Terunobu ICHIMURA* and Makoto M. WATANABE*: An Axenic Clone of *Microcystis aeruginosa* KÜTZ. emend. ELENKIN from Lake Kasumigaura

市村輝宜*・渡辺　信*: 霞ケ浦産アオコの無菌培養

Among the planktonic blue-green algae forming water blooms during summer and fall in eutrophicated waters, such as Lake Kasumigaura, *Microcystis aeruginosa* KÜTZ. emend. ELENKIN has been reported to cause many obnoxious and nuisance problems in various ways, for instance, sudden death of carps in cultivation nets and disagreeable stench attacking the near shore people.

It is prerequisite for controlling the nuisance water blooms to know physico-chemical conditions for the optimal growth of this alga. As far as we know, however, only two strains of this alga are at present available from the culture collections[1),2)]. The two strains[3-5)] are originally from North America and both have been purified through ultraviolet irradiation, which is known to have a mutagenic effect on many organisms.

In this paper we shall describe the isolation method and morphological characteristics of an axenic clonal culture of this alga which was obtained from Lake Kasumigaura and purified by the pipette washing method.

Materials and Methods

In August 1974, a liquid sample (KAS-1) which contained blue-green algae forming a water bloom was collected from the shore of Lake Kasumigaura at the vicinity of Tsuchiura City, Ibaraki-ken.

For isolation and purification, a single colony was washed by a fine Pasteur-type pipette under a dissecting microscope[6),7)]. The growth media and bacteriological test media are listed in Tables 1 and 2. Cultures were illuminated for 16 hours daily by fluorescent lamps at a light intensity of approximately 4,000 lux. Temperatures were kept at 25±1 C except in bacteriological tests.

* Institute of Applied Microbiology, University of Tokyo, Bunkyo-ku, Tokyo, 113 Japan.
東京大学応用微生物研究所 (113 東京都文京区弥生1丁目1の1)

Bull. Jap. Soc. Phycol. **25**: 177–181. 1977.

Table 1. Growth media

Salt	C medium[8]	NaCB medium
$Ca(NO_3)_2 \cdot 4H_2O$	15 mg	15 mg
KNO_3	10 mg	—
$NaNO_3$	—	10 mg
$MgSO_4 \cdot 7H_2O$	4 mg	4 mg
βNa_2glycero-phosphate. $5H_2O$	5 mg	5 mg
Vitamin B_{12}	0.01 μg	0.01 μg
Biotin	0.01 μg	0.01 μg
Thiamin HCl	1 μg	1 μg
P IV metals*	0.3 ml	0.3 ml
Tris buffer	50 mg	—
Bicine (DOTITE)	—	50 mg
Distilled H_2O	99.7 ml	99.7 ml
pH adjusted with 1 N HCl or NaOH	7.5	9.0

* 1 ml of P IV metals contains:

Na_2EDTA	1 mg	Fe (as Cl^-)	0.04 mg
Co (as Cl^-)	0.001 mg	Mn (as Cl^-)	0.01 mg
Mo (as Na^+)	0.005 mg	Zn (as Cl^-)	0.005 mg

Table 2. Bacteriological test media

Medium	Nutrient	Amount*
I	Proteose-peptone (Difco)	1
II	Yeast-extract (Difco)	5
III	Peptone (Difco)	5
	Beef extract (Difco)	3
IV	Glucose	1
	Peptone (Difco)	1
V	Na acetate. $3H_2O$	0.5
	Glucose	0.5
	Tryptone (Difco)	0.5
	Yeast extract (Difco)	0.3

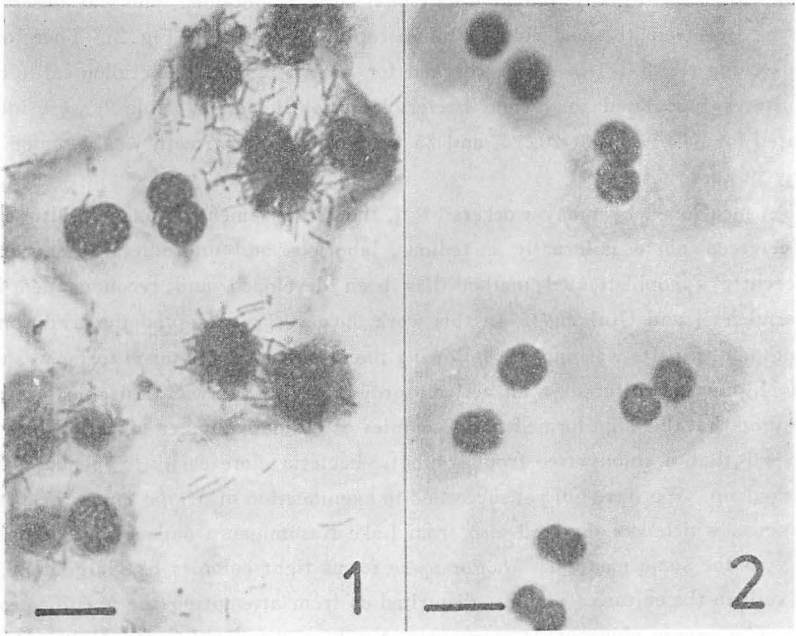
* Gram per liter of distilled water or NaCB medium.

Result and Discussion

From the liquid sample, a crude culture composed mainly of *Microcystis aeruginosa* was obtained by inoculating a single colony of this alga into a test

tube containing 10 ml of C medium[8]: Two weeks after the inoculation, a rather compact large colony was observed by naked eyes, although the growth was poor and slow in C medium. Considering the suspected contamination of epiphytic algae, the colony was broken into small pieces by strongly shaking the test tube. After washing several times in sterile distilled water, a 10-20-celled colonial piece was inoculated into a test tube containing C medium. In this way unialgal clone KAS-1-1 was obtained.

Meanwhile, a nutritional study was initiated, using the clonal culture of KAS-1-1, and it became known that this alga grew much better when C medium was modified by replacing KNO_3 with $NaNO_3$ and buffering at pH 9.0 with Bicine instead of at pH 7.5—8.5 with Tris. Thus, NaCB medium was used for further purification and maintenance of the culture. In NaCB medium, this alga grew considerably well, forming many small amorphous colonies of 4 to



Figs. 1 and 2. *Microcystis aeruginosa* KÜTZ. emend. ELENKIN cultured in NaCB medium.
1. Unialgal culture. Colonial matrix and rod-shaped bacteria are stained with basic fuchsin.
2. Axenic culture. Colonial matrix and algal cells are heavily stained with basic fuchsin, but there is no indication of the presence of epiphytic bacteria. A bar in each figure indicates 10μm.

16 cells embedded in a transparent mucilaginous matrix. The matrix of polysaccharide nature was stained well by basic fuchsin (Figs. 1 and 2).

A week after inoculating a small quantity of the culture into fresh medium, 4- or 8-celled colonies of early exponential growth phase were washed successively five times in sterile distilled water and inoculated individually into a test tube containing 10 ml of the medium. Three weeks after the inoculation, five fast growing clonal cultures were discarded because it was considered that they started from a large colony and were highly suspected to be contaminated, but three comparatively slowly growing clonal cultures were saved. All of these three clones saved, however, were turned out to be contaminated with bacteria by scrutinizing under a compound microsope. The same purification procedure as described above was undertaken again using KAS-1-1-3, one of the three clones which was observed to be contaminated with rod-shaped bacteria of one kind (Fig. 1). Finally, out of nine clonal cultures obtained, one was observed to be free from the bacterium by microscopic scrutinization (Fig. 2). Therefore, this clone (KAS-1-1-3-3) was checked for purity by the bacteriological tests. Cultures inoculated in various bacteriological test media (Table 2) were incubated for 1—4 weeks at 20, 25, and 35 C. No bacterial growth was detected in any of these tests.

As mentioned by many workers[2),6),9)], the establishment of axenic culture in blue-green algae is usually a tedious, laborious and unpredictable exercise. Recently a sophisticated method has been developed and recommended by Carmichael and Gorham[10)]. In this work, however, we adopted the traditional method of pipette-washing[6),7)], following the rule that the organism to be washed should be pre-cultured in an actively growing state. It was desirable for this method that this alga formed loose colonies of a small number of cells, since it helped that a colony free from epiphytic bacteria more easily happened to be picked up. We have not yet succeeded in axenification of *M. flos-aquae* (WITTR.) KIRCHN. which was obtained also from Lake Kasumigaura and cultured unialgally in the same medium. *M. flos-aquae* forms tight colonies of a large number of cells in the culture. This has dispirited us from attempting the axenification.

The axenic clone of *M. aeruginosa* has been deposited in the Culture Collection of Algae at the Institute of Applied Microbiology, University of Tokyo under the designation of M-176. Cell dimension of this clone is as follows; minimum 5.0μm, mean 5.5μm, maximum 6.4μm, standard deviation 0.3μm and

coefficient of variability 5.4%. These statistical values are based on 100 cells cultured in NaCB medium for one week.

We wish to express our sincere thanks to Dr. T. TSUDA and Dr. A. HAMADA, Freshwater Fisheries Experiment Station, Ibaraki Prefecture and Dr. S. KASEBAYASHI, Ibaraki Prefectural Office for their encouragement and assistance.

Literature cited

1) GEORGE, E. (1971) *Culture Collection of Algae and Protozoa. List of Strains.* Culture Centre of Algae and Protozoa, Cambridge, England: 1-73.
2) STANIER, R.Y., KUNISAWA, R., MANDEL, M. and COHEN-BAZIRE, G. (1971) Purification and properties of unicellular blue-green algae (Order Chroococcales). Bact. Rev. **35**: 171-205.
3) BISHOP, C.T., ANET, E.F.L.J. and GORHAM, P.R. (1959) Isolation and identification of the fast-death factor in *Microcystis aeruginosa* NRC-1. Can. J. Biochem. and Physiol. **37**: 453-471.
4) HUGHES, E.O., GORHAM, P.R. and ZEHNDER, A. (1958) Toxicity of a unialgal culture of *Microcystis aeruginosa.* Can. J. Microbiol. **4**: 225-236.
5) GERLOFF, G.C., FITZGERALD, G.P. and SKOOG, F. (1950) The isolation, purification and culture of blue-green algae. Am. J. Bot. **37**: 216-218.
6) PRINGSHEIM, E.G. (1946) *Pure Culture of Algae.* Cambridge University Press. London: 1-119.
7) HOSHAW, R.W. and ROSOWSKI, J.R. (1973) Methods for microscopic algae. In J.R. STEIN *Handbook of Phycological Methods: Culture Methods and Growth Measurements.* Cambridge Univ. Press, Cambridge: 53-67.
8) ICHIMURA, T. (1971) Sexual cell division and conjugation-papilla formation in sexual reproduction of *Closterium strigosum.* Proc. Intl. Seaweed Symp. **7**: 208-214.
9) FOGG, G.E., STEWART, W.D.P., FAY, P. and WALSBY, A.E. (1973) *The Blue-green Algae.* Academic Press, London: 130-132.
10) CARMICHAEL, W.W. and GORHAM, P.R. (1974) An improved method for obtaining axenic clones of planktonic blue-green algae. J. Phycol. **10**: 238-240.