Acclimation and stress response of *Prochlorococcus* to low salinity

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Prochlorococcus, transcriptome, low salinity acclimation, low salinity stress, RNAseq

**Introduction**

Cyanobacterium *Prochlorococcus* is the smallest and most abundant photosynthetic, oxygen-evolving organism on Earth, playing a significant role in carbon fixation and biogeochemical cycles in the ocean (Guillard et al., 1985; Goericke and Welschmeyer, 1993; Liu et al., 1997). The prokaryotic *Prochlorococcus* cells contain divinyl-chlorophyll a and both monovinyl and divinyl-chlorophyll b as their primary photosynthetic pigments, which are unique to other cyanobacteria that contain chlorophyll a and phycobiliprotein...
Prochlorococcus is believed to be an obligate marine organism that is predominantly found in oligotrophic open oceans, as well as in some coastal waters, but barely seen in low salinity estuarine waters (Flemming et al., 2013). Prochlorococcus thrives throughout the euphotic zone in the tropical and subtropical oceans from 45° N to 40° S (Scanlan et al., 2009). This genus of marine picocyanobacteria is divided into high-light (HL) adapted and low-light (LL) adapted ecotypes, which are also phylogenetically distinct (Ferris and Palenik, 1998; Moore and Chisholm, 1999). HL ecotypes are usually distributed in upper euphotic zone, while LL ecotypes are generally distributed in the lower to bottom euphotic zone (Johnson et al., 2006; Zinser et al., 2007). Besides the light-related niche partitioning of HL and LL ecotypes, two HL ecotypes, HL I and HL II, also display temperature-related niche partitioning that HL II ecotypes dominate the warmer oceans between 30° N and 30° S while HL I ecotypes dominate the higher latitude oceans (West et al., 2001; Rocap et al., 2003; Mühling, 2012; Voigt et al., 2014). Despite comprising diverse phylogenetic lineages, Prochlorococcus is monophyletic on the phylogenetic tree built on 16S rRNA sequences of cyanobacteria (Rocap et al., 2002). Synechococcus is the sister genus of Prochlorococcus. However, Synechococcus is a provisional genus containing polyphyletic clusters which are scattering on the phylogenetic tree of cyanobacteria (Robertson et al., 2001). Marine Synechococcus is affiliated with cluster 5, which comprises subclusters 5.1, 5.2 and 5.3. In contrast to Prochlorococcus, marine Synechococcus is much more widely distributed, existing from estuary to open ocean and from equatorial to polar regions (Partensky et al., 1999; Zwinglermaier et al., 2008).

Salinity is a crucial factor affecting the growth and biogeography of cyanobacteria (Scanlan et al., 2009). There were plenty of studies on cyanobacteria’s salt acclimation and salt stress response (Hagemann, 2011). However, most of those studies were conducted mainly on freshwater cyanobacteria such as the euryhaline Synechocystis strain PCC 7002 and moderately halotolerant Synechocystis strain PCC 6803 rather than typical marine cyanobacteria such as Prochlorococcus or marine Synechococcus (Hagemann, 2011). For example, when growing at high salinity, Synechococcus PCC 7002 had increased expression of genes involved in compatible solute biosynthesis and electron transport, while only minor changes were observed when cells were grown at low salinity (Ludwig and Bryant, 2012). It also has been revealed that 200–300 genes were up-regulated and a comparable number of genes were down-regulated after the addition of salt in Synechocystis PCC 6803 (Kanasaki et al., 2002; Marin et al., 2003). Secondly, very few studies focus on the acclimation and stress response of marine cyanobacteria to low salinity. Lastly, compared to Synechococcus, salinity-related physiological studies on Prochlorococcus are even more seldom. A recent study showed that Prochlorococcus strain AS9601 could be acclimated to a high salt concentration of 5% (w/v; Al-Hosani et al., 2015). The authors compared the growth rate and transcriptome of AS9601 at salinities 3.8% (w/v) and 5% (w/v), and found that, under high salt concentration, approximately one-third of the genome expressed differentially.

The strict biogeographic distribution of Prochlorococcus in oceanic waters suggests that this organism cannot be adapted to low salinity. However, what is the lowest salinity that Prochlorococcus can survive and what is the stress response of Prochlorococcus cells to low salinity are still unclear. In this study we first tested the salinity range of two Prochlorococcus strains, NATL1A and MED4, and then acclimated the two strains under different salinities. We found that the lowest acclimation salinity is 25 psu for MED4 and 28 psu for NATL1A. Both NATL1A and MED4 cells were stressed when growing in salinities lower than 34 psu. We also found that the transcriptomic response of the two strains to low salinity stress were highly different.

### Materials and methods

#### Strains and growth conditions

Prochlorococcus strains MED4 and NATL1A were obtained from Jiao Nianzhi Lab, Xiamen University. Cultures were maintained in Pro99 natural seawater medium with a salinity of 34 psu, at 21°C and under a constant light intensity of 10μEm⁻²s⁻¹. We used canted neck polystyrene flasks (Corning Inc., Corning, NY, United States) of different volumes to culture the Prochlorococcus strains.

#### Experiment setup and growth rate calculation

Preparation of Pro99 medium followed the protocol from the Chisholm Laboratory. The seawater from the South China Sea basin was filtered through 0.22 μm polycarbonate membrane, and the salinity was pre-adjusted to 22 psu – 60 psu with a 2 psu interval using ddH₂O or NaCl. Salinity was measured using an ATAGO PAL-06S refractometer (ATAGO, Japan). These seawaters were autoclaved at 121°C for 15 min. Macronutrient (NH₄Cl and NaH₂PO₄) stocks and the trace metal stock were prepared in advance, and they were added into the above seawater base. Prochlorococcus cultures growing in the Pro99 medium of salinity 34 psu were inoculated into the salinity gradient mediums. The salinity was finally adjusted to 22 psu – 60 psu using the ddH₂O with Pro99 nutrients. Prochlorococcus growth was monitored every day for 2 weeks by measuring the OD₄₄₀ absorbance using a multimode plate reader (PerkinElmer, Waltham, MA, United States) and measuring the cell abundance using a flow cytometer (BD Accuri C6, BD Biosciences, CA, United States). Three biological replicates were set up for the experiment. Growth rate was calculated based on the two monitoring methods.

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1. [https://chisholmlab.mit.edu](https://chisholmlab.mit.edu)
respectively. Growth rate was calculated according to Mackey et al. (2013): 
\[ T_d = \ln(\frac{N_{i+1}}{N_i}) \]
where \( N_i \) is the number of cells on day \( i \), \( N_{i+1} \) is the number of cells on day \( i+1 \), and \( T_d \) is the growth rate of cells. The average growth rate of cells was calculated during the logarithmic phase.

**Low salinity acclimation**

*Prochlorococcus* strains MED4 and NATL1A were acclimated to different salinities (24 psu, 25 psu, 26 psu, 27 psu, 28 psu, 30 psu, 32 psu, 34 psu) by consecutive transfers from exponential growing cultures to fresh media. Three biological replicates were set up for each salinity. Five rounds of transfer were conducted for each strain. Using flow cytometry, cell abundance was monitored at day 0, day 5 and day 10. To assess the stress to low salinity, each strain’s dark-adapted photochemical efficiency (\( F_{v}/F_{m} \)) was monitored on day 10 in each round, using a handheld fluorometer (AquaPen AP 110/C, Photon Systems Instruments). To measure \( F_{v}/F_{m} \), 1 ml culture was dark-adapted in the sample cuvette for 15–30 min. The maximal fluorescence levels (\( F_{m} \)) were measured in the dark and under bright purple light (455 nm, 100 \( \mu \)Em\(^{-2}\)s\(^{-1}\)), where \( F_{i} \) is the basal fluorescence level and \( F_{r} \) is the variable fluorescence. The PSII quantum yield was calculated as \( \eta = \frac{F_{v}}{F_{m}} = \frac{F_{m} - F_{0}}{F_{m}} \).

**RNAseq analysis**

To acclimate the *Prochlorococcus* strains, MED4 and NATL1A were growing in the Pro99 medium of salinity 28 psu and 34 psu for five rounds of inoculation. Then the acclimated cultures were inoculated in fresh medium of salinity 28 psu and 34 psu, with salinity 34 psu being the control. Three biological replicates were set up. During the exponential growth phase, 100 ml cultures were filtered onto 0.22 \( \mu \)m polycarbonate membrane to collect cells and the membranes were immediately flash frozen in RNAlater by liquid nitrogen and stored at –80°C until RNA extraction. Total RNA was extracted from the membrane using the MagZol Reagent (Magen Biotech, Guangzhou, China). Sequencing libraries were prepared using VAHITS™ Stranded mRNA-seq Library Prep Kit for Illumina® (Vazyme biotech co., Ltd., Nanjing, China) following the manufacturer’s instructions. Libraries were multiplexed and sequencing was carried out on an Illumina HiSeq system with the 2 × 150 paired-end (PE) configuration (GENEWIZ). Cutadapt (v1.9.1) was used to remove adapters, primers, and reads with a base quality <20 based on FASTQ files. Clean data were aligned to the MED4 and NATL1A genomes via Bowtie2 software (v2.1.0). HTSeq (v0.6.1p1) was used to estimate gene expression levels from clean data. Differential expression analysis was performed using the DESeq Bioconductor package, a model based on negative binomial distribution. After adjusting using Benjamini and Hochberg’s approach for controlling the false discovery rate, differentially expressed genes were considered significant at value of \( p < 0.05 \). Highly induced or suppressed genes were considered as meeting both false discovery rate \( p < 0.05 \) and magnitude of log2fold change with values greater than 1 (highly induced) or less than –1 (highly suppressed). These two different criterions were also used in a previous study (Al-Hosani et al., 2015). Transcriptomic data have been deposited in NCBI Gene Expression Omnibus (GEO) under the accession number GSE195946.

**Results and discussion**

**Salinity range and acclimation to low salinity of *Prochlorococcus***

High-light adapted *Prochlorococcus* strain MED4 and low-light adapted strain NATL1A were tested for growth in different salinities ranging from 22 psu to 60 psu. Cell counting through flow cytometry (Figure 1A) and absorbance measurement

![FIGURE 1](https://example.com/figure1.png)

**FIGURE 1**

Growth rate of *Prochlorococcus* strains NATL1A and MED4 growing in Pro99 medium with salinities from 22 psu to 60 psu. Flow cytometry (A) and absorbance (OD440) measurement of chlorophyll (B) were used to monitor growth.
at 440 nm (Figure 1B) were used to monitor the growth of Prochlorococcus cells. MED4 could grow in the salinity range from 22 psu to 50 psu and NATL1A could grow in the range from 26 psu to 50 psu. The optimal salinity ranges of MED4 and NATL1A were similar, from 30 psu to 40 psu. This result came from the first transfer of cultures from salinity 34 psu to other salinities. Under the same growing temperature (21 °C) and light intensity (10 μE m⁻² s⁻¹), the LL strain NATL1A grew faster than the HL strain MED4.

During the acclimation experiment, NATL1A could grow in salinities higher than 26 psu in the first round, but could not survive in salinity lower than 28 psu in the last round (Figure 2B). Interestingly, MED4 showed a gradually changing growth rate in the salinity gradient from 25 psu to 28 psu, while NATL1A showed a sharp change between salinity 27 psu and salinity 28 psu (Figures 2C,D). The effective quantum yield of PSII photochemistry (Fv/Fm) was measured on the 10th day at the end of each incubation round (Figure 3). Both strains showed reduced yield when growing in low salinities from 24 psu to 32 psu, compared to the yield when growing in salinity 34 psu, and the lower the salinity resulted in lower yield. The yield of MED4 growing in salinity 24 psu was not detectable after round 4, while the yield of NATL1A growing in salinities 27 psu and below was not detectable after round 2. Together, these data showed that Prochlorococcus MED4 and NATL1A could be acclimated in salinities 25 psu and 28 psu, respectively. Interestingly, the high-light adapted strain MED4 and low-light adapted strain NATL1A showed different tolerance to low salinity.

It is well known that Prochlorococcus is an oceanic microorganism (Partensky et al., 1999), although a few studies claimed that Prochlorococcus-like populations existed in estuarine and even freshwater environments (Corzo et al., 1999; Shang et al.,...
However, these studies all only depended on flow cytometry investigation, and could not confirm that those “populations” on the flow cytometry diagram were indeed Prochlorococcus. Our acclimation study suggests that Prochlorococcus cannot live in salinity lower than 25 psu for a long time period (50 days in this study). This study provides evidence supporting that Prochlorococcus is an oceanic organism.

Differentially expressed genes in low salinity acclimated Prochlorococcus cells

RNA-seq was performed to assess the response of acclimated Prochlorococcus cells to low salinity (28 psu), with the salinity 34 psu being the control. When the filter criteria of significance meet the value of $p < 0.05$, there were 525 differentially expressed genes in the low salinity acclimated cells of NATL1A, with 286 genes being induced and 239 genes being repressed (Table 1). By contrast, MED4 appears to be less fluctuant under low salinity stress, with only 277 differentially expressed genes, among which 146 were induced and 131 were repressed (Table 1). A previous study compared the transcriptomes of Prochlorococcus AS9601 under high salt stress (5.0%) and under normal salt concentration (3.8%), and found 627 differentially expressed genes (Al-Hosani et al., 2015). Together, these results suggest that Prochlorococcus is sensitive to salinity changes.

Subsequently, the differentially expressed genes of these two strains were functionally classified according to Cyanobase definitions (Fujisawa et al., 2014). Firstly, the numbers of induced and repressed genes were equal for most functional modules

| Function categories                                      | Total no. | Total no. differential expressed | No. induced | No. repressed | Prevalent expression profile |
|-----------------------------------------------------------|-----------|----------------------------------|-------------|---------------|------------------------------|
|                                                          | NATL1A    | MED4                             | NATL1A      | MED4          | NATL1A                      | MED4                      |
| Translation, ribosomal structure and biogenesis          | 128       | 130                              | 38          | 25            | 5                            | 24                        | 33                        | 1                          |
|                                                          |            |                                  |             |               | Repressed                   | Induced                   |                           |
| Transcription                                            | 26        | 27                               | 10          | 6             | 7                            | 5                         | 3                         | 1                          |
|                                                          |            |                                  |             |               | Induced                      | Induced                   |                           |
| Signal transduction mechanisms                           | 26        | 24                               | 7           | 3             | 3                            | 2                         | 4                         | 1                          |
|                                                          |            |                                  |             |               | Equal                        | Equal                     |                           |
| Secondary metabolites biosynthesis, transport, and catabolism | 18       | 22                               | 4           | 7             | 3                            | 2                         | 1                         | 5                          |
|                                                          |            |                                  |             |               | Equal                        | Repressed                 |                           |
| Replication, recombination, and repair                   | 4          | 4                                | 2           | 1             | 2                            | 1                         | 0                         | 0                          |
|                                                          |            |                                  |             |               | Equal                        | Equal                     |                           |
| Posttranslational modification, protein turnover, chaperones | 87       | 82                               | 23          | 19            | 13                           | 5                         | 10                        | 14                         |
|                                                          |            |                                  |             |               | Induced                      | Repressed                 |                           |
| Nucleotide transport and metabolism                      | 48        | 50                               | 14          | 5             | 8                            | 4                         | 6                         | 1                          |
|                                                          |            |                                  |             |               | Equal                        | Induced                   |                           |
| Lipid metabolism                                         | 29        | 31                               | 10          | 6             | 3                            | 3                         | 7                         | 3                          |
|                                                          |            |                                  |             |               | Repressed                    | Equal                     |                           |
| Intracellular trafficking and secretion                  | 14        | 12                               | 4           | 2             | 2                            | 1                         | 2                         | 1                          |
|                                                          |            |                                  |             |               | Equal                        | Equal                     |                           |
| Inorganic ion transport and metabolism                   | 58        | 57                               | 8           | 6             | 5                            | 2                         | 3                         | 4                          |
|                                                          |            |                                  |             |               | Equal                        | Equal                     |                           |
| Energy production and conversion                         | 77        | 78                               | 28          | 14            | 19                           | 8                         | 9                         | 6                          |
|                                                          |            |                                  |             |               | Induced                      | Equal                     |                           |
| DNA replication, recombination, and repair               | 68        | 65                               | 12          | 7             | 7                            | 5                         | 5                         | 2                          |
|                                                          |            |                                  |             |               | Induced                      | Equal                     |                           |
| Defense mechanisms                                       | 17        | 16                               | 2           | 1             | 2                            | 0                         | 0                         | 1                          |
|                                                          |            |                                  |             |               | Equal                        | Equal                     |                           |
| Coenzyme metabolism                                      | 105       | 101                              | 20          | 11            | 6                            | 4                         | 14                        | 7                          |
|                                                          |            |                                  |             |               | Repressed                    | Repressed                 |                           |
| Cell wall/ membrane/envelope biogenesis                  | 1          | 2                                | 1           | 1             | 1                            | 0                         | 0                         | 1                          |
|                                                          |            |                                  |             |               | Equal                        | Equal                     |                           |
| Cell envelope biogenesis, outer membrane                 | 93        | 91                               | 30          | 10            | 16                           | 6                         | 14                        | 4                          |
|                                                          |            |                                  |             |               | Equal                        | Equal                     |                           |
| Cell division and chromosome partitioning                | 14        | 16                               | 4           | 3             | 3                            | 1                         | 1                         | 2                          |
|                                                          |            |                                  |             |               | Equal                        | Equal                     |                           |
| Cell cycle control, cell division, chromosome partitioning | 2         | 2                                | 0           | 1             | 0                            | 1                         | 0                         | 0                          |
|                                                          |            |                                  |             |               | Equal                        | Equal                     |                           |
| Carbohydrate transport and metabolism                    | 46        | 48                               | 10          | 10            | 4                            | 6                         | 6                         | 4                          |
|                                                          |            |                                  |             |               | Equal                        | Equal                     |                           |
| Amino acid transport and metabolism                      | 128       | 118                              | 38          | 17            | 19                           | 11                        | 19                        | 6                          |
|                                                          |            |                                  |             |               | Equal                        | Induced                   |                           |
| Function unknown                                         | 86        | 88                               | 18          | 13            | 10                           | 4                         | 8                         | 9                          |
|                                                          |            |                                  |             |               | –                            | –                         | –                         | –                          |
| General function prediction only                         | 137       | 132                              | 44          | 13            | 23                           | 8                         | 21                        | 5                          |
|                                                          |            |                                  |             |               | –                            | –                         | –                         | –                          |
| Others                                                   | 6          | 5                                | 2           | 0             | 0                            | 0                         | 2                         | 0                          |
|                                                          |            |                                  |             |               | –                            | –                         | –                         | –                          |
| Not in COGs                                              | 1,021     | 841                              | 196         | 96            | 125                          | 43                        | 71                        | 53                         |
|                                                          |            |                                  |             |               | –                            | –                         | –                         | –                          |
| Total                                                    | 2,239     | 2042                             | 525         | 277           | 286                          | 146                       | 239                       | 131                        |

2007; Mitbavkar et al., 2012; Zhang et al., 2013). However, these studies all only depended on flow cytometry investigation, and could not confirm that those “populations” on the flow cytometry diagram were indeed Prochlorococcus. Our acclimation study suggests that Prochlorococcus cannot live in salinity lower than 25 psu for a long time period (50 days in this study). This study provides evidence supporting that Prochlorococcus is an oceanic organism.
(60–70%) in each of the two strains. Secondly, compared to the control group, the changed prevalent expression profiles between the two strains were different. In low salinity acclimated NATL1A, some genes involved in translation, ribosomal structure and biogenesis, lipid metabolism and coenzyme metabolism were down-regulated, while genes involved in transcription, posttranslational modification, protein turnover, chaperones and energy production and conversion were up-regulated (Table 1). However, in MED4, regulation profile of the functions mentioned above is different from NATL1A, except for coenzyme metabolism.

### Contrasting regulation between NATL1A and MED4

Most interestingly, among the genes involved in translation, ribosomal structure and biogenesis, five were up-regulated and 33 were down-regulated in low salinity acclimated NATL1A cells compared to control (Tables 1, 2). However, in MED4, 24 genes of those genes were up-regulated and only one was down-regulated (Tables 1, 3). Strikingly, the regulation of genes involved in energy production and conversion were also in distinct patterns between NATL1A and MED4. In low salinity acclimated NATL1A cells, the ATP-producing genes were down-regulated (atpA, atpC, atpD, atpH and other ATP synthase genes), while many genes involved in photosynthesis (psaC, psb27, rbcS), cytochrome oxidation (cyoA, cyoB, ctaE), NADH dehydrogenase (ndhA, ndhH) were up-regulated (Table 2). However, in low salinity acclimated MED4 cells, genes for photosynthesis were down-regulated, such as photosystems II (psbA, psbB, psbD, psbN), cytochrome F (petA), and electron transport chain intermediate (ndhD), while most ATP-producing genes (acnB, atpG, atpF, atpH, atpD) were up-regulated. This striking contrasting transcriptional regulation indicated the two strains processed different response mechanisms to low salinity stress. It is likely that, to respond to low salinity stress, NATL1A enhanced photosynthesis but repressed ATP production and translation and biosynthesis. In contrast, MED4 repressed photosynthesis but enhanced ATP production, translation and biosynthesis. The reason is possible that NATL1A and MED4 were in different stress level under the salinity 28 psu, which appears to be slightly stressful for MED4, but extremely stressful for NATL1A. This is the reason why the differentially expressed genes of NATL1A were more than those of MED4.

It has been pointed out that the response of photosystem gene expression to high salt stress might be dependent on the organism under study, based on the investigations on Prochlorococcus strain AS9601, Synechocystis PCC 6803 and Synechococcus PCC 7002 (Al-Hosani et al., 2015). In high salt acclimated AS9601 cells, many genes coding for components of Photosystem I, Photosystem II and chlorophyll were down-regulated. By contrast, in high salt acclimated PCC 7002, PSI genes were down-regulated but PSII genes were not changed significantly (Ludwig and Bryant, 2012). Similarly, in this study, NATL1A and MED4 also showed heterogeneity in response to low salinity stress.

### Compatible solute and transporters

Cyanobacteria generally use the salt-out strategy for salt acclimation, in which cells maintain low intracellular ion concentration and accumulate compatible solutes to establish turgor (Hagemann, 2011). Compatible solutes are low-molecular-weight organic compounds, with sucrose, glucosylglycerol (GG), glucosylglycerate (GGA) and glycine betaine (GB) being the most common ones utilized by cyanobacteria (Klähn and Hagemann, 2011). Prochlorococcus cells probably use GGA and sucrose as their main compatible solutes (Scanlan et al., 2009). In NATL1A, we observed significant decrease in transcript abundance of the GGA synthesis genes (gpgS, encoding glucosyl-phosphoglycerate phosphatase, and gpgS, encoding glucosyl-phosphoglycerate synthase) in the low salinity acclimated cells compared to control cells (Table 4). However, we did not observe significant change on the sucrose synthesis gene spsA (encoding sucrose phosphate synthase). Moreover, in MED4, all the three genes did not show significant change in transcript abundance. These results suggest that, to cope with low salinity stress, NATL1A probably reduced the concentration of intracellular compatible solute GGA, while MED4 did not reduce the concentration of the compatible solutes. Again, different observations may be due to that the two strains were at different stress level when growing in the medium with salinity 28 psu. In another study, high salt acclimated Prochlorococcus AS9601 cells up-regulated the gpgS gene and a sodium transporter, suggesting that active extrusion of sodium ions and accumulation of GGA are involved in AS9601 acclimation to high salt stress (Al-Hosani et al., 2015). Together, these results suggest that compatible solute GGA may play an important role in the adaptation of Prochlorococcus to salinity changes.

Na+/H+ antiporter is closely related to plant salinity tolerance, and it is one of the critical factors of plant salt tolerance. To adapt to a high salt environment, plants will reduce the plasma membrane Na+ level through Na+/H+ antiporter (Apse et al., 1999; Hasegawa et al., 2000). Besides, cyanobacteria cells involved in salt stress tolerance was correlated with the activity of Na+/H+ antiporter (Allakhverdiev et al., 1999, 2000). However, in this study, the transcript level of Na+/H+ antiporter (nhaP) was increased under low salinity stress in NATL1A cells (Table 2). It is not clear what is the mechanism involved in this phenomenon. Perhaps the increasing expression of Na+/H+ antiporter would help to reduce the cytoplasm Na+ level which has already adapted to high salinity level of seawater.

### Iron transporter and molecular chaperone

Interestingly, a periplasmic ABC-type Fe3+ transporter (afaA/idA/futA) was up-regulated in low salinity acclimated cells of
| Gene ID     | Gene name      | Product                                      | p-Value  | log_2 FC |
|------------|----------------|----------------------------------------------|----------|----------|
| gene-NATL1_05001 | cyoA          | putative cytochrome c oxidase, subunit 2      | < 0.001  | 1.249    |
| gene-NATL1_17081 | acoA          | Pyruvate dehydrogenase E1 alpha subunit      | < 0.001  | 0.979    |
| gene-NATL1_04991 | cyoB          | Cytochrome c oxidase, subunit I              | < 0.001  | 0.897    |
| gene-NATL1_06051 | rbcS          | Ribulose bisphosphate carboxylase, small chain | 0.010    | 0.780    |
| gene-NATL1_02471 | ndhH          | putative NADH dehydrogenase subunit          | < 0.001  | 0.770    |
| gene-NATL1_05651 | psb27         | possible Photosystem II reaction center Psb27 protein | < 0.001  | 0.6925   |
| gene-NATL1_05981 | chiN          | Light-independent protochlorophyllide reductase subunit N | < 0.001  | 0.650    |
| gene-NATL1_20591 | psaC          | Photosystem I subunit PsaC                   | 0.004    | 0.633    |
| gene-NATL1_04561 | pdhC          | Dihydrolipoamide acetyltransferase           | 0.001    | 0.607    |
| gene-NATL1_04981 |ctaE          | Cytochrome c oxidase, subunit III            | 0.002    | 0.580    |
| gene-NATL1_20451 | icoD         | Isocitrate dehydrogenase                     | 0.006    | 0.532    |
| gene-NATL1_04171 | petB          | Cytochrome b6                                | 0.006    | 0.498    |
| gene-NATL1_02331 | ndhA          | putative respiratory chain NADH dehydrogenase subunit | 0.024    | 0.484    |
| gene-NATL1_17231 | NATL1_17231   | FAD/FMN-containing dehydrogenases            | 0.0158   | 0.453    |
| gene-NATL1_03751 | rub           | probable rubredoxin                         | 0.0188   | 0.394    |
| gene-NATL1_03311 | psb1          | photosystem II reaction center Pshl protein  | 0.0263   | −0.446   |
| gene-NATL1_19381 | NATL1_19381   | Fe-S oxidoreductase                         | 0.021    | −0.482   |
| gene-NATL1_18501 | atpH          | ATP synthase, delta (OSCP) subunit           | 0.008    | −0.497   |
| gene-NATL1_18491 | atpA          | ATP synthase F1, alpha subunit               | 0.006    | −0.565   |
| gene-NATL1_19601 | psaI          | photosystem I subunit VIII (PsaI)            | 0.008    | −0.574   |
| gene-NATL1_18481 | NATL1_18481   | Flavoprotein, FldA                          | 0.009    | −0.647   |
| gene-NATL1_18511 | NATL1_18511   | ATP synthase gamma subunit                   | < 0.001  | −0.668   |
| gene-NATL1_18381 | atpD          | ATP synthase F1, beta subunit                | < 0.001  | −0.671   |
| gene-NATL1_14931 | gldA          | putative glycerol dehydrogenase             | 0.004    | −0.755   |
| gene-NATL1_18391 | atpC          | ATP synthase, Epsilon subunit                | < 0.001  | −1.089   |
| gene-NATL1_16181 | afuA          | putative iron ABC transporter, substrate binding protein | < 0.001  | 1.208    |
| gene-NATL1_19031 | NATL1_19031   | Ferric uptake regulator family               | < 0.001  | 0.835    |
| gene-NATL1_05281 | nhpP          | putative Na+/H+ antiporter, CPAI family       | 0.005    | 0.597    |
| gene-NATL1_20831 | mgtE          | MgtE family, putative magnesium transport protein | 0.009    | 0.501    |
| gene-NATL1_03411 | amtB          | Ammonium transporter family                  | 0.022    | 0.414    |
| gene-NATL1_03071 | metS          | ATP-sulfurylase                              | 0.002    | −0.582   |
| gene-NATL1_15081 | petH          | ferredoxin-NADP oxidoreductase (FRN)         | < 0.001  | −0.687   |
| gene-NATL1_09851 | NATL1_09851   | Molecular chaperone DnaK, heat shock protein hsp70 | 0.001    | 0.668    |
| gene-NATL1_21861 | NATL1_21861   | Molecular chaperone DnaK2, heat shock protein hsp70-2 | 0.010    | 0.624    |
| gene-NATL1_18631 | NATL1_18631   | PtK cell division protein: S4 domain-Hemolysin A | 0.001    | 0.837    |
| gene-NATL1_04781 | NATL1_04781   | rRNA/rRNA methyltransferase (SpoU)           | 0.006    | 0.573    |
| gene-NATL1_00131 | NATL1_00131   | rRNA-dihydrouridine synthase                 | 0.040    | 0.566    |
| gene-NATL1_04521 | lrtA          | light repressed protein A-like protein       | 0.002    | 0.563    |
| gene-NATL1_03171 | ileS          | Isoleucyl-tRNA synthetase                    | 0.016    | 0.425    |
| gene-NATL1_04021 | rps1a         | 30S ribosomal protein S1, protein A          | 0.036    | −0.379   |
| gene-NATL1_17711 | rplU          | 50S ribosomal protein L21                    | 0.025    | −0.445   |
| gene-NATL1_16641 | rpsN          | 30S Ribosomal protein S14                    | 0.011    | −0.455   |
TABLE 2 (Continued)

| Gene ID      | Gene name | Product                                                                 | p-Value | logFC |
|--------------|-----------|--------------------------------------------------------------------------|---------|-------|
| gene-NATL1_05781 | frr       | Ribosome recycling factor                                                | 0.022   | -0.457|
| gene-NATL1_19971 | rpsC      | 30S ribosomal protein S3                                                 | 0.016   | -0.480|
| gene-NATL1_07951 | glyS      | Glycyl-tRNA synthetase beta subunit                                      | 0.013   | -0.485|
| gene-NATL1_19921 | rp5X      | 50S ribosomal protein L24                                                | 0.022   | -0.490|
| gene-NATL1_07891 | rpsB      | 30S ribosomal protein S2                                                 | 0.015   | -0.497|
| gene-NATL1_02771 | rpL       | 50S ribosomal protein L7/L12                                             | 0.004   | -0.564|
| gene-NATL1_19481 | rpsl      | 30S ribosomal protein S10                                                | 0.001   | -0.568|
| gene-NATL1_21821 | aspS      | Aspartyl-tRNA synthetase                                                 | 0.005   | -0.570|
| gene-NATL1_09331 | gatA      | Glutamyl-tRNA (Gln) amidotransferase A subunit                           | < 0.001 | -0.631|
| gene-NATL1_05331 | map       | putative methionine aminopeptidase                                       | < 0.001 | -0.635|
| gene-NATL1_16221 | glyQ      | glycyl-tRNA synthetase- alpha subunit                                    | 0.034   | -0.637|
| gene-NATL1_20021 | rpD       | 50S ribosomal protein L4                                                 | < 0.001 | -0.705|
| gene-NATL1_19881 | rpsR      | 50S ribosomal protein L18                                                | < 0.001 | -0.736|
| gene-NATL1_19951 | rpmC      | 50S ribosomal protein L29                                                | < 0.001 | -0.752|
| gene-NATL1_00581 | aliS      | Alanyl-tRNA synthetase                                                   | 0.002   | -0.756|
| gene-NATL1_10481 | fmt       | putative Methionyl-tRNA formyltransferase                                | 0.008   | -0.777|
| gene-NATL1_17561 | tyrS      | Tyrosyl-tRNA synthetase                                                  | 0.026   | -0.777|
| gene-NATL1_03281 | pth       | Peptidyl-tRNA hydrolase                                                 | 0.050   | -0.785|
| gene-NATL1_20011 | rpsW      | 50S ribosomal protein L23                                                | < 0.001 | -0.791|
| gene-NATL1_07901 | tsf       | putative Elongation factor Tis                                           | < 0.001 | -0.821|
| gene-NATL1_19511 | rpsG      | 30S ribosomal protein S7                                                 | < 0.001 | -0.868|
| gene-NATL1_21311 | rpT       | 50S ribosomal protein L20                                                | < 0.001 | -0.883|
| gene-NATL1_06131 | tdxF      | Putative translation initiation inhibitor - yjgF family                  | 0.001   | -0.939|
| gene-NATL1_10131 | rpsR      | 30S Ribosomal protein S18                                                | 0.001   | -1.140|
| gene-NATL1_10191 | cspR      | putative tRNA/rRNA methyltransferase (SpoU family)                       | 0.009   | -1.690|

Both NATL1A and MED4, compared to control cells. Moreover, NATL1A also up-regulated a ferric uptake regulator (NATL1_19031). It has been demonstrated that the transcript levels of idA gene in Synechococcus PCC 6301 and Prochlorococcus MED4 were increased under iron deficiency conditions (Michel et al., 1999; Webb et al., 2001; Thompson et al., 2011). This suggests that cells may be iron-limited under low salt-stress. Previously, afuA was found to be down-regulated in high salt-stressed cells of Prochlorococcus AS9601 (Al-Hosani et al., 2015). The authors attributed this to the reduced expression of iron required proteins under high salt condition. They also concluded that AS9601 was not iron limited because no difference in ferredoxin expression level was found between salt acclimated cells and control cells. It has been also revealed that iron requirement and siderophore production in cells is lower under high salinity (Boyle et al., 1977; Ruebsam et al., 2018). Together, these results indicates that there is a tight link between iron requirement and salt conditions in Prochlorococcus. However, the gene isiB (flavodoxin), which was induced in low iron stress (Erdner and Anderson, 1999; McKay et al., 1999), was not observed to be up-regulated in this study (Table 3). Hence, the specific relationship between low salinity stress and iron homeostasis remains to be investigated.

Up-regulated expression of dnaK was observed in both MED4 and NATL1A, which suggests that this gene could play a role in low salinity acclimation (Tables 2, 3). However, the molecular chaperone dnaK is one of the key factors for salt stress tolerance in halophiles, and over expression of dnaK can greatly reduce the growth lag period of the bacteria, allowing them to grow normally under salt stress (Sugimoto et al., 2003). Fukuda et al. (2001, 2002) cloned the dnaK gene from Tetragenococcus halophilica JCM5888 and introduced it into E. coli, and found that the dnaK transcript abundance was increased approximately 3.5-fold under salt stress. Meanwhile, dnaK was also found to be present in the halotolerant cyanobacterium Aphanothece halophytica (Hibino et al., 1999). The gene product of dnaK, heat shock protein hsp70, likely plays an important role in stress resistance, no matter it is low salinity stress or high salt stress.
TABLE 3 List of a part of differentially expressed genes ($p < 0.05$) in low salinity acclimated *Prochlorococcus* MED4.

| Gene ID   | Gene name | Product                                                                 | $p$-Value | log$_2$ FC |
|-----------|-----------|-------------------------------------------------------------------------|-----------|------------|
| gene-PMM0930 | pdhB   | Pyruvate dehydrogenase E1 beta subunit                                  | < 0.001   | 0.691      |
| gene-PMM317 | psbM    | possible Photosystem II reaction center M protein (PsbM)                 | 0.033     | 0.590      |
| gene-PMM544 | chiB    | Light-independent photosystem II reaction center B subunit               | 0.004     | 0.507      |
| gene-PMM1452 | atpH,atpD | ATP synthase, delta (OSCP) subunit                                     | 0.042     | 0.476      |
| gene-PMM0785 | prkbB    | phosphoribulokinase                                                     | 0.007     | 0.475      |
| gene-PMM1700 | axrB    | Acetate hydratase B                                                     | 0.009     | 0.460      |
| gene-PMM1454 | atpG    | ATP synthase B′/CF (0)                                                  | 0.019     | 0.411      |
| gene-PMM1453 | atpF    | ATP synthase B′/CF (0)                                                  | 0.038     | 0.392      |
| gene-PMM223 | psbA    | Photosystem II PsbA protein (D1)                                        | 0.018     | −0.427     |
| gene-PMM1517 | psbD    | Photosystem II PsbD protein (D2)                                        | 0.008     | −0.470     |
| gene-PMM315 | psbB    | Photosystem II reaction center N protein (psbN)                         | 0.014     | −0.476     |
| gene-PMM1454 | atpG    | ATP synthase B′/CF (0)                                                  | 0.041     | −1.707     |
| gene-PMM1229 | PMM1229 | Dehydrogenase, E1 component                                             | 0.009     | −0.577     |
| gene-PMM594 | ndbD    | putative NADH Dehydrogenase (complex I) subunit (chain 4)               | 0.001     | −0.663     |
| gene-PMM252 | psbN    | Photosystem II reaction center N protein (psbN)                         | 0.001     | −0.919     |
| gene-PMM366 | PMM0366 | Type-1 copper (blue) domain                                             | 0.002     | −0.960     |
| gene-PMM316 | PMM0316 | possible ferredoxin                                                     | < 0.001   | −1.358     |
| gene-PMM926 | psb28   | possible Photosystem II reaction center Pb28 protein                    | 0.041     | −1.707     |
| gene-PMM1032 | PMM1032 | ABC transporter, substrate binding protein, possibly Mn.                | 0.006     | 0.751      |
| gene-PMM1164 | ftaA/ftaA/ftaA | putative iron ABC transporter, substrate binding protein               | < 0.001   | 0.950      |
| gene-PMM808 | PMM0808 | Rieske iron–sulfur protein 2Fe-2S subunit                              | 0.019     | −0.501     |
| gene-PMM227 | cydD    | ATP-sulfurylase                                                         | < 0.001   | −0.893     |
| gene-PMM1701 | PMM1701 | putative chloride channel                                               | < 0.001   | −0.984     |
| gene-PMM504 | PMM0504 | CutA1 divalent ion tolerance protein                                    | 0.004     | −2.817     |
| gene-PMM1704 | dnaK2   | Molecular chaperone DnaK2, heat shock protein hsp70-2                    | 0.047     | 0.360      |
| gene-PMM1537 | rps3, rpsM | 30S ribosomal protein S13                                               | 0.001     | 1.774      |
| gene-PMM1538 | rpm9, rpm8 | 50S Ribosomal protein L36                                               | < 0.001   | 1.081      |
| gene-PMM1688 | aspS    | Aspartyl-tRNA synthetase                                                | 0.002     | 0.952      |
| gene-PMM1507 | rpoC, rps10 | 30S ribosomal protein S10                                               | < 0.001   | 0.844      |
| gene-PMM068 | def     | putative formylmethionine deformylase                                    | 0.025     | 0.745      |
| gene-PMM1661 | rpm35, rpm1 | 50S ribosomal protein L55                                               | 0.006     | 0.724      |
| gene-PMM1534 | rpm17, rpmQ | 50S ribosomal protein L17                                               | 0.002     | 0.697      |
| gene-PMM1550 | rpl29, rpmC | 50S ribosomal protein L29                                               | 0.041     | 0.609      |
| gene-PMM1545 | rps8, rpsH | 30S ribosomal protein S8                                                | 0.001     | 0.599      |
| gene-PMM1911 | pnp     | polyribonucleotide nucleotidyltransferase                               | 0.003     | 0.592      |
| gene-PMM997 | thrS    | Threonyl-tRNA synthetase                                                | 0.016     | 0.561      |
| gene-PMM312 | rps11a, rpsA1 | 30S ribosomal protein S1 (homolog A)                                   | 0.002     | 0.560      |
| gene-PMM1548 | rpl14, rplN | 50S Ribosomal protein L14                                               | 0.047     | 0.539      |
| gene-PMM1280 | PMM1280 | putative bifunctional enzyme tRNA methyltransferase, 2-C-methyl-D-erythritol 2-4-cyclodiphosphate synthase | 0.029 | 0.551 |
| gene-PMM0202 | rpl10, rpmI | 50S ribosomal protein L10                                               | 0.002     | 0.507      |
| gene-PMM1662 | rpl20, rplT | 50S ribosomal protein L20                                               | 0.017     | 0.498      |
| gene-PMM0870 | rpl33, rpmG | 50S Ribosomal protein L33                                               | 0.030     | 0.467      |
| gene-PMM1706 | rps6, rpe | 30S ribosomal protein S6                                                | 0.041     | 0.465      |
| gene-PMM1308 | tufA    | Elongation factor Tu                                                   | 0.006     | 0.459      |
| gene-PMM258 | ileS    | Isoleucyl-tRNA synthetase                                               | 0.027     | 0.429      |
| gene-PMM1532 | rpl13, rpmM | 50S ribosomal protein L13                                               | 0.044     | 0.425      |
| gene-PMM203 | rpl1, rplA | 50S ribosomal protein L1                                                | 0.021     | 0.420      |
| gene-PMM1546 | rps5, rplE | 50S ribosomal protein L5                                                | 0.028     | 0.384      |
| gene-PMM1309 | fuaA    | Elongation factor G                                                    | 0.030     | 0.370      |
Highly differentially expressed genes

When the filter criterion was changed from only meeting the value of \( p \) \((p < 0.05)\) to meeting both value of \( p \) and \( \log_{2}\text{fold change} \) with values greater than 1 (high induction) or less than -1 (high inhibition), there were 81 and 30 highly differentially expressed genes in NATL1A and MED4, respectively. These number are comparable to the previous study on Prochlorococcus AS9601, in which 69 highly differentially expressed genes were found in high salt acclimated cells compared to control cells (Al-Hosani et al., 2015). In NATL1A, 22 genes were down-regulated and 59 were up-regulated, while in MED4, 17 genes were down-regulated and 13 were up-regulated. There was no apparent gene enrichment pattern observed among these highly differentially expressed genes (Supplementary Tables S1, S2). For example, in low-salinity stress cells of NATL1A, many genes were highly inhibited, which were related to posttranslational modification (NATL1_02111 and NATL1_13731), signal transduction mechanisms (typA), cell envelope biogenesis, outer membrane (NATL1_08371 and NATL1_04491), translation, ribosomal structure and biogenesis (rpsR), coenzyme metabolism (foiE), energy production and conversion (atpC), and amino acid transport and metabolism (proA). Nevertheless, in salinity acclimated cells of MED4, some other genes appear to be repressed, which were those involved in energy production and conversion (PMM0316), secondary metabolites biosynthesis, transport, and catabolism (PMM0280), DNA replication, recombination, and repair (ruvC), lipid metabolism (des, yocE) and posttranslational modification (PMM1006).

Conclusion

Prochlorococcus is the most abundant phototroph in the ocean. This organism has been adapted to open ocean areas with stable salt concentrations, and barely found in nearshore and estuarine waters with lower and variable salt concentrations. In this study, we showed that the lowest salinities for acclimation of high-light adapted Prochlorococcus strain MED4 and low-light adapted strain NATL1A were 25 psu and 28 psu, respectively. The optimal growing salinity of both MED4 and NATL1A were from 30 to 40 psu. Global transcriptome analysis showed that the two strains responded differently to low salinity stress. First, far more genes of NATL1A were impacted than those of MED4 in low salinity acclimated cells, suggesting NATL1A was more intensively stressed than MED4 under salinity 28 psu. Second, compared to control, low salinity acclimated cells of NATL1A repressed the expression of genes involved in translation, ribosomal structure and biogenesis and ATP production, but enhanced photosynthesis, while MED4 regulated these pathways in an opposite way. To cope with low salinity, NATL1A also reduced the transcript abundance of genes involved in compatible solute GGA, while MED4 did not. Interpreting from a previous study and this study, a tight link between iron transportation and salt condition was verified, with high salinity stressed cells coupling with up-regulation of iron transporters and low salinity stressed cells coupling with down-regulation of iron transporters. This study demonstrated the regulations of global transcriptome of Prochlorococcus under low salinity stress and the mechanisms within those regulations warrant further investigation.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE195946.

Author contributions

SH, JD, and LL designed the experiments. XH and HL performed the experiments and analyzed the data. SH and XH wrote the manuscript. JD and LL provided resources and supervision. All authors contributed to the article and approved the submitted version.

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TABLE 4 Expression change on genes responsible for compatible solute biosynthesis.

| Locus     | p-Value | log.FC | Locus     | p-Value | log.FC |
|-----------|---------|--------|-----------|---------|--------|
| gpg      | 0.001   | -0.809 | PMM0515   | 0.367   | 0.214  |
| gpg      | 0.012   | -0.431 | PMM0962   | 0.476   | -0.132 |
| pspA     | 0.421   | -0.147 | PMM1711   | 0.143   | -0.371 |
Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2022.1038136/full#supplementary-material
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