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Effects of Silicon, Calcium or Boron on Cell Growth and Lipid Accumulation in *Pinnularia gibba* var. Linearis

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Abstract. Effects of silicon, calcium or boron on cell growth and lipid accumulation in diatoms were studied. The growth of *Pinnularia gibba* var. linearis showed a log phase and a stable phase when cultured under silicon-rich condition. Cells accumulated more pectin and showed greater growth but contained less lipid and chrysolaminaran in log phase than in stable phase. Silicon deficiency treatment inhibited pectin production and cell growth. Silicon depletion after log phase did not affect pectin production and cell growth but stimulated lipid formation and inhibited chrysolaminaran accumulation, resulting the highest level of lipid production among all treatments. Effects of calcium or boron on the above measurements were similar but not as effective as silicon.

Keywords: silicon, calcium, boron, lipids, pectin, chrysolaminaran, *Pinnularia gibba* var. linearis

1. Introduction

Diatoms are unique in accumulating high level of neutral lipids during their growth [1], [2]. Silicon is essential for diatom growth and depleting silicon from cultural solution increased neutral lipid formation [2], [3], [4], [5], [6], [7], [8] and reduced chrysolaminarin accumulation [6], [7], [8], [9] as well.

Except lipids, diatoms also accumulate chrysolaminarin, a major storage polysaccharide [10], and pectin, the dominant component in cell wall. A feed control mechanism among assimilates exists widely in plant kingdom. How silicon regulates these assimilate allocation in diatoms, however, is not clear. Calcium and boron are important component in cell walls of other plants including many microalgae, the effects of replacing silicon with calcium or boron on assimilate allocation and lipid accumulation in diatoms has not been studied. Here we report our study on this approach by using *Pinnularia gibba* var. linearis.

2. Plants and materials

2.1. Strain of *Pinnularia gibba* var. linearis

Pinnularia gibba var. linearis was collected from the seashore 40 mile north of Seattle. A strain with higher neutral lipid production rate was separated and prepared for experiment.

2.2. Cultural conditions

Strain of *Pinnularia gibba* var. linearis was cultured in a basic sea water solution at 25℃, 12D/12L light period with a light intensity at 3500-4000lx. The cultural solution contained: sea water, NaHCO3 0.15g/L, Na2SiO3(9H2O)0.20g/L, NaNO31.00g/L, KH2PO4 0.02g/L, Vitamin B1 2.7mg/L, and Vitamin B12 1.5μg/L.

2.3. ¸ Treatments

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(1) Silicon-rich: strain was inoculated into a basic solution containing sufficient silicon, which included de-ionized water, NaHCO3 0.15g/L, Na2SiO3(9H2O) 0.20g/L, NaNO31.00g/L, KH2PO4 0.02g/L, Vitamin B1 2.7mg/L, and Vitamin B12 1.5μg/L.

(2) Silicon-deficient: strain was inoculated into a solution containing zero silicon, which included everything in treatment 1 but silicon. They were de-ionized water, NaHCO3 0.15g/L, NaNO3 1.00g/L, KH2PO4 0.02g/L, Vitamin B1 2.7mg/L, and Vitamin B12 1.5μg/L.

(3) Calcium-rich: strain was inoculated into a solution containing zero silicon but sufficient calcium, which included de-ionized water, NaHCO3 0.15g/L, CaCl2(2H2O)0.20g/L, NaNO31.00g/L, KH2PO4 0.02g/L, Vitamin B1 2.7mg/L, and Vitamin B12 1.5μg/L.

(4) Boron-rich: strain was inoculated into a solution containing zero silicon but sufficient boron, which included de-ionized water, NaHCO3 0.15g/L, Na2B4O7•(10H2O) 0.20g/L, NaNO31.00g/L, KH2PO4 0.02g/L, Vitamin B1 2.7mg/L, and Vitamin B12 1.5μg/L.

(5) Silicon-depletion: strain was first incubated in silicon-rich solution (treatment 1) for 3 days, then was transferred into a silicon-deficient solution (treatment 2) and incubated for 4 days.

All treatments were carried out in a self-made 10 L-bioreactor and inoculates were incubated at 25℃ with 12D/12L light period and a light intensity of 3500-4000lx. Each treatment had 3 replicates. Samples of 100 mL were taken every day, centrifuged at 6000 r/min for 10 min, freeze dried, and stored in a freezer for further use.

2.4. Biomass measurement

The incubation solution was mixed thoroughly, 100 mL of the solution was centrifuged 6000 r/min., supernatant was discarded and the precipitate was washed with distilled water twice, centrifuged at 6000 r/min. for 10 min., dried in an oven at 80℃ for 5 hr., placed in a desiccator for 10 hr. and weighted. The biomass of *Pinnularia gibba* var. linearis was represented as mg dry weight/L incubation solution.

2.5. Neutral lipid, chrysolaminarin and pectin measurement

Neutral lipids were measured as described by Roesller [7]. Chrysolaminarin measurement was carried out by the method of Beattie et al [11] and the method of Meijer et al [12] was used to measure pectin.

Data were subjected to analysis of variance (ANOVA) and regression procedures using the SAS Statistical Software (SAS Institute Inc. NC, USA). In figures, means were compared by Turkey's Studentized Range Test (HSD procedure) at $p \leqslant 0.05$.

3. Results

3.1. Effects of silicon, calcium and boron on biomass production

Under silicon-rich condition, the growth of *Pinnularia gibba* var. linearis showed a log phase in the first 4 days, and reached stable phase thereafter, both linear and quadratic regression were significant (Table 1). When silicon was deprived from the solution (silicon-deficient treatment), there was a significant reduction in biomass production. Silicon-depletion treatment (silicon-rich solution for 3 days and silicon-deficient condition for 4 days) had more biomass than silicon-deficient treatment but the same biomass with the silicon-rich treatment.

Calcium or boron treatments resulted slower growth than silicon-rich treatment but faster growth than silicon-deficient treatment. No difference was found between calcium and boron treatments in affecting biomass production.

Table 1: Effects of Si, Ca, and B on biomass (mg D.W./L) production of *Pinnularia gibba* var. linearis

Days	Si-rich	Si-deficient	Si-depletion	Ca-rich	B-rich
1	6.7	7.2	6.2	6.9	7.9

2	21.2	19.8	22.2	19.7	22.1
3	32.5	21.0	34.7	29.8	27.0
4	45.9	23.9	47.6	33.8	32.7
5	47.5	27.6	51.9	39.8	37.8
6	54.8	28.1	57.7	40.2	38.9
7	56.9	29.8	59.8	40.8	39.5
Regression					
Linear	****	****	****	****	****
Quadratic	***	***	***	***	***
Cubic	ns	ns	ns	ns	ns

3.2. Effects of silicon, calcium and boron on neutral lipid accumulation

Cells of *Pinnularia gibba* var. linearis accumulated more neutral lipids in the stable phase than in the log phase under silicon-rich condition (data not shown). Lipid contents were higher under silicon-deficient condition than under silicon-rich but similar to silicon-depletion condition (Fig. 1). When compared with the total neutral lipid production from the treatments, however, silicon-rich treatment produced more lipids than silicon-deficient culture. Silicon-depletion culture produced highest level of total lipids among all the treatments.

Lipid contents or total lipids were similar between calcium-rich and boron-rich treatments, which were lower than silicon-rich or silicon-depletion treatments. When compared with silicon-deficient treatment, however, cells had lower lipid content and similar total lipids in calcium or boron treatments.

3.3. Effects of silicon, calcium and boron on neutral lipid accumulation

Pectin formation showed a log phase increase after 2 days, and reached stable phase after 4 days in silicon-rich solution, which is parallel to cell growth (data not shown). Total pectin in silicon-deficient culture was the lowest among all the treatments. Total pectin in silicon-depletion treatment was similar to that in silicon-rich treatment, and both were higher than that in silicon-deficient treatment.

Pectin accumulation between calcium-rich and boron-rich solution was similar, it was lower than that in silicon-rich or silicon-depletion treatments but higher than that in silicon-deficient treatment.

3.4. Effects of silicon, calcium and boron on chrysolaminarin accumulation

Under silicon rich condition, cells of *Pinnularia gibba* var. linearis accumulated more chrysolaminarin in the stable phase than in the log phase (Data not shown). Chrysolaminarin was similar between silicon-deficient and silicon-depletion treatments, which were lower than that in silicon-rich treatment.

No difference was found in chrysolaminarin accumulation between calcium-rich and boron-rich treatments, which was similar to that in silicon-rich treatment and higher than that in silicon-deficient or silicon-depletion treatment.

4. Discussion

Our results showed that the growth of *Pinnularia gibba* var. linearis had two phases, a log phase and a stable phase (Table 1). Silicon is more important for cell growth in log phase than in stable phase and depletion of silicon in stable phase stimulated lipid accumulation without affecting cell growth. This founding could be used as a guide in diatom culture to produce more neutral lipids.

Pectin accumulated faster in log phase than in stable phase (data not shown), while both lipids and chrysolaminarin accumulated more rapidly in stable phase than in log phase (data not shown) under silicon-rich condition. Depletion of silicon in stable phase did not affect pectin production but reduced chrysolaminarin formation and increased lipid accumulation (Fig. 1, 2). These results indicate a shift in assimilate allocation during cell growth of *Pinnularia gibba* var. linearis and a feed control regulation may exist among the three major assimilates. Depletion of silicon in stable phase caused more assimilates shifted to lipid than to chrysolaminarin formation, which is consistent with the reports [7], [8], [9] that under silicon deficient condition, chrysolaminarin synthase activity decreased by 31% and ACCase (a key enzyme for lipid biosynthesis) activity was increased by 2-4 folds.

Compared with silicon, calcium or boron were less effective in promoting cell growth, pectin production, lipid accumulation and chrysolaminarin formation (Table 1; Fig 1,2) in *Pinnularia gibba* var. linearis, indicating silicon is not simply a stabilizer for pectin or simply as a component of cell wall, it plays an important role in regulating assimilate allocation in *Pinnularia gibba* var. linearis.

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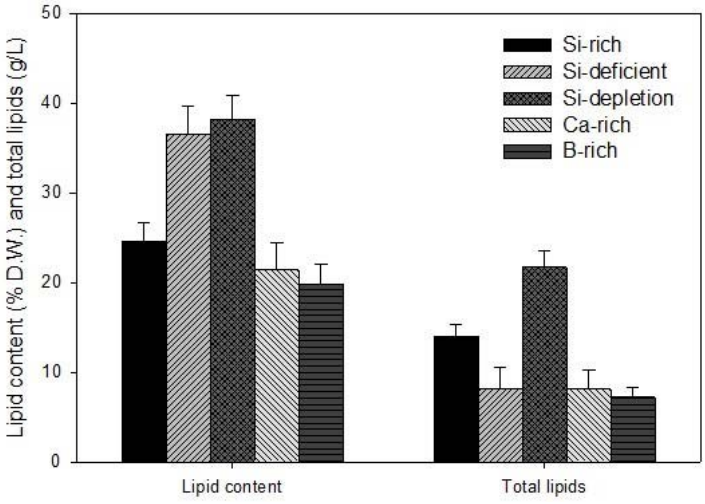

Fig. 1: Effects of Si, Ca or B on lipid accumulation.

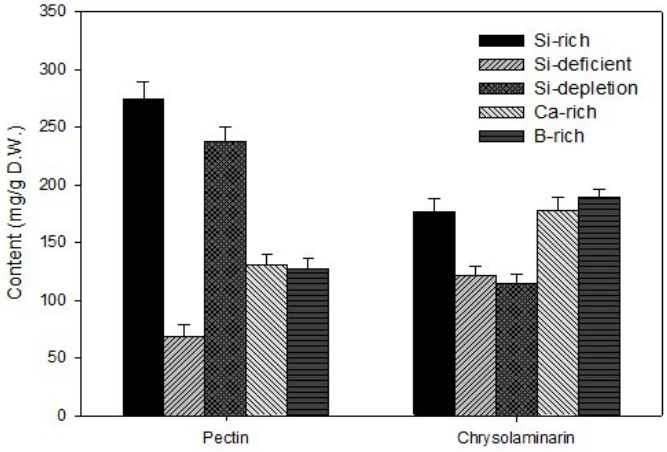

Fig. 2: Effects of Si, Ca or B on pectin and chrysolaminarin accumulation.