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Algal growth stimulation by heterotrophic bacteria with lake sediment extract

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Algal growth stimulation by heterotrophic bacteria with lake sediment extract was researched by two method of bottle tests. A stimulatory effect was higher enriching with lake sediment extract added to unfiltrated lake water in bottle test than that of enriching with inorganic nutrients. Some bacterial strains isolated from lakes stimulated algal growth in coexistant culture with sediment extract. Maximum growth of *Chlorella pyrenoidosa*, *Scenedesmus bijuga*, and *Microcystis aeruginosa* in coexistant culture were 3.4, 3.6, and 4.1-fold as large as algal yield in unialgal culture.

From these results, it is suggested that coexistant heterotrophic bacteria promoted algal growth with stimulator produced from sediment extract by them except for stimulation with inorganic nutrients.

Key index words: *algal growth stimulation*; *freshwater algae*; *heterotrophic bacteria*; *Lake Barato*; *lake sediment extract*; *organic substance.*

Algal growth is influenced by various parameters. Since SHAPIRO (1970) suggested water bloom and red tide were caused by a supply of phosphorus, many workers have been researching algal growth stimulation from the point of inorganic nutritional concentration.

On the other hand, it is reported that organic substances such as purine, pirimidine, vitamins, and hormones stimulate algal growth (IWASAKI 1979, ADAIR and MILLER 1982, HINO and ANDO 1983). Moreover, organic substances derived from lake sediment and sewage water stimulate algal growth (PRAKASH and RASHID 1968, MCDONALD and CLESCERI 1973, PRAKASH *et al.* 1973, COOKSEY and COOKSEY 1979, HINO and ANDO 1981, 1983).

Although inorganic nutrients are necessary to algal growth, other factors, for instance biological interaction, may associate with algal growth in natural water. In previous papers, it is reported that heterotrophic bacteria in lake water produced algal growth stimulator when sugars are added to natural water (KUENTZEL 1969, TEZUKA and HAYASHI 1975). Heterotrophic bacteria and organic substances in natural water may take part in algal growth stimulation.

The aim of the present study was research on the interaction among algae, heterotrophic bacteria, and substances derived from sediment on algal growth stimulation. We attempted to isolate heterotrophic bacteria from lake, and researched experimentally by bottle test using lake water and coexistant culture enriched with sediment extract.

Materials and Methods

Sediment and water samples:

Sediment and surface water samples were collected on 24 April 1980, in Lake Barato located around the central Hokkaido of Japan. Lake Barato is a crescent lake formed by changing of River Ishikari in 1930, which is 9 m in maximum depth and 4.37 km^2 in

Table 1. Chemical nature of surface water in Lake Barato and the sediment extract used in culture experiments.

Item	Unit	Surface water	Sediment extract
pH		7.3	6.5
Dissolved oxygen	(mg $O_2 \cdot l^{-1}$)	6.9	—
Ammonium nitrogen	(mg $N \cdot l^{-1}$)	3.72	1.26
Nitrite nitrogen	(mg $N \cdot l^{-1}$)	0.15	0.01>
Nitrate nitrogen	(mg $N \cdot l^{-1}$)	1.41	0.01>
Total organic nitrogen	(mg $N \cdot l^{-1}$)	0.98	34.8
Phosphate	(mg $P \cdot l^{-1}$)	0.058	0.200
Total phosphorus	(mg $P \cdot l^{-1}$)	0.104	0.310
Carbonhydrate	(mg· l^{-1})	1.89	274
Free-amino acids	(mg· l^{-1})	0.17	18
Proteins	(mg· l^{-1})	2.03	115
Acetic acid	(mg· l^{-1})	—	6.8
Purine and Pyrimidine	(μg· l^{-1})	3.8	630

area. Sediment extract solution was obtained by the same method of previous paper (HINO and ANDO 1983) except for using sterile distilled water. The samples' chemical qualities are shown in Table 1.

Algal materials:

Axenic *Chlorella pyrenoidosa* (C-28) and *Scenedesmus bijuga* (C-347) which were supplied by the Institute of Applied Microbiology, Tokyo University, were used. *Microcystis aeruginosa* was isolated from surface water of Lake Barato by micropipette washer method, but this strain was not axenic. Culture medium was modified 1/2 ASM medium (HINO and ANDO 1983).

Isolation of heterotrophic bacteria:

Heterotrophic bacteria were isolated by agar plate method from suspension of lake water and sediment mud of Lake Barato. One liter of nutrient agar medium contained 1 g meat extract, 0.5 g polypeptone, 0.5 g NaCl, and 18 g Bact-agar. Agar plates were incubated at 25°C for 14 days. The growth of isolated bacteria were tested in sterile sediment extract solution.

Bottle test using lake water:

Five hundred m*l* of unfiltrated lake water was poured into four one liter, sterile glass bottles. One bottle sample was enriched with 200 m*l* of sediment extract. Another two bottle samples were enriched with 200 m*l* of inorganic nutrients mixture containing 62 μg KH_2PO_4 and 7.2 mg $NaNO_3$ filtrated by sterile membrane filter (0.22 μm average pore size). In second samples, amount of phosphorus and nitrogen is similar to those in sediment extract. The last bottle sample was added 200 m*l* of sterile distilled water as a control. The bottles were incubated for 15 days at 25°C. Illumination was provided by fluorescent lamps (ca 5,000 lux) and 16 : 8 hr L : D cycle. The samples were agitated by magnetic stirrer during the incubation. They were drawn at intervals and subjected to biological and chemical analysis.

Test of algal growth stimulation by heterotrophic bacteria:

Each alga was pre-incubated in modified 1/2 ASM medium for 8 days by the same condition as bottle test. Each heterotrophic bacterium was pre-incubated in the sterile sediment extract for 14 days at 25°C.

For coexistant culture of heterotrophic

bacterial strains and algal strains, each preculture was centrifuged and washed three times with sterile distilled water. Then, algal and bacterial suspension were mixed. Half m*l* of mixed suspension was inoculated into glass flasks containing 200 m*l* of sediment extract diluted twice with modified ASM medium or modified 1/2 ASM medium. Each culture was incubated for 12-17 days under the same condition as bottle test, and was shaked by Monosin-shaker. Controls were prepared as follows: C_1 control, enriched with sediment extract, and C_2 control, modified 1/2 ASM medium. These controls were not inoculated for heterotrophic bacteria.

Measurement of bacterial numbers and algal growth:

Five m*l* of culture solution was daily drawn out by sterile syringe for determination of bacterial and algal growth. Bacterial numbers were measured by agar plate method. Algal growth were assayed with chlorophyll *a* and pheophytin *a* content with Tanner 111 Fluorometer.

Results

The bottle test on enriched with inorganic nutrients or sediment extract:

Fig. 1 shows the changes in chlorophyll *a* (chl *a*) concentration. Chl *a* in the control sample (lake water plus distilled water) increased slowly and reached 42 μg chl $a \cdot l^{-1}$. Chl *a* in the sample enriched with inorganic nutrients increased slowly and reached 86 μg chl $a \cdot l^{-1}$. All the while, bacterial numbers in the two samples did not increase significantly. However, chl *a* in the sample containing sediment extract increased rapidly and reached 340 μg chl $a \cdot l^{-1}$. In this sample, bacterial numbers also increased from 2.3×10^4 cells$\cdot$ml^{-1} to 3.4×10^6 cells$\cdot$ml^{-1}. When sediment extract was added after 5 days to the sample enriched with inorganic nutrients, the concentration of chl *a* was increased rapidly in 290 μg chl $a \cdot l^{-1}$.

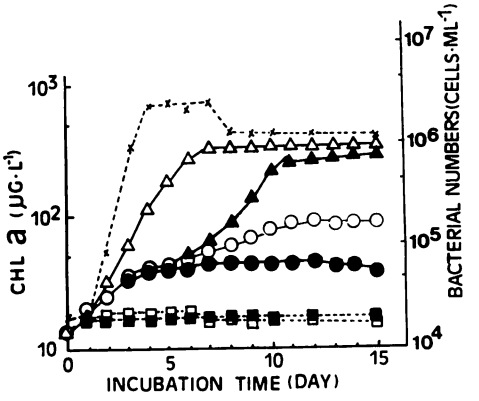

Fig. 1. Changes in chlorophyll *a* concentration and bacterial cells in lake water used in bottle test. ●; chlorophyll *a* in lake water (control), ○; chlorophyll *a* in lake water added of inorganic nutrients, △; chlorophyll *a* in lake water added of sediment extract, ▲; chlorophyll *a* in lake water added of sediment extract after 5 days of culturing of lake water added of inorganic nutrients, □; bacterial numbers in lake water, ■; bacterial numbers in lake water with inorganic nutrients, ×; bacterial numbers in lake water with sediment extract.

Isolation of heterotrophic bacteria:

Forty eight strains of heterotrophic bacteria were isolated from poor nutrient agar medium, while only eleven strains could grow in sediment extract medium.

Algal growth stimulation by heterotrophic bacteria:

As shown in Table 2, when alga and bacterium were coexisted with medium without sediment extract, algal growth (A/C_1, A/C_2) were not promoted. Algal growth effects enriching with sediment extract were higher than that of heterotrophic bacteria.

When alga and bacterium were coexisted in the medium enriched with sediment extract, algal growth as compared with C_1 control were promoted at 1.2~3.4-fold in *C. pyrenoidosa*, at 1.1~4.1-fold in *S. bijuga*, and at 1.4~3.3-fold in *M. aeruginosa*. As compared with sediment extract-free C_2 control, algal growth were promoted at 1.9~7.3-fold in *C. pyrenoidosa*, at 2.7~12.2-fold in *S. bijuga*, and at 2.5~8.3-fold in *M. aeruginosa*. Bacteria strains 2, 3, 6, 9 and

Table 2. Algal growth stimulation in coexistant or unialgal cultures.

	Chlorella pyrenoidosa				*Scenedesmus bijuga*				*Microcystis aeruginosa*			
	Ratio of final algal yield											
No. \	A/C_1	A/C_2	B/C_1	B/C_2	A/C_1	A/C_2	B/C_1	B/C_2	A/C_1	A/C_2	B/C_1	B/C_2
1	0.48	1.0	0.95	2.0	0.33	1.0	1.4	4.2	0.38	0.96	1.0	2.5
2	0.52	1.1	3.4	7.3	0.33	1.0	2.3	6.8	0.40	1.0	1.6	4.0
3	0.44	0.93	2.3	4.9	0.32	0.97	2.5	7.4	0.44	1.1	2.0	5.0
4	0.45	0.95	1.2	2.6	0.33	0.99	1.1	3.3	0.44	1.1	1.0	2.5
5	0.48	1.0	1.0	2.1	0.33	1.0	1.0	3.0	0.40	1.1	1.1	2.8
6	0.48	1.0	2.6	5.6	0.37	1.1	3.1	9.2	0.40	1.1	2.2	5.5
7	0.48	1.0	1.1	2.4	0.37	1.1	0.90	2.7	0.38	0.96	1.0	2.5
8	0.47	0.98	0.98	2.1	0.31	0.94	0.92	2.7	0.39	0.97	1.0	2.5
9	0.48	1.0	2.4	5.2	0.33	0.98	3.6	10.7	0.40	0.99	3.3	8.3
10	0.45	0.95	3.3	7.1	0.32	0.97	4.1	12.2	0.39	0.98	2.7	6.3
11	0.52	1.1	0.90	1.9	0.33	1.0	1.2	3.6	0.44	1.1	1.1	2.8

No. ; Bacterial strain number
Culture condition
C_1; + sediment extract, − bacterial strain, C_2; − sediment extract, − bacterial strain
A; − sediment extract, + bacterial strain, B; + sediment extract, + bacterial strain

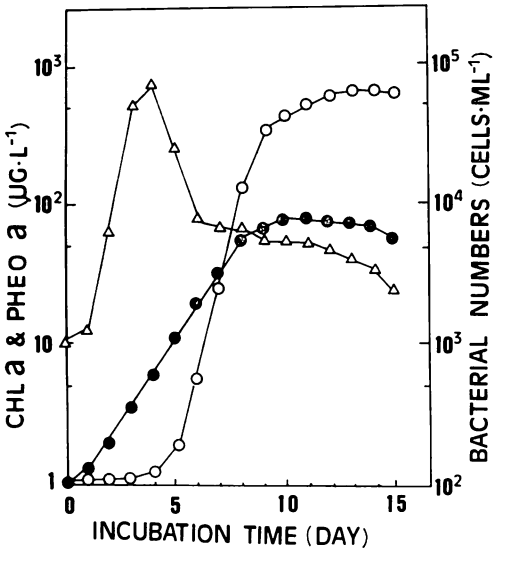

Fig. 2. Growth curves of *Chlorella pyrenoidosa* and bacteria (strain 2) in coexistant culture and unialgal culture. ●; *Chlorella pyrenoidosa* in unialgal culture, ○; *Chlorella pyrenoidosa* in coexistant culture, △; bacterial strain 2 in coexistant culture.

10 promoted of algal growth in three species.

Algal (*C. pyrenoidosa*) and bacterial (strain 2) growth curves are shown in Fig. 2. When only *C. pyrenoidosa* was incubated in sediment extract diluted with modified ASM medium, the alga grew without lag phase. While, in the coexistant culture, the algal growth showed lag phase for 3 days and did not increase until bacterial growth reached plateau, maximum yield of the algal growth was much higher than that of unialgal culture.

Discussion

Algal growth was stimulated with sediment extract containing organic substances in a bottle test using lake water (Fig. 1). KUENTZEL (1969) and, HARTE and LEVY (1983) reported that suitable addition of organic substances to lake water stimulated algal growth and the effect was due to mineralization from organic substances by heterotrophic bacteria. However, Fig. 1 shows algal growth stimulation is not caused

by inorganic nutrients in sediment extract and inorganic nutrients mineralized by bacteria. Because, the stimulatory effect of enriching with sediment extract was more higher than that of enriching with inorganic nutrients of the same concentration of total nutrients in the sediment extract. Therefore, we hypothesized that heterotrophic bacteria were related to algal growth stimulation with sediment extract.

When heterotrophic bacteria isolated from lake and algal strains coexisted with sediment extract, algal growth were stimulated at 3.4~4.1-fold compared with their unialgal cultures, and at 7.3~12.2-fold compared with sediment extract-free unialgal cultures (Table 2). This result suggests that heterotrophic bacteria are related to algal growth stimulation except for bacterial mineralization of organic substances.

LANGE (1971) reported that algal growth was stimulated by addition of both bacteria and many kind of sugars, and suggested that algal growth stimulator was produced from sugars by coexistant bacteria. Moreover, TEZUKA and HAYASHI (1975) reported the final yield of phytoplankton on bottle test was more higher addition of both glucose and inorganic nutrients than addition of glucose or inorganic nutrients alone. Therefore, they suggested that the stimulatory effect might be due to supply of some growth stimulator except for carbon dioxide by coexistant bacteria.

Although LANGE (1971) and, TEZUKA and HAYASHI (1975) added sugars in their experiments, we added sediment extract containing organic substances (Table 1) to consider possibility of releasing from sediment (AUSTIN and LEE 1973, OCHIAI *et al.* 1978). While, we obtained the similar results to enriching with sugars (LANGE 1971, TEZUKA and HAYASHI 1975).

Except for stimulator and mineralizing of organic substances, we hypothesized that heterotrophic bacteria decomposed algal autoantagonism and autoinhibitor named by LEFÈVRE (1964) and SATOMI (1967), and stimulated indirectly algal growth. But, as shown in Table 2, coexistant heterotrophic bacteria without sediment extract did not stimulate algal growth. Moreover, Fig. 3 suggests that algal growth is promoted by stimulator released from coexistant bacterium after it utilizes substances in sediment extract and grow quicker than the alga. Therefore, we concluded that the stimulator produced from the substances in sediment extract promoted algal growth.

Although inorganic nutrients are necessary for algal growth, they are rather regulating factors on algal yield than algal growth stimulator. Except for inorganic nutrients, it is reported that algal growth stimulator in the lake and sea sediment promote algal growth (PRAKASH and RASHID 1968, PRAKASH *et al.* 1973, COOKSEY and COOKSEY 1978, HINO and ANDO 1981 1983). Moreover, our research showed that the algal growth stimulator produced from sediment extract by heterotrophic bacteria promoted algal growth (Fig. 1, 2 and Table 2). When precursor of the algal growth stimulator released from the sediment, the stimulator produced from precursor by heterotrophic bacteria as same as the stimulator in the sediment might asociate with algal growth stimulation with inorganic nutrients in natural environment.

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日野修次： 底泥抽出物共存下における従属栄養細菌による藻類増殖促進効果について

茨戸湖より分離された従属栄養細菌および底泥抽出物が藻類増殖におよぼす影響を調べるため，二種類の培養実験を行った。

その結果，底泥抽出物および従属栄養細菌が存在すると，*Chlorella pyrenoidosa*, *Scenedesmus bijuga*, *Microcystis aeruginosa* の三種の藻類増殖は，従属栄養細菌が存在しない時と比較して3.4～4.1倍となり，従属栄養細菌が藻類増殖に影響を与えることが明らかとなった。またこれらの効果は，湖水に対する底泥抽出物および栄養塩類の添加実験による結果より，従属栄養細菌による底泥抽出物質からの生産物質によるものと推定された。(060 札幌市北区北19条西12丁目 北海道公害防止研究所水質部)