AN AUTOLYTIC SUBSTANCE IN A FRESHWATER CYANOBACTERIUM *PHORMIDIUM TENUE*

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An autolytic substance in an axenic cyanobacterium, *Phormidium tenue*, was identified as a mixture of fatty acids, by use of cultured cells in the laboratory. Among them, linoleic acid and linolenic acid were potent growth inhibitors.

KEYWORDS axenic; cyanobacteria; autolytic substance; *Phormidium tenue*; fatty acid mixture; unsaturated fatty acid

Recently blooms of algae (Mizu no hana), especially the ones induced by cyanobacteria, have occurred in many nutrient lakes and water reservoirs. Since these blooms often produced some toxins[1] and earthy-musty odors,[2] they have been studied from various points of view. *Phormidium tenue* is distributed widely in Japan and is one of the typical cyanobacteria that produce the earthy-musty odor in drinking water. So far the substance causing the earthy-musty odor of this species has proved to be 2-methylisoborneol.[3] An axenic clone of this species was obtained by the capillary pipetto method and the odor was found to be produced not by symbiotic bacteria but by the cyanobacterium itself. Further, it was found that the bacteria-associated *P. tenue* grew for a long time, whereas the axenic one died suddenly after a definite time.[4] These findings indicated that *P. tenue* produced extracellar metabolites that inhibit its own growth. Thus, we were interested in examining its growth inhibiting substances. In this paper, we report on the chemical elucidation of the autolytic substances of the cyanobacterium *P. tenue*.

An axenic *P. tenue* used for this experiments was isolated from the moat around the Nagoya Castle (Aichi Prefecture) in 1981 and was maintained in CT-medium[5] adjusted to pH 8.0 at 25 °C with cool-white fluorescent illumination of 1000 lx. Large scale cultivation was carried out in the following manner. The axenic culture was grown for three weeks in CT-medium in 5-l Erlenmeyer flasks at 25°C, illuminated continuously with cool-white fluorescent lights (1000 lx) and aerated vigorously with sterilized-air passed through a 0.2-μm membrane filter (Millipore, Mirex FG-50) at the rate of 0.5-l per minute. The alga was harvested by centrifugation at 20,000 x g from the combined 35-l culture and lyophilized.

The lyophilized alga (6.5 g) was homogenized and extracted with acetone at 25°C for 24 h, then with 80% MeOH. The acetone extract exhibited the growth inhibitory activity. The activity was monitored by separating the acetone extract by silica gel and ODS (YMC AQ-120-S50) column chromatography which gave a potent growth-inhibiting substance (5.0 mg). The ^{1}H NMR spectrum of this substance showed several signals due to primary methyl groups and a broad signal assignable to olefinic protons (δ5.39, m), whereas the IR spectrum exhibited an absorption band due to a carboxylic group (3400-2600cm^{-1}, 1700 cm^{-1}). In the EI-MS spectrum, the active substance did not show a molecular ion peak because of its poor volatility, while its methyl ester exhibited molecular ion peaks at 242($C_{14:0}$), 268($C_{16:1}$), 270($C_{16:0}$), 292($C_{18:3}$), 294($C_{18:2}$), and 296($C_{18:1}$) respectively. So, the autolytic substance was proved to be a mixture of some fatty acids. The composition of the fatty acids was determined by gas-liquid chromatography-mass spectra (GC-MS) analysis to be a mixture of methyl myristate, methyl palmitate, methyl palmitoleate, methyl oleate, methyl *cis*-vaccenate, methyl linoleate, and methyl linolenate in a ratio of 5 : 4 : 5 : 4 : 1 : 47 : 31.[6]

We next examined the growth inhibiton by these fatty acids. Various extracts from *P. tenue* and purified commercially available fatty acids were assayed as follows. Algal cultures for bioassay were grown to the late logarithmic growth phase for 10 days. Portions (20 ml) of the algal cultures were transferred to 50-ml Erlenmeyer flasks. Test samples were dissolved in MeOH or DMSO, then these solution were sterilized through membrane filters (Bio-Rad, Micro Prep-Disc, 0.2-μm of each pore size) and 0.2 ml of them were added to the culture flasks. The controls were cultured with only 0.2 ml of MeOH added. Individual cultures were allowed to grow for 3 days, each culture being treated with a fatty acid. Then, they were bioassayed in duplicate. The minimum growth inhibiton was determined by measuring chlorophyll a as described by Strickland.[7] The relative growth rate of the alga was expressed as a percentage of the control. The minimum growth-inhibiting concentration of each fatty acid is shown in Table I. A mixture of fatty acids isolated from *P. tenue* inhibited growth at 0.5 ppm. Among these fatty acids, unsaturated fatty acids such as linoleic and linolenic acid

Table I. Minimum Growth Inhibitory Concentrations of Fatty Acids (ppm)

Myristic acid ($C_{14:0}$)[8]	>100
Palmitic acid ($C_{16:0}$)[8]	>100
Palmitoleic acid ($C_{16:1}$)	2.5
Oleic acid ($C_{18:1}$)	1.0
cis-Vaccenic acid ($C_{18:1}$)	5.0
Linoleic acid ($C_{18:2}$)	0.5
Linolenic acid ($C_{18:3}$)	0.5
fatty acids isolated from *P. tenue*	0.5

were inhibitory at 0.5 ppm, while saturated ones were inactive even at 100 ppm.

There are a few reports on the growth inhibitory activity of fatty acids against some algae.[9] However, it should be noted that fatty acids were first isolated as autolytic substances from natural cyanobacteria. The extinction mechanism of *P. tenue* by unsaturated fatty acids is under investigation.

ACKNOWLEDGEMENT The authors are grateful to Mr. T. Ohki of the Bio-Dynamics Research Institute for running the GC-MS. We thank Prof. H. Ikezawa of Nagoya City University for his valuable advice throughout this work.

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6)The composition of these fatty acids was scarcely changed under the same cultivation condition. Oleic and *cis*-vaccenic acids were shown to be identical with authentic samples by capillary GC analysis of their methyl esters.

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(Received December 25, 1989)