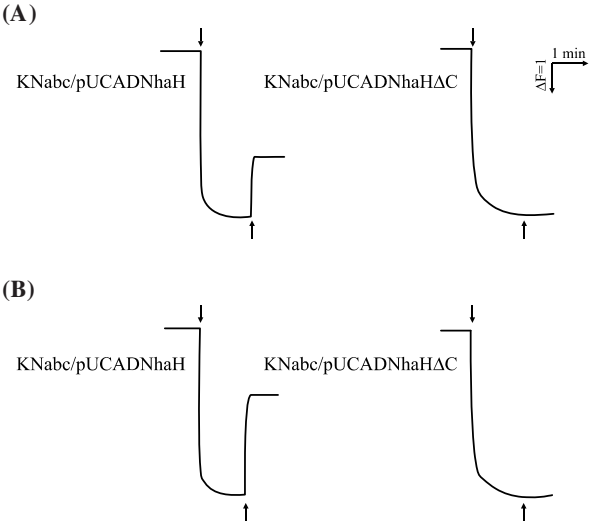


**Fig. 6.** Assays of $Na^+/H^+$ antiporter and $Li^+/H^+$ antiporter activities in membrane vesicles at pH 8.5. The activities measurements for $Na^+/H^+$ antiporter (A) and $Li^+/H^+$ antiporter (B) were performed in everted membrane vesicles prepared from cells of *E. coli* KNabc/pUCADNhaHΔC or KNabc/pUCADNhaH. Fluorescence quenching is shown in arbitrary units.

from *H. aidingensis* studies provided more evidence for investigation of the mechanism of ion transport for moderately halophilic bacteria. It has been reported that the NhaA antiporter of *E. coli* is highly pH-dependent and possesses amino residues responsible for pH sensing (Gerchman *et al*., 1993), and the pH profiles for the NhaHs of *H. aidingensis* and *H. dabanensis* are similar to that of NhaA. Thus, it is likely that the NhaHs from these strains possess pH sensing amino acid residues.

### Deletion of the C-terminal tail

The hydropathy profile indicated that eight hydrophilic amino-acid residues (395 VIKKLGLA 403) are present at the C-terminal region of NhaH (Fig. 2). Previously, it has been reported that the long C-terminal tails are considered to play an important role in the regulation of transport activity in animals. However, long C-terminal tails are found in a few bacteria; for example, NhaG from *B. subtilis* ATCC 9372 possesses a hydrophilic segment with more than 100 amino-acid residues at the carboxyl terminal region (Gouda *et al.*, 2001), the C-terminal cytosolic region of SynNhaP from *Synechocystis* sp. PCC6803 has 120 amino-acid residues, and NhaP in *Aphanothece halophytica* has 128 amino-acid residues (Hamada *et al*., 2001). In *B. subtilis*, the 26 C-terminal amino acid residues of NhaG were truncated, which severely damaged its function (Gouda *et al.*, 2001). Through construction of a chimera between ApNhaP and SynNhaP, it was shown that the ion specificity was affected by the C-terminal tail (Rungaroon *et al*., 2001). At one time, it also has been described that the partial deletion of the C-terminal tail of NhaP from *Synechocystis* sp. PCC 6803 decreased its $Na^+/H^+$ antiporter activity (Hamada *et al.,* 2001). However, the effect of a short C-terminal tail on in the $Na^+/H^+$ antiporter has not been reported yet. So we constructed a expression vector carrying the NhaH with the C-terminal tail deleted, and named pUCADNhaHΔC. The *E. coli* KNabc cells transformed with pUCADNhaHΔC could not grow on the LBK medium containing 0.2 M NaCl. This is indicated that the deletion of hydrophilic C-terminal amino acid residues inhibited the complementation ability of NhaH. In addition, both $Na^+/H^+$ and $Li^+/H^+$ antiporter activities were measured in membrane vesicles from KNabc/pUCADNhaH and KNabc/pUCADNhaHΔC. As shown in Fig. 6, the mutant has no antiporter activity. It was also shown that a short C-terminal tail is vital for $Na^+/H^+$ antiporter activity.

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