Analysis of seven putative Na⁺/H⁺ antiporters of Arthrospira platensis NIES-39 using transcription profiling and in silico studies: an indication towards alkaline pH acclimation

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Abstract Na⁺/H⁺ antiporters mediated pH regulation is one of the known mechanism(s), which advocates a possible role of the antiporters in the alkaline pH tolerance of Arthrospira platensis NIES-39. Seven putative Na⁺/H⁺ antiporters have been reported in A. platensis NIES-39. Based upon the in silico analysis, the seven putative antiporters were characterized into two different superfamilies, where A1, Q2, L2, and L6 belonged to the CPA1 family whereas C5, D5 and O6 belonged to CPA2 family. The orientation of functionally important residues in both CPA1 and CPA2 subfamily are conserved in modeled Q2 and C5 antiporters. Conserved domain analysis of the seven putative antiporters indicated the presence of nine different kinds of domains. Out of these nine domains, six domains function as monovalent cation-proton antiporters and two as the universal stress protein (Usp) category. Transcription profile of these seven antiporters was also generated at three different pH (7, 9 and 11) and time frames which showed a significant difference in the mRNA levels along with a temporal pattern of the expression profile. The in silico and the real-time PCR analysis put together, suggest the active participation of these seven putative Na⁺/H⁺ antiporters in alkaline pH homeostasis of this cyanobacterial strain where CPA1 subfamily antiporters play a major role.

Keywords Arthrospira platensis NIES-39 · CPA family · Na⁺/H⁺ antiporters · pH · Real-time PCR

Abbreviations
A1 NIES39_A01730
C5 NIES39_C00590
CPA Cation proton antiporter
CDD Conserved domain database
D5 NIES39_D05350
L2 NIES39_L02650
L6 NIES39_L06050
NIES National Institute for Environmental Studies
O6 NIES39_O06850
PCR Polymerase chain reaction
Q2 NIES39_Q02770

Introduction
Alkali and salt are two major abiotic stresses experienced by all prokaryotic and eukaryotic organisms. In this context, Na⁺ and H⁺ are two most important ions whose adequate concentration plays a crucial role in cellular functioning. Hence, all living cells are critically dependent on ion and pH homeostasis. It is also reported that for both prokaryotic and eukaryotic organisms, Na⁺/H⁺ antiporters actively participate in this homeostasis mechanism (Padan and Schuldiner 1994; Shi et al. 2000; Burckhardt et al. 2002; Brett et al. 2005; Banciu and Sorokin 2013; Padan 2014; Calinescu et al. 2014). Antiporters maintain a stable pH inside the cell under alkaline conditions (Padan et al. 2005) and maintain...
appropriate cell volume (Nanatani et al. 2005). All the Na\(^{+}/\)H\(^{+}\) antiporters ranging from prokaryotes to human belong to CPA superfamily which is further subdivided into CPA1 and CPA2 subfamily (Brett et al. 2005). The structure and function of Na\(^{+}/\)H\(^{+}\) antiporters were first studied in *E. coli* (Goldberg et al. 1987; Dover and Padan 2001). The role of the nhaA gene (Na\(^{+}/\)H\(^{+}\) antiporter) in pH regulation and salt stress is examined through chromosome deletion study (Padan et al. 1989). Further, gene knockout studies of Na\(^{+}/\)H\(^{+}\) antiporters in *Synechocystis sp. PCC 6803* strongly supported their active participation in pH regulation along with salinity stress (Wang et al. 2002). Deletion of plant genes which encodes the vacuolar and cytoplasmic membrane Na\(^{+}/\)H\(^{+}\) antiporters decreases the plant’s salt tolerance (Apse et al. 2003; Qiu et al. 2003) whereas overexpression is used to produce salt-resistant plants (Apse et al. 1999). The structures of several prokaryotic antiporters have revealed detailed mechanism of antiporter function and the specific role of amino acid residues in ion transportation (Williams et al. 1999; Lee et al. 2013; Paulino et al. 2014). Hence, it is considered that antiporters play an essential role in the survival and growth of alkalophiles which could tolerate a pH of 9 and above.

*Arthrospira platensis* NIES-39 is an alkalophilic cyanobacterium which tolerates a pH range of 6–11 with optimum growth observed between pH 9–9.5. Its genome is composed of a single, circular double-stranded chromosome of 6.8 Mb, without any plasmids (Fujisawa et al. 2010). Though seven putative Na\(^{+}/\)H\(^{+}\) antiporters are reported within *Arthrospira platensis* NIES-39 genome, the mechanism behind its survival under such a high pH condition is not entirely understood. In this report, we first characterize seven putative Na\(^{+}/\)H\(^{+}\) antiporters using in silico analysis and identify important residues which are possibly directly involved in ion transport activity. Among the seven putative antiporters, four antiporters belong to CPA1 subfamily whereas the remaining three belongs to CPA2. The differential and temporal transcript expression profile of the putative Na\(^{+}/\)H\(^{+}\) antiporters under different pH regimes was also studied to appraise their possible role during pH stress. We observed that all the seven genes are significantly highly expressed at pH 11 as compared to pH 7 and 9, some within 1 h of treatment and some after 4 or 6 h of treatment, subtly indicating the role of these genes/gene products in alkaline pH homeostasis.

**Materials and methods**

**Sequence analysis and domain search**

Seven putative protein sequences of the *Arthrospira platensis* NIES-39 antiporters were retrieved from NCBI database (Geer et al. 2010) and abbreviated for this article as A1 (NIES39_A01730; WP_014273993.1), C5 (NIES39_C00590; WP_043468050.1), D5 (NIES39_D05350; WP_006618914.1), L2 (NIES39_L02650; WP_006616525.1), L6 (NIES39_L06050; WP_014276668.1), O6 (NIES39_O06850; WP_006618272.1) and Q2 (NIES39_Q02770; WP_014277492.1). Pairwise and Multiple sequence alignments for Na\(^{+}/\)H\(^{+}\) antiporters were conducted using ClustalW servers (Thompson et al. 1994). The domains and motifs of these antiporters were analyzed using the conserved domain (CD) search tools, NCBI (Marchler-Bauer et al. 2015). The conservation patterns of the amino acid positions, in the CPA1 and CPA2 family, were analyzed using sequence logos (Crooks et al. 2004). Gneg-mPLoc (Chou and Shen 2010) and PSORTb (Yu et al. 2010) software were used to identify the subcellular localization of all the putative Na\(^{+}/\)H\(^{+}\) antiporters. Phobius (Käll et al. 2007) was used for prediction of the transmembrane helical region and its topology. Phobius and SignalP-5.0 (Petersen et al. 2011) were used to predict the presence of the signal peptide on the membrane protein.

**Three-dimensional models of *Arthrospira platensis* NIES-39 antiporters**

Swiss-model server was used to obtain full-length three-dimensional structures of antiporters of *Arthrospira platensis* NIES-39 (Waterhouse et al. 2018). Swiss-model server allows us to generate high-quality three-dimensional structural models of the target sequence by homology modeling procedure. The generated models were then subjected to five thousand cycles of energy minimization using AMBER 18 (Case et al. 2018) suite of programs to remove possible very high energy conformation and steric clashes. Structural validation of three-mol dimensional models was conducted by ERRAT (Colovos and Yeates 1993), PROVE (Pontius et al. 1996), WHATCHECK (Hooft et al. 1996) and QMEAN (Benkert et al. 2011) servers.

**Maintaining *Arthrospira platensis* NIES-39**

*Arthrospira platensis* NIES-39 was procured from National Institute for Environmental Studies, Japan, and maintained aseptically in SOT medium (pH 9–9.5) at 30 °C temperatures with 4 Klux light intensity and a photoperiod of 16:8 (dark: light) with continuous shaking. pH 9–9.5 is the preferred pH for growing this cyanobacterium.

**Sample collection at different pH**

*Arthrospira platensis* NIES-39 was grown, and the mid-log phase culture was inoculated at different pH, i.e., pH 7, 9 and 11. pH 9 is the optimum pH for the growth whereas
pH 7 and 11 were the points used to study the pH-dependent response of the antiporters. The treated cells were collected at 0th, 1st, 4th and 6th hour. The buffered medium was used to maintain a stable pH and the fluctuations were timely monitored.

RNA Isolation, cDNA synthesis, and real-time PCR

The pH treated cells were collected in RNA protect Bacteria Reagent (QIAGEN). Total RNA was extracted using the RNeasy mini kit (QIAGEN) and quantified using GE Healthcare Nanodrop. cDNA was synthesized using the Quantitect Reverse Transcription Kit (QIAGEN). iQ SYBR Green Supermix (BIO-RAD) to a final concentration of 1 × was used to study the expression profile by semi-quantitative real-time PCR. iQ5 multicolor real-time PCR detection system from BIO-RAD was used for the PCR. 16 s RNA was used as an internal control for normalizing the calculations. Primers were designed using Primer3 (Untergasser et al. 2012) and the stability parameters were analyzed using IDT Oligoanalyzer 3.1. Primers were synthesized from SIGMA-ALDRICH (details given in Supplementary Table S1). The PCR conditions used for the real-time PCR starts with an initial denaturation of 3 min at 95 °C followed by 45 cycles of 95 °C-10 s, 56 °C-30 s, 72 °C-50 s each, after which the fluorescence of the product was recorded at 72 °C at the end of every cycle. The final extension was given for 10 min at 72 °C. The fold values were calculated using the ΔΔCt method (Livak and Schmittgen 2001).

Results and discussion

In silico characterization of putative antiporters of Arthrospira platensis NIES-39

The amino acid sequence length of the seven antiporters from Arthrospira platensis NIES-39 varied from 409 to 691 (Table 1). Pairwise global sequences alignment showed that on an average, 17.54% (Standard deviation of 3.58) sequence identity existed among all seven antiporters with maximum 29% sequence identity between D5 and O6 antiporters. Lowest sequence identity of 13.3% was observed between Q2 and D5. Conserved domain database (CDD) search tool identified nine (among them six are unique) different domains among seven antiporters of Arthrospira platensis NIES-39 (Table 1). Coverage span analysis of each domain revealed that each antiporter of Arthrospira platensis NIES-39 contained at least one domain belonging to NhaP, Na+/H+ exchanger, KefB and Asp-A1a exchanger superfamily which exchanges alkali cations such as Na+, Li+, K+, Rb+, Ca2+ and NH4+ against protons (Resch et al. 2011) across the plasma membrane. Universal stress protein (USP) like domain along with USP A domain, Na+/H+ antiporter C and KefA domain along with TrkA-N were the other three domains present among the antiporters of Arthrospira platensis NIES-39. It has been shown that the expression of USP is enhanced when the bacterial cell is exposed to stress agents (Sousa and McKay 2001). TrkA is a constituent of K+ uptake systems and is peripherally bound to the inner side of the cytoplasmic membrane (Bosslemeyer et al. 1989; Schlosser et al. 1993). The presence of all stress related domains within the seven antiporters indicates the possible role of these antiporters in the alkaline stress tolerance mechanism.

Protein subcellular localization prediction tools (GnegmPLoc and PSORTb) predicted that all the seven putative Na+/H+ antiporters from Arthrospira platensis NIES-39 are located on the cytoplasmic membrane with very high localization scores. However, none of the prediction tools could detect any signal peptide sequence associated with putative Na+/H+ antiporter sequences. Even the absence of signal peptide sequence in known Na+/H+ antiporters of E. coli, Pyrococcus abyssi, Methanocaldococcus jannaschii and Thermus thermophilus, probably indicate that membrane-bound Na+/H+ antiporters of Arthrospira platensis NIES-39 do not contain localization signals. All the tools identified several transmembrane helices with excellent probability scores. Results from the subcellular protein localization, transmembrane topology, and signal peptide predictors are summarized in Table 2 which demonstrated that these proteins are located on the cytoplasmic membrane with a substantial number of transmembrane helices.

To further characterize the antiporters, the sequences of seven antiporters were compared with the four prokaryotic Na+/H+ antiporters whose crystal structures are available. The sequence comparison revealed that all antiporters of Arthrospira platensis NIES-39 do not have much sequence similarity with Na+/H+ antiporter of E. coli (EcNhaA, PDB ID: 1ZCD) (Taglicht et al. 1993), which is one of the well-studied antiporters of cation-proton antiporter subfamily 2 (CPA2). PaNhaP, the Na+/H+ antiporter NhaP from Pyrococcus abyssi (PDB ID: 4CZA) (Wöhler et al. 2014), had significant similarity with N-terminal domains of Q2 and A1 antiporters. N-terminal end of Q2 and A1 antiporters housing Na+/H+ exchanger domain (~ 400 residues) was homologous to PaNhaP, which indicates that A1 and Q2 antiporters belong to CPA1 subfamily. MjNhaP1, NhaP1 antiporter from Methanocaldococcus jannaschii (PDB ID: 4CZB) (Paulino et al. 2014) was quite similar to L6 and L2. N-terminal end of NhaP domain present in L2 and L6 antiporters was homologous to both PaNhaP and MjNhaP1 with expectation (E) value
This makes L2 and L6 antiporters to be, possibly belonging to CPA1 subfamily. NapA, the antiporter from *Thermus thermophiles* (PDB ID: 4BWZ) (Lee et al. 2013) had significant similarity with the N-terminal domain of C5, D5, O6 antiporters. The domain comparison of these antiporters is given in Table 3. C5, D5 and O6 antiporters containing KefB domain, belonged to CPA2 subfamily as these antiporters are homologous to NapA with an E-value of less than $1 \times 10^{-17}$. In addition to KefB domain, D5 contains Na$^+/H^+$ antiporter C

### Table 1

| Antiporters | Abbreviation used for the antiporters | Protein length | Conserved domains | Domain position |
|-------------|--------------------------------------|----------------|------------------|----------------|
| NIES39_A01730 | A1 | 534 | NhaP, Na$^+/H^+$ exchanger, USP like | 1–405 |
| NIES39_C00590 | C5 | 474 | KefB, Na$^+/H^+$ exchanger | 42–473 |
| NIES39_D05350 | D5 | 689 | KefB, Na$^+/H^+$ exchanger, Na$^+/H^+$ antiporter C | 10–390, 422–553 |
| NIES39_L02650 | L2 | 524 | NhaP, Na$^+/H^+$ exchanger | 9–426 |
| NIES39_L06050 | L6 | 642 | NhaP, Na$^+/H^+$ exchanger, USP like | 1–417, 563–680 |
| NIES39_O06850 | O6 | 691 | KefB, Na$^+/H^+$ antiporter C | 29–415 |
| NIES39_Q02770 | Q2 | 409 | Na$^+/H^+$ exchanger, Asp-Ala exchanger superfamily | 1–407 |

### Table 2

| Antiporters | Number of predicted Transmembrane helices | Presence of signal peptide sequence (scale between 0 and 1) | Cytoplasmic membrane localization score (scale between 1 and 10) |
|-------------|------------------------------------------|----------------------------------------------------------|-------------------------------------------------|
| A1          | 11                                       | 0.0287                                                   | 10                                             |
| C5          | 12                                       | 0.2873                                                   | 10                                             |
| D5          | 12                                       | 0.0079                                                   | 10                                             |
| L2          | 13                                       | 0.0009                                                   | 10                                             |
| L6          | 13                                       | 0.0071                                                   | 10                                             |
| O6          | 13                                       | 0.0041                                                   | 10                                             |
| Q2          | 11                                       | 0.0155                                                   | 10                                             |
| 4CZA        | 13                                       | 0.0006                                                   | 10                                             |
| 4CZB        | 13                                       | 0.0101                                                   | 10                                             |
| 4BWZ        | 11                                       | 0.0093                                                   | 10                                             |
| 1ZCD        | 11                                       | 0.1084                                                   | 10                                             |

*a* Using Phobius (Käll et al. 2007)

*b* Using SignalP-5.0 (Petersen et al. 2011)

*c* Using PSORTb (Yu et al. 2010)
Comparison of seven putative Na\(^{+}/\text{H}^{+}\) antiporters from <i>Arthrospira platensis</i> NIES-39 against crystal structures of CPA1 (PDB ID: 4CZA and 4CZB) and CPA2 (PDB ID: 4BWZ) subfamily

| Antiporters | Reference protein | Query coverage (%) | E-value | Identity (%) |
|------------|------------------|--------------------|---------|--------------|
| A1         | PaNhaP (4CZA)    | 64                 | 3e−14   | 29           |
| Q2         | PaNhaP (4CZA)    | 85                 | 2e−16   | 25           |
| L2         | MjNhap1 (4CZB)   | 63                 | 1e−10   | 25           |
| L6         | MjNhap1 (4CZB)   | 45                 | 4e−21   | 25           |
| C5         | NapA (4BWZ)      | 88                 | 2e−62   | 36           |
| D5         | NapA (4BWZ)      | 58                 | 2e−17   | 24           |
| O6         | NapA (4BWZ)      | 43                 | 1e−18   | 25           |

The amino acid sequence comparison of the seven antiporters with available crystals structures (PDB ID: 4CZA and 4CZB) of Na\(^{+}/\text{H}^{+}\) antiporters revealed that the critical residues of both CPA1 and CPA2 subfamilies are conserved among these seven antiporters. The detailed analysis of the Na\(^{+}/\text{H}^{+}\) antiporter from <i>Pyrococcus abyssi</i> (PaNhaP, PDB ID: 4CZA) and <i>Methanocaldococcus jannaschii</i> (MjNhap1, PDB ID: 4CZB) states that Asp130, Asp159, Thr129 and Ser155 (residues are indicated according to PaNhaP crystal structure numbering) are involved in direct or water-mediated indirect ion binding. The oxygen atoms of the side chain or main chain carbonyl oxygen atoms are responsible for this protein-substrate ion interaction. The multiple sequence alignment (MSA) of these two crystal structures with A1, Q2, L2, and L6 antiporters showed that these four residues (except Ser155 in L6 antiporter) were conserved (or replaced with similar amino acids) in CPA1 subfamily indicating a prominent role of these residues in substrate ion binding. Both the crystal structures (PDB ID: 4CZA and 4CZB) of CPA1 subfamily have also revealed that Glu73, Thr129, Asn158, Glu154, and Arg337 are part of the polar cavity which facilitates ion transport across the membrane. Our sequence analysis showed that Arg337 was conserved entirely among A1, Q2, L2 and L6 antiporters whereas most of the other polar residues were either conserved or replaced with another polar side chain. Only in case of L6 antiporters, Asn158 was replaced with non-polar residues. Interestingly, even the residues (Ile151, Phe355, and Gly359) whose structural rearrangement is responsible for blocking of ion transportation (Wöhler et al. 2014) at low pH were conserved among all CPA1 family antiporters. From the sequence analysis, it is clear that both ion binding cavity, as well as the polar cavity of ion transportation, were highly conserved among A1, Q2, L2, and L6 antiporters indicating that these antiporters belong to CPA1 subfamily.

The sequence comparison of NapA (4BWZ) with EcNhaA and Human Nha2 (available crystal structures of other two CPA2 family members) showed 22.6% and 18.9% sequence identity respectively which possibly indicates that for the function of this class of protein, overall topology and relative orientation of transmembrane helices are more important than the sequence conservation. The pairwise sequence alignment of the existing crystal structure of bacterial CPA2 antiporters and C5, D5, O6 antiporters resulted into only 20–32% sequence identity with Lys305 conserved among all the three antiporters whereas functionally important Asp156 residue in D5 and O6 was replaced with polar Thr side chain. In the case of O6 antiporter, the functionally essential Asp157 was replaced with polar Asn. The sequence analysis also showed the occurrence of a considerable amount of acidic and basic side chains (13–16%) which is a characteristic feature of CPA2 family protein. Interestingly, it is observed that protein three dimensional structure prediction servers like SWISS-MODEL (Waterhouse et al. 2018), I-TASSER (Zhang 2008), PHYRE (Kelley et al. 2015) automatically consider PaNhaP (PDB ID: 4CZA) and MjNhap1 (PDB ID: 4CZB) protein as a template to model C5, D5, and O6 antiporter sequences while NapA (PDB ID: 4BWZ) protein as a template to model C5, D5, and O6 antiporter sequences. This observation further reinforces our classification of A1, Q2, L2, and L6 as a member of CPA1 subfamily while C5, D5, and O6 belong to CPA2 subfamily.

Three-dimensional structural models of Q2 and C5 antiporters (belonging to CPA1 and CPA2 subfamily respectively) were constructed through homology modeling techniques. The stereochemical quality and accuracy of the models were tested using different servers and was found to be comparable to that of template structures (shown in Table 4). About 97.7% residues of modeled Q2 antiporter were in the allowed regions of Ramachandran plot while 98.1% residues of C5 antiporters were in the
Table 4  Quality of homology modeled Q2 and C5 antiporters from *Arthrospira platensis* NIES-39 is compared against their respective template structure. The values within parenthesis represent the quality of template structures.

| Modeled protein | Template used (PDB ID) | Organism                  | Identity (%) | PROVE score | ERRAT score | Q-MEAN score | WHATCHECK |
|----------------|------------------------|---------------------------|--------------|-------------|-------------|--------------|-----------|
| Q2             | 4CZA                   | *Pyrococcus abyssi*       | 26           | 5.6 (5.3)   | 97 (91)     | −5.39 (−3.57) | PASS      |
| C5             | 4BWZ                   | *Thermus thermophilus*    | 39           | 5.8 (5.3)   | 98 (99)     | −4.6 (3.29)  | PASS      |

Many studies have reported the positive correlation of the Na\(^+\)/H\(^+\) antiporter involvement during alkaline stress tolerance mechanism. A similar role of the antiporters could be related in this cyanobacterium. Transcription profile based upon the real-time PCR for these seven putative Na\(^+\)/H\(^+\) antiporters showed a differential temporal expression pattern with a significant enhancement at pH 11 which was recorded as- A1 showed 40-fold; C5, 11-fold; D5, 7-fold; L2, 10-fold; L6, 49-fold; O6, 11-fold; Q2, 14-fold increase (Fig. 2a–g). After 1 h of incubation, D5 showed a significant expression while antiporters A1, C5,
O6, and Q2 were expressed at the 4th hour of incubation. Antiporters L2 and L6 showed their considerable expression on the 6th hour of incubation. Exceptionally, L2 showed 14-fold higher expression at pH seven as compared with the other antiporters. Differential expression was found for different antiporters at pH 7, 9 and 11. We have also observed that the expression of these antiporters under pH stress is temporally regulated. This also throws light on the actual importance of the seven antiporters in alkaline stress tolerance mechanism. These seven antiporters based upon their temporal transcription expression profiles towards higher alkaline pH (pH 11) could be labeled under early responsive antiporter, intermediate responsive antiporter, and late responsive antiporter categories. D5 (CPA1 subfamily) falls into the early responsive antiporter category indicating this antiporter to be the most sensitive towards the pH change, as it got upregulated within an hour after alkaline pH treatment. Intermediate responsive antiporters are A1, C5, O6, and Q2 belonging to both CPA1 and CPA2 family. Late responsive antiporter is L6 belonging to CPA1 family.

Fig. 2 a–g Expression profile of differential transcripts of A1, C5, D5, L2, L6, O6 and Q2 Na+/H+ Antiporters from Arthrospira platensis NIES-39. Relative gene expression values were normalized using Arthrospira platensis NIES-39 16 s rRNA levels as an internal loading control. Three biological replicates were taken and the results are mean ± SD of duplicates for each sample.
A1 and L6 showed a higher level of expression upon subjecting the cyanobacteria to pH 11 as compared to pH 9. Our in silico analysis shows that A1 houses NhaP, Na\(^+/\)H\(^+\) Exchanger, USP like, Usp A domains and antiporters L6 house NhaP, TrkA-N, TrkA domains. Interestingly, only the A1 antiporter shows the unique UspA domain among the 177 Na\(^+/\)H\(^+\) antiporters studied from 37 sequenced and annotated cyanobacteria (data not given). Many reports (Nachin et al. 2005; Liu et al. 2007; Shokry et al. 2014) categorically suggest the role of UspA in metabolic, oxidative and temperature stresses. UspA is also found to be associated with pH stress, where its expression is upregulated under an alkaline condition in *E. coli* K-12 (Yohannes et al. 2004). Our experiments show that exposing the cyanobacteria to pH 7 lead to 14-fold higher expression of the antiporter L2, housing NhaP and Na\(^+/\)H\(^+\) exchanger domains (Fig. 2d). However, this result could suggest a possible dual role of the antiporter in pH regulation (acidification and alkalinization). Our in silico analysis indicates that the antiporters of *Arthrospira platensis* NIES-39 belong to both the CPA1 and CPA2 subfamily and probably play an active role in pH homeostasis.

Conclusion

The seven putative Na\(^+/\)H\(^+\) antiporters work together amicably keeping the primary goal of pH regulation as their target. *Arthrospira platensis* NIES-39 contains seven antiporters which belong to two different families, i.e., CPA1 and CPA2 as evident from the in silico sequence and modeling analysis, where the critical amino acid residues are well conserved for their function and structure. NhaP, KefB and UspA domains could be tagged as the primary domains for the antiporters involved in the pH regulation as per our findings and the available literature. The temporal transcriptional expression profile of the seven antiporters suggest that all the antiporters are active in pH regulation and perform similar kind of work but still, they are different from each other. This could be traced from their low identity percentage and the presence of nine different types of domains.

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Compliance with ethical standards

Conflict of interest Authors declare that they have no conflict of interest.

References

Apse MP, Aharon GS, Snedden WA, Blumwald E (1999) Salt tolerance conferred by overexpression of a vacuolar Na\(^+/\)H\(^+\) antiport in Arabidopsis. Science 285:1256–1258

Apse MP, Sotossanto JB, Blumwald E (2003) Vacuolar cation/H\(^+\) exchange, ion homeostasis, and leaf development are altered in a T-DNA insertional mutant of AtNHX1, the Arabidopsis vacuolar Na\(^+/\)H\(^+\) antiporter. Plant J 36:229–239

Banciu HL, Sorokin DY (2013) Adaptation in halokalkaliphiles and natronophilic bacteria. In: Seckbach J, Oren A, Stan-Lotter H (eds) Polyextremophiles. Cellular origin, life in extreme habitats and astrobiology. Springer, Dordrecht, pp 121–178

Benkert P, Biasini M, Schwede T (2011) Toward the estimation of the absolute quality of individual protein structure models. Bioinformatics 27:343–350

Bosslemeyer D, Borchard A, Dosch DC, Helmer GC, Epstein W, Booth IR et al (1989) K\(^+\)-transport protein TrkA of *Escherichia coli* Is a peripheral membrane protein that requires other trk gene products for attachment to the cytoplasmic membrane. J Biol Chem 264:16403–16410

Brett CL, Donowitz M, Rao R (2005) Evolutionary origins of eukaryotic sodium/proton exchangers. Am J Physiol Cell Physiol 288:223–239

Burchhardt G, Di Sole F, Helmele-Kolb C (2002) The Na\(^+/\)H\(^+\) exchange gene family. J Nephrol 5:3–21

Calinescu O, Paulino C, Kuhlbrandt W, Fendler K (2014) Keeping it simple, transport mechanism and pH regulation in Na\(^+/\)H\(^+\) exchangers. J Biol Chem 289:13168–13176

Case DA, Ben-Shalom IY, Brozell SR, Cerutti DS, Cheatham TE III, Cruziero VWD (2018) AMBER 2018. University of California, San Francisco

Chou K, Shen H (2010) Cell-PLoc 2.0: an improved package of web-servers for predicting subcellular localization of proteins in various organisms. Nat Sci 2:1090–1103

Colovos C, Yeates TO (1993) Verification of protein structures: patterns of nonbonded atomic interactions. Protein Sci 2:1511–1519

Crooks GE, Hon G, Chandonia JM, Brenner SE (2004) WebLogo: a sequence logo generator. Genome Res 14:1188–1190

Dover N, Padan E (2001) Transcription of nhaA, the Main Na\(^+/\)H\(^+\) Antiporter of *Escherichia coli*, Is Regulated by Na\(^+\) and Growth Phase. J Bacteriol 183:644–653

Fujisawa T, Narikawa R, Okamoto S, Ehira S, Yoshimura H, Suzuki I et al (2010) Genomic structure of an economically important cyanobacterium, *Arthrospira* (*Spirulina*) *platensis* NIES-39. DNA Res 17:85–103

Geer LY, Marchler-Bauer A, Ger R, Han L, He J, He S et al (2010) The NCBI BioSystems database. Nucl Acids Res 38:492–496

Goldberg EB, Arbel T, Chen J, Karpel R, Mackie GA, Schuldiner S et al (1987) Characterization of a Na\(^+/\)H\(^+\) antiporter gene of *Escherichia coli*. Proc Natl Acad Sci 84:2615–2619

Hooft RWW, Vriend G, Sander C, Abola EE (1996) Errors in protein structures. Nature 381:272–272

Käll L, Krogh A, Sonnhammer ELL (2007) Advantages of combined transmembrane topology and signal peptide prediction-the Phobius web server. Nucl Acids Res 35:429–432

Kelley LA, Mezulis S, Yates CM, Wass MN, Sternberg MJE (2015) The Phyre2 web portal for protein modeling, prediction, and analysis. Nat Protocols 10:845–858

Lee C, Kang HJ, van Ballmoos C, Newstead S, Uzdavinsys P, Dotson DL et al (2013) A two-domain elevator mechanism for sodium/ proton antiport. Nature 501:573–577

Liu WT, Karavolos MH, Bulmer DM, Allioui A, Hormaeche RD, Lee JJ et al (2007) Role of the universal stress protein UspA of
