Physiological response and transcriptome analysis of *Emiliana huxleyi* to different nitrogen concentrations

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Abstract

Coccolithophores are phytoplankton widely distributed in the global oceans and play a key role in biological carbon fixation. Particulate organic carbon (POC) is produced through photosynthetic carbon fixation, and particulate inorganic carbon (PIC) is produced during calcification and coccoliths formation. In addition, the dense coccoliths can increase the sinking rate of POC, and accelerate the output of POC driven by biological pumps to the deep ocean. In this study, we investigated the physiological and molecular responses of cells under different nitrogen concentrations using two strains of coccolithophores, *Emiliana huxleyi* (BOF-92 and RCC1266). It was found that the pigment abundance of the two strains decreased, but the inorganic carbon content of the cells increased significantly under nitrogen limitation. For the RCC1266, which had higher levels of calcification, nitrogen limitation also increased cellular organic carbon content. High nitrogen concentration had no obvious effect on cell growth, pigment content and cell carbon content. We found that the low nitrogen concentration group had the highest number of differentially expressed genes (DEGs), and nitrogen limitation induced more gene expression changes. More down-regulated genes were found at high nitrogen concentrations, and more up-regulated genes were found at low nitrogen concentrations. Compared with the BOF-92, the RCC1266 was more sensitive to changes in nitrogen concentration and had more DEGs. Under nitrogen limitation, calcification related genes were extensively up-regulated, including Ca²⁺ and inorganic carbon related transporter genes. The RCC1266 had more up-regulated genes. This study provides us with a fundamental understanding of changes in cellular carbon content and molecular mechanisms of coccolithophores under nitrogen limitation. It is an important basis for exploring how marine nutrient limitation affects the efficiency of phytoplankton biological pumps in the future.

Table 1 The number of differentially expressed genes (DEGs) in different nitrogen concentration groups was compared

<table>
<thead>
<tr>
<th>Comparative group</th>
<th>P-value</th>
<th>P&lt;0.05</th>
<th>L vs C</th>
<th>H vs C</th>
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<th>Log2FoldChange</th>
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Table 2 The number of differentially expressed genes (DEGs) in different nitrogen concentration groups was compared

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Fig. 1 Change of nitrate concentration and growth curve of medium under different nitrogen concentrations

(a) Change of nitrate concentration in BOF-92 medium, (b) change of nitrate concentration in RCC1266 medium, (c) growth curve of BOF-92, (d) growth curve of RCC1266

Fig. 2 Cytochrome content at different nitrogen concentrations

(a) chlorophyll a content, (b) carotenoid content. Letters are the results of Tukey test for pairwise comparisons. Different letters indicate significant differences (P < 0.05).

Fig. 3 Specific growth rates at different nitrogen concentrations

Letters are the results of Tukey test for pairwise comparisons. Different letters indicate significant differences (P < 0.05).

Fig. 4 Cell carbon content at different nitrogen concentrations

(a) Particulate organic carbon (POC) content of BOF-92, (b) POC content of RCC1266, (c) Particulate organic carbon (POC) content of BOF-92, (d) POC content of RCC1266, (e) POC/POC ratio of BOF-92, (f) POC/POC ratio of RCC1266. Letters are the results of Tukey test for pairwise comparison. Different letters indicate significant differences (P < 0.05).

Fig. 5 Cell nitrogen content at different nitrogen concentrations

(a) Particulate organic nitrogen (PON) content of BOF-92, (b) PON content of RCC1266, (c) PON/PON ratio of BOF-92, (d) PON/PON ratio of RCC1266.

Fig. 6 The number of differentially expressed genes (DEGS) in different nitrogen concentration groups was compared. The number of up-regulated genes is shown in red, and the number of down-regulated genes is shown in green. In the interaction region, the common up-regulated genes are represented in red, the common down-regulated genes are represented in green, and the genes with opposite up-down-regulated patterns are represented in gray. L vs C: low N and moderate N concentration comparison group; H vs C: high N and moderate N concentration comparison group; L vs H: low N and high N concentration comparison group. (a) BOF-92, (b) RCC1266.

Fig. 7 The number of differentially expressed genes (DEGS) in different nitrogen concentration groups was compared. Up-regulated genes are shown in red and down-regulated genes are shown in green. (a) BOF-92, (b) RCC1266.

Conclusion

- Low nitrogen concentration inhibited the growth and cytochrome content. But the content of PIC increased. Low nitrogen concentration promoted the calcification of *Emiliana huxleyi*. There was no significant decrease in PON content under low nitrogen condition.
- *Emiliana huxleyi* were more sensitive to nitrogen limitation conditions, but high nitrogen concentration had no obvious effect. The transcriptional regulation of the two strains showed more common characteristics under low nitrogen concentration. The regulation difference is greater at high nitrogen concentration.
- Nitrogen restriction enhanced the expression of calcification related genes in *Emiliana huxleyi*. The RCC1266, which had higher levels of calcification, had more up-regulated calcification related genes.