Diatoms as a Model System in Studying Lipid Biosynthesis Regulation

Zhiguo Ju, Lixiao Ding, Qingwu Zheng, Zhigang Wu, and Faxin Zheng

***Abstract*—Effects of silicon, calcium or boron on cell growth and lipid accumulation in diatoms were studied. Growth of *Chaetoceros gracilis* showed a log phase and a stable phase under silicon-rich condition. Cells produced more pectin in log phase while accumulated more lipids and chrysolaminaran in stable phase when cultured in silicon-rich solution. Silicon deficient treatment reduced pectin production and cell growth, increased lipid contents, but reduced total lipid production compared to silicon-rich condition. Silicon depletion by the end of log phase did not affect pectin production and cell growth but increased lipid production and reduced chrysolaminaran accumulation. No differences were found between calcium and boron treatments, both increased pectin production, cell growth, total lipid production and chrysolaminaran accumulation compared to silicon-deficient treatment but they were not as effective as silicon-rich treatment. Results showed that diatoms could be used as a model system in studying lipid biosynthesis and its regulation.**

***Index Terms*—Silicon, lipids, pectin, chrysolaminaran, *Chaetoceros gracilis*.**

I. Introduction

Diatoms are unique in that they do not synthesize cellulose but accumulate neutral lipids [1, 2], chrysolaminarin[3], a major storage polysaccharide, and pectin, one of the dominant components in cell wall, during their growth. Silicon is essential for diatom growth and depleting silicon from cultural solution increased neutral lipid formation[4, 5, 6, 7, 8,9,10] and reduced chrysolaminarin accumulation [6, 7, 8, 9], indicating a feed control mechanism in regulating assimilate allocation in diatoms.

Our early results [11] showed that silicon played an important role in regulating cell growth and assimilate allocation in strain from Pin*nularia gibba* var. linearis. If this is confirmed, diatoms could be used as a useful model system in studying the regulation of lipid biosynthesis and accumulation. Here we report our results using stain from *Chaetoceros gracilis*.

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Z. Ju, L. Ding, Q. Song, Z. Wu and are F. Zheng with Rizhao Polytechnic College, Rizhao,Shandong, China (e-mail: tanggufusang@ 163.com; lixiaoding65@ 163.com; songqingwu123@ 163.com; wzqlittle@ 126.com; faxinzheng@ 163.com).

II. Plants and Materials

A. Strain of Chaetoceros Gracilis

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

B. Cultural Conditions

Strain of *Chaetoceros gracilis* was cultured in a basic sea water solution at 25C, 12 D/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.

C. Treatments

(1) Silicon-rich (Si-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 (Si-def.): 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 (Ca-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 (B-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 (Si-dep.): strain was first incubated in silicon-rich solution (treatment 1) for 4 days, then was transferred into a silicon-deficient solution (treatment 2) and incubated for 3 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 withdraw every day, centrifuged at 6000 r/min for 10 min, freeze dried, and stored in a freezer for further use. The same amount of de-ionized water was added to the bioreactor whenever samples were taken to keep the constant volume of incubation solution.

D. 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 desiccators for 10 hr. and weighted.

The biomass of *Chaetoceros gracilis* was represented as mg dry weight/L incubation solution.

E. Neutral Lipid, Chrysolaminarin and Pectin Easurement

Neutral lipids were measured as described by Roesller [8]. Chrysolaminarin measurement was carried out by the method of Beattie et al [12] and the method of Meijer et al [13] 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 $p \leq 0.05$.

III. Results

A. Effects of Silicon, Calcium and Boron on Biomass Production

Under silicone-rich condition, the growth of *Chaetoceros gracilis* showed a log phase in the first 4 days and a stable phase thereafter, both linear and quadratic regression were significant (Table 1). Silicone deficient treatment resulted significant reduction in biomass production. Biomass from silicone depletion treatment was similar to silicone-rich treatment but higher than that from silicone-deficient treatment.

Biomass production between calcium-rich and boron-rich treatments was similar; both were lower than in Si-rich solution but higher than in silicone-deficient solution.

TABLE I: Effects of Si, Ca, and B on biomass (mg D.W./L) production of *Chaetoceros gracilis* #

Days	Si-rich	Si-def.	Si-dep.	Ca-rich	B-rich
1	4.4	5.6	4.8	4.7	4.9
2	11.1	7.9	12.2	11.4	11.8
3	23.8	11.9	22.8	19.3	16.7
4	39.2	14.6	38.3	21.8	21.3
5	43.5	17.2	44.4	25.8	24.2
6	49.8	22.2	47.7	36.3	32.8
7	58.1	23.5	57.2	38.9	36.3
Regression					
Linear	****	****	****	****	****
Quadratic	***	***	***	***	***
Cubic	ns	ns	ns	ns	ns

#: **** significant at $p \leq 0.0001$; *** significant at $p \leq 0.001$; ns: not significant

B. Effects of Silicon, Calcium and Boron on Neutral Lipid Accumulation

In silicon-rich treatment, cells of *Chaetoceros gracilis* accumulated more neutral lipids in the stable phase than in the log phase (data not shown). Lipid contents were higher under silicone-deficient condition than under silicon-rich but similar to silicon-depletion condition (Fig. 1). When compared with the total neutral lipid production from the cultures, however, silicon-rich treatment produced more lipids than silicon-deficient culture. But the silicon-depletion treatment produced the highest level of lipids among all the treatments. Neutral lipids in calcium-rich or boron-rich treatment were similar and were lower than that in silicon-rich or silicon-depletion treatments.

C. Effects of Silicon, Calcium and Boron on Pectin Accumulation

In silicon-rich solution, pectin content increased linearly after 2days, and reached stable phase after 4 days (data not shown). Fig. 2 showed the effects of silicon, calcium and boron on pectin accumulation. 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. Calcium or boron treatment showed similar effects on pectin formation. Pectin contents in calcium or boron treatments were lower than that in silicon-rich or silicon depletion treatments but higher than that in silicon-deficient solution.

D. Effects of Silicon, Calcium and Boron on Chrysolaminarin Accumulation

Cells of *Chaetoceros gracilis* accumulated more chrysolaminarin in stable phase than in log phase when cultured under silicon-rich condition (Data not shown). According to Fig 2., chrysolaminarin contents were similar between silicon-deficient and silicon-depletion treatments, which were lower than that in silicon-rich treatment. Chrysolaminarin contents in calcium-rich or boron-rich treatments were similar; they were similar to that in silicon-rich treatment but higher than that in silicon-deficient or silicon-depletion treatment.

IV. Discussion

Our results showed two phases of diatom growth, a log phase and a stable phase (Table 1), which is similar to *Pinnularia gibba* var. linearis [11].. Silicon is essential for pectin formation and cell growth (Table 1, Fig. 2). Depletion of silicon after log phase stimulated lipid accumulation without affecting cell growth (Table 1). These results indicate that silicone could be used as an effective tool in stimulating lipid biosynthesis and to increase lipid production in diatoms.

Similar to *Pinnularia gibba* var. linearis[11], cells accumulated pectin in log phase and lipids and chrysolaminarin in stable phase during normal growth of *Chaetoceros gracilis*. Depletion of silicon after log phase did not affect pectin production but reduced chrysolaminarin production and increased lipid accumulation (Fig. 1, 2), indicating a shift in assimilates allocation during growth and there is a feed control mechanism among the three major assimilates in diatoms. The different effects of silicon on lipids and chrysolaminarin may be explained by the finding

that depletion of silicon from solution reduced chrysolaminarin synthase activity by 31% and increased ACCase (an key enzyme for lipid biosynthesis) activity by 2-4 folds [9,10].

Compared with silicon, calcium or boron were less effective in promoting cell growth, pectin production, lipid accumulation or reducing chrysolaminarin formation (Table 1; Fig 1,2) in *Chaetoceros gracilis*, indicating silicon is not just a stabilizer for pectin or simply as a component of cell wall, it plays an important role in regulating assimilate allocation in *Chaetoceros gracilis*. Together with the early findings from *Pinnularia gibba* var. linearis[11], this study showed that diatoms is unique in assimilate allocation and could be used as a model system in studying lipid biosynthesis and regulation.

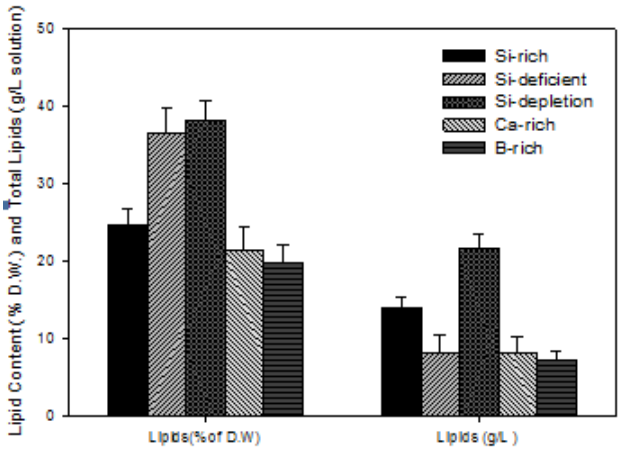

Fig.1. Effects of Si, Ca or B on lipid content and total lipid production

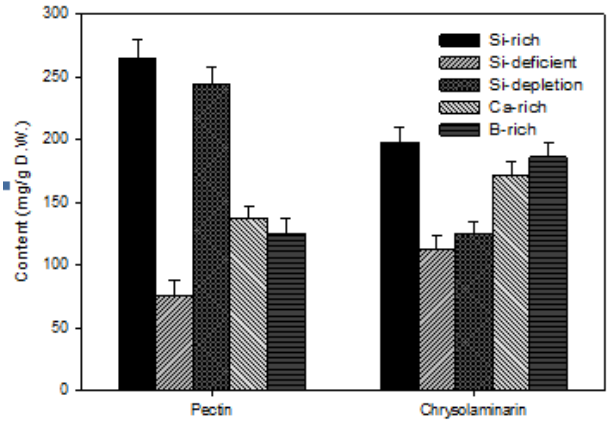

Fig.2. Effects of Si, Ca or B on pectin and chrysolaminarin accumulation

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Zhiguo Ju received his Bachelor degree from Laiyang Agricultural College, Master degree from Beijing University, China and Ph. D from the University of Massachusetts at Amherst, USA. He used to be a Post-Doc at the University of California, Davis, a research scientist at USDA-ARS Tree Fruit Research Lab in Wenatchee, Washington State, and a research scientist at Valent BioScience Corp in Chicago. He was a professor at Laiyang Agricultural College, and currently is a Faculty member professor at Rizhao Polytechnic College in China. His interests include plant physiology and biochemistry, bio-pesticide development, and bio-energy research.