Relation between Diatom Growth and Bacterial Population in Semi Mass Culture Tanks of Diatom

SUMINTO and Kazutsugu HIRAYAMA

The practical semi mass culture of diatoms was conducted at the Yuya Laboratory of Nippon Saibai Suisan Co., Ltd., Yamaguchi Prefecture, using cylindrical fiberglass tanks (500 *l* of water capacity). At the start of the culture, to the tanks inorganic fertilizers were introduced, but no starter of the diatom species cultured was inoculated.

Either growth pattern of diatoms or bacterial had the common characteristics in three culture trials. The bacterial population (CFU/m*l*) increased with the growth of diatoms in early period of the culture but the population ratio of the bacterial to the diatoms rapidly declined. At the peak of diatom population (the 3rd or 4th day), the bacterial population made a bottom and the population ratio showed the lowest value. After that, diatom density sharply declined and on the contrary bacterial population rapidly increased. These facts suggest that the actively growing population each of diatom and bacteria may exhibit the suppressive effect on the growth of another population and that artificial control of bacterial population (so-called "bio-control method") could be effective on the improvement in the technique of diatom mass culture.

Key words: Diatom; mass culture; bacteria; growth pattern.

For mass culture of the larvae of kuruma prawn *Penaeus japonicus*, diatoms are used as the most effective food organisms. Practical mass production of diatoms is usually conducted in the open tanks with addition of some nutrients into the culture water. In the case of no special treatment, mass culture sometimes meets unstable production. Several researchers reported the environmental bacterial role on the diatom growth and contrary role of diatom to the bacterial growth under culture conditions.[1-4] Siebuth[5] and Rheinheimer[6] proposed diverse relationships between bacterial and phytoplanktons in the aquatic ecosystem.

As a primary research on the relationship between diatom growth and bacterial population, the diatoms were cultured in semi mass culture scale, and the growth patterns of the both organisms of diatom and bacteria were investigated, with the measurement of several environmental factors which may influence the diatom growth.

Materials and methods

Three trials of the semi mass cultures of diatoms were conducted at the Yuya Laboratory of Nippon Saibai Suisan Co., Ltd., Yamaguchi Prefecture, in 1992, using cylindrical fiberglass tanks (500 *l* of water capacity), according to the practical method of the Yuya Laboratory. Trials in May and in June and July were made in duplicate and triplicate, respectively. Three culture trials continued for 5, 5 and 6 days in May, June and July, respectively, until the growth of diatom ceased to the declining phase.

Seawater taken from near Yuya Laboratory was pumped into the reservoir after sand filtration. From the reservoir, seawater which was filtered through nylon net with 80 mesh size screen (261μm) was filled into the experimental tanks. To each tank was added a mixture of fertilizers of KNO_3, Na_2HPO_4, and K_2SiO_3 (assay: 27-29 %). They were diluted as 200 mg,

40 mg, and 0.02 ml per 1 l seawater, respectively. No seed of cultured diatom cells was inoculated as a starter into the experimental tanks. These tanks were then placed under the direct sun light. Week aeration was given during culture period.

The water sample was taken with a 1 l glass beaker three times a day in the daytime. The number of diatom cells was counted by haemocytometer using microscope at every collection. Daily diatom cell concentration was calculated as an average of three counts. Identification of dominant diatoms was conducted twice during culture period at the 2nd day in the growing phase and at the 5th day in the declining phase. For investigation of bacterial population, the water samples were collected every day at 1 pm. They were diluted with sterilized seawater and spread onto Zobell's 2216E agar plates, which were incubated for 2 days at 25°C. Then, bacteria colony-forming units of the samples collected were determined. Genera of the bacterial strains appeared on the agar plates were identified on the basis of the Simidu's schema[7] and Bargey's Manual of Systematic Bacteriology Vol. 1 and 2.[8,9] Viable cell number of dominant bacterial genera was percentaged against total number colony forming unit (CFU) at the growing or declining phase of diatoms in every trial.

Water temperature, salinity, and pH were recorded once at 1 pm, and light intensity was recorded 5 times a day in the daytime.

Results

Changes in diatom cell density (cells/ml) and viable bacterial population (CFU/ml) in each trial were illustrated in Fig. 1. Growth patterns of diatoms in the duplicate or triplicate tanks in each trial were all similar. Growth patterns in three trials had common characteristics, especially, in trials in May and June. The growths in trials in May and June reached the peak density of 3-4 $\times$ 10^5 cells/ml at the 3rd day. The peak density in trial in July reached of about 9 $\times$ 10^4 cells/ml at the 4th day. After the peak,

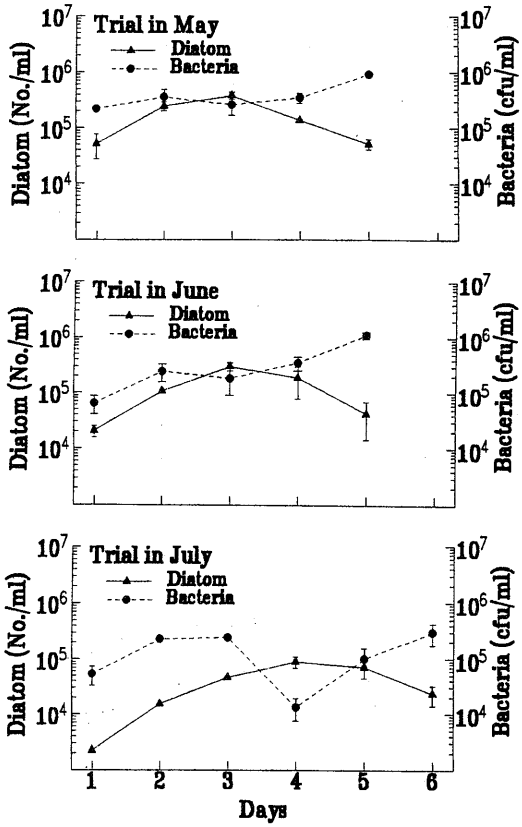

Fig. 1. The growth patterns of diatom and viable bacterial colony during the culture period in each trial. Plotted points and bars indicate mean and standard deviation of duplicate or triplicate tanks, respectively.

they immediately enter into the declining phase. Bacterial populations in all trials increased in the early period for 2 or 3 days, then once declined at following day and from the 4th or 5th day they rapidly increased again. The day showing bottom of bacterial population was the same day of the peak in diatom cell density.

The changes in ratio of bacterial population to diatom cell density in each trial were illustrated in Fig. 2. Diatom cell density and bacterial population were represented as the average density for duplicate or triplicate tanks. The ratio rapidly declined at the early period of culture, and then sharply increased at the late period of culture. The day at the lowest value of the ratio was the same day at the peak of diatom population.

The diatom composition in duplicate or tripli-

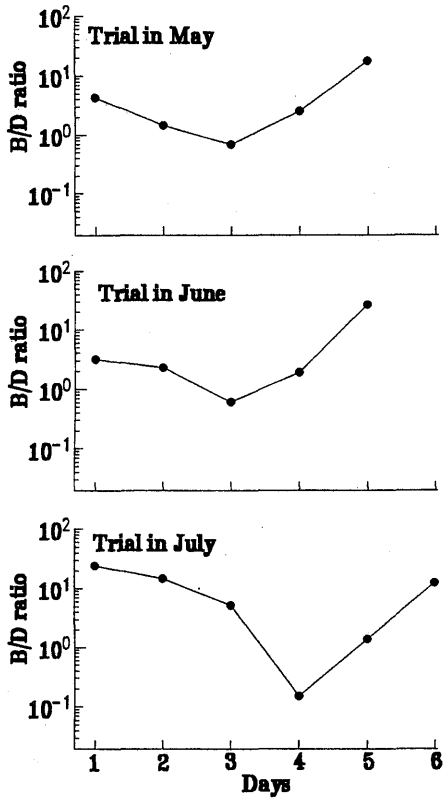

Fig. 2. Patterns of the ratio of viable bacterial colony to diatom cells density (B/D ratio) during the culture period in each trial. Ratio was calculated from average values of both cell densities of duplicate or triplicate tanks, respectively.

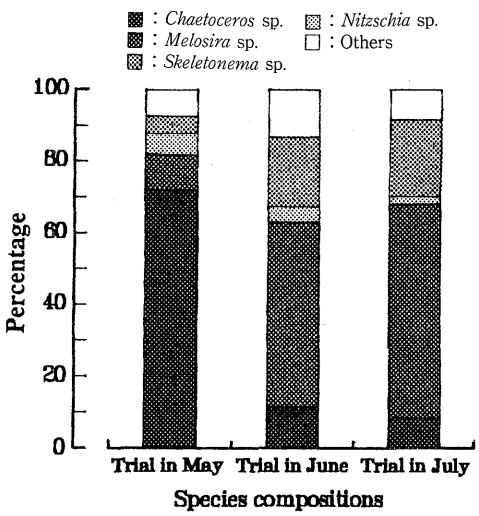

Fig. 3. Species compositions of diatom cells in each trial. Percentages are calculated from the average density from the densities at growing and declining phases.

cate culture tanks underwent so similar that an average density of each diatom species was used for estimation of cell composition of the diatom species in each trial. In Fig. 3, percentages of cell number of diatom species appeared in three trials are shown. In every trial, a few species is so predominated during the culture period, that dominant four species only were shown in pictures. The single species occupied the larger percentage more than 50 % in diatom cell composition in every trial; *Chaetoceros* sp. in trial in May, *Melosira* sp. in trials in June and July.

In order to compare the bacterial compositions in the growing and declining phases of diatom growth, the genus compositions of bacte-

Table 1. Composition of bacterial genera at the growing and the declining phases of diatom growth in each trial

Trial	Genus composition of bacteria (%)									Total
	A	B	C	D	E	F	G	H	I	
in May										
Day 1 to Day 2	49.5	5.0	20.8	5.8			4.1	4.7	10.1	100
Day 4 to Day 5	33.9	7.8	20.2	15.6			7.3	4.7	10.0	100
in June										
Day 1 to Day 2	13.5	17.3	20.0	3.5	36.2			1.1	8.4	100
Day 4 to Day 5	20.0	3.7	6.7	26.3	33.7			0.3	9.3	100
in July										
Day 1 to Day 3	4.1	3.0	58.9		17.5	8.5		0.9	7.1	100
Day 5 to Day 6	4.7	7.3	52.2		17.0	9.8		0.5	8.5	100

Precentage data are from average value of duplicate tanks in May; and triplicate tanks in June and July, respectively. A, *Alteromonas*; B, *Flavobacterium*; C, *Bacillus*; D, *Micrococcus*; E, *Moraxella*; F, *Alcaligenus*; G, *Coryneforms*; H, *Vibrio*; I, Unknown.

Table 2. Average values of light intensity, water temperature, salinity, and pH in each trial

Trial	Light intensity (x1000 lux)	Temperature (°C)	Salinity (ppt)	pH (unit)
in May	*	22.9 (19.8—25.5)	34.9 (33.0—36.8)	8.5 (8.5—8.9)
in June	24.8 (12.7—33.3)	25.7 (23.7—28.0)	36.1 (34.5—37.5)	8.5 (8.2—8.8)
in July	8.5 (4.9—13.0)	24.9 (24.3—25.6)	34.3 (33.5—35.0)	8.5 (8.3—8.6)

*, No data.
Ranges are in parentheses.

rial cells in both phases were calculated from the average CFU number in duplicate or triplicate tanks in each of the growing phases (Table 1). Dominant genera were similar in all tanks at every trial, but different in three trials. The difference in bacterial compositions between two phases had no similar tendency in three trials.

In table 2 was listed the averages and ranges of light intensities, water temperatures, salinities and pH for every trial. Out of environmental conditions measured, only light intensity showed the difference between in trials in June and July.

Discussion

In present experimental semi mass culture of diatoms (Figs. 1 and 2), the bacterial population increased with the growth of diatoms in the early period of the culture, but the population ratio of bacteria to diatom rapidly declined. At the peak of diatom population (the 3rd or 4th day) the bacterial population made a bottom and population ratio showed the lowest value. After that diatom density sharply declined and on the contrary bacterial population rapidly increased. These facts suggest the existence of the close interaction between the diatom growth and the bacterial population. Actively growing population each of diatom and bacteria may exhibit the suppressive effect on the growth of other population.

In each trial, the duplicate or triplicate cultures had almost the identical growth pattern of diatom or bacteria. But, in trial in May a dominant species of diatoms (*Chaetoceros* sp.) was different from other trials (*Melosira* sp.). In the trial in July, the diatom population was different in the level of maximum density and the peak day of population (Figs. 1 and 2) from other trials. Genus composition of bacterial population had the different faces each other in three trials, but no special tendency was shown in the growing and declining phases of diatom (Table 1).

These differences in three trials might be caused from the seasonal succession of environmental conditions, though light intensity only showed the distinct difference among trials in the environmental parameters measured in the experiment.

The results obtained in the present study suggest that artificial control of bacterial population (so-called "bio-control method") could be effective method on the improvement in the technique of diatom mass culture.

Acknowledgments

We would like to express our thanks to the Nippon Saibai Suisan Co., Ltd., for supporting this research. This study was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

References

1) K. Kogure, U. Simidu, and N. Taga: *J. Exp. Mar. Biol. Ecol.*, **36**, 201-215 (1979).

2) T. Sakata, Y. Fujita, and H. Yasumoto: *Nippon Suisan Gakkaishi*, **57**, 1147－1152 (1991).
3) A. Mitsutani, K. Tatesue, M. Kirita, and Y. Ishida: *Nippon Suisan Gakkaishi*, **58**, 2159-2169 (1992).
4) C. E. Riquelm, K. Fukami, and Y. Ishida: *Bull. Jap. Soc. Microb. Ecol.*, **3**, 29－34 (1988).
5) J. McN. Siebuth: Advances in Microbiology of the Sea (eds. M. R. Droop & E. J. F. Wood), Academic Press, London & New York, 1968, pp. 63-94.
6) G. Rheinheimer: Aquatic Microbiology (ed. G. Rheinheimer), John Willy Sons. Inc. Publ., New York, U. S. A., 1992, pp. 169-173.
7) U. Simidu: Marine Microbiology (ed. N. Taga), Tokyo University Press, Tokyo, Japan, 1974, pp. 45-65.
8) N. R. Krieg, and J. G. Holt: Bargey's Manual of Systematic Bacteriology, Vol. 1., Williams and Wilkins Publ., Baltimore, U. S. A., 1984.
9) P. H. A. Sneath, and J. G. Holt: Bargey's Manual of Systematic Bacteriology, Vol. 2., Williams and Wilkins Publ., Baltimore, U. S. A., 1986.

珪藻大量培養槽における珪藻と環境細菌との関係

ス ミ ン ト，平山　和次

日本栽培水産株式会社油谷研究所において1992年５，６，７月の３回500ℓの水槽を用いて珪藻の野外大量培養を試みた．使用した海水は261μmのメッシュのナイロンネットで沪過したものである．培養開始時に無機栄養塩は添加したが，培養珪藻を種として添加することはしなかった．増殖した珪藻が減少するまで５－６日間培養は継続した．珪藻は最初の２－３日間で速やかに増殖したが３－４日目にはピークに達し，以後減少した．一方，細菌濃度（CFU／ml）は珪藻の増殖時にも増大したが，珪藻細胞数に対する細菌数の比は速やかに減少した．珪藻濃度がピークに達した３－４日目には細菌濃度は一旦減少し，細菌の珪藻に対する細胞比は最低となった．以後珪藻濃度は減少するが細胞濃度は急速に増大したので，細胞比は著しく増大した．時期の異なる３回の培養でこの傾向は一致した．

以上の結果は珪藻培養槽内では珪藻と細菌とは競争関係にあることを示している．今後，環境細菌制御（いわゆるバイオコントロール法）よる珪藻大量培養方法の改善の可能性を追究する．