# CELL GROWTH INHIBITION IN MOUSE LEUKEMIA L5178Y CELLS *IN VITRO* BY CRUDE EXTRACTS FROM FRESHWATER BLUE-GREEN ALGAE

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## Introduction

Industrial interest in algae as potential sources for biologically active natural products has increased in recent years. Cytotoxic and/or antitumor activity of crude extracts or purified compounds obtained from algae have been uncovered by several investigators.[2,4,6-8,10] Recently, the National Cancer Institute[9] began a screening program of blue-green algae (cyanobacteria) as a promising resource of anticancer natural products. There have been relatively few past studies of freshwater species of blue-green algae. In this context, the purpose of the present study is to examine the potential cytotoxic activity of crude extracts of five species of freshwater blue-green algae, which are generally allowed to grow rapidly and to be readily cultivated in the laboratory, by testing inhibition of cell growth against mouse leukemia L5178Y cells *in vitro*.

## 1. Materials and Methods

### 1.1 Cultivation of algae

The following freshwater blue-green algae, obtained from the Algal Culture Collection in the Institute of Applied Microbiology, University of Tokyo, were used in this study: *Anacystis nidulans* IAM M-6, *Anabaena variabilis* IAM M-2, *Anabaena variabilis* IAM M-205, *Calothrix brevissima* IAM M-7, *Oscillatoria neglecta* IAM M-82. All algae strains were grown photoautotrophically in 500-ml cotton-stoppered oblong flat flasks containing modified Detmer medium[11] at 30 °C and at a light intensity of 5 klux. The cultures were aerated by plain air.

### 1.2 Preparation of crude extracts

After 3 to 5 days the algae were harvested by filtration and freeze-dried. Since the aqueous and alcoholic extracts proved to contain cytotoxic substances, and the chloroform extract of some species displayed cytotoxic activity[1,5,8,10], three kinds of crude extracts were prepared from each algal specimen as follows. The dried algae (1g) were extracted initially with a mixture of chloroform and methanol (1:2 by volume) at room temperature for 24 hr. Water was added to the filtrate and the chloroform layer was washed repeatedly with water. The marc was dried and then subjected to extraction with 70% ethanol at room temperature for 24 hr and an ethanol-soluble extract was obtained. Finally, aqueous extracts were prepared by boiling with water for 4 hr from the resultant marc. Most of the solvent was removed from each extract by a rotary evaporator. Final drying was carried out under a stream of $N_2$ for the chloroform (extract "**C**") and ethanol (extract "**E**") extracts. Aqueous (extract "**A**") extract was freeze-dried. Sterile instruments were used throughout in preparing all extracts.

### 1.3 *In vitro* cytotoxicity testing

*In vitro* tests using the mouse leukemia cell line L5178Y, RCB135, which was obtained from the Riken Gene Bank, Ibaraki, Japan, were carried out by the method of tissue culture. The cells were cultured in RPMI 1640 medium supplemented with 10% calf serum, $1 \times 10^5$ units/l penicillin, and $1 \times 10^{-4}$ kg/l streptomycin in a $CO_2$ incubator at 37°C. L5178Y cells ($3\text{-}5 \times 10^7$ cells/l) were cultured for 48 hr after addition of algae extracts. Lipophilic extracts were injected into medium solution by using dimethyl sulfoxide (its final concentration (1-0.01 wt%) was less than a predetermined noncytotoxic level (1 wt%) ) and aqueous extracts were dissolved or suspended in medium. Three dose levels at one-log interval ($1 \times 10^{-4}$, $1 \times 10^{-5}$, and $1 \times 10^{-6}$ kg/l) with two tubes per level were tested according to the NCI protocol[3]. The extracts were handled as if sterile, and sterile techniques were used throughout weighing and dosing procedures. Cell growth was determined by counting the viable cells (dead cells were stained by trypan blue). Cytotoxic activity was evaluated as the ratio of the mean cell number of the treated tubes ($T$) to the mean cell number of the control tubes ($C$), $T/C \times 100$, and $IC_{50}$, the concentration of test substance required to inhibit growth to 50% of the growth of the controls[12].

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**Table 1.** Cytotoxic activity of crude extracts from blue-green algae against mouse leukemia L5178Y cells

| Algal specimen | Type of crude extract* | Cell growth (T/C %) concentration($10^{-6}$kg/l) 1 | 10 | 100 |
|---|---|---|---|---|
| *Anacystis nidulans* IAM M-6 | C | 104 | 96 | 5 |
| | E | 98 | 96 | 6 |
| | A | 112 | 116 | 124 |
| *Anabaena variabilis* IAM M-2 | C | 101 | 73 | 4 |
| | E | 106 | 100 | 24 |
| | A | 102 | 136 | 103 |
| *Anabaena variabilis* IAM M-205 | C | 99 | 86 | 3 |
| | E | 101 | 106 | 4 |
| | A | 92 | 106 | 118 |
| *Calothrix brevissima* IAM M-7 | C | 105 | 113 | 5 |
| | E | 118 | 126 | 26 |
| | A | 120 | 143 | 161 |
| *Oscillatoria neglecta* IAM M-82 | C | 89 | 93 | 9 |
| | E | 105 | 93 | 9 |
| | A | 98 | 113 | 133 |

*C: chloroform extract; E: ethanol extract; A: aqueous extract

## 2. Results and Discussion

The cytotoxic activity of the chloroform, ethanol, and aqueous extracts from five algae against L5178Y cells are reported in **Table 1**. The data show that all chloroform and ethanol extracts had *T/C* (test/control) values of less than 50% at a dosage of $1 \times 10^{-4}$ kg/l. Of these extracts, chloroform extracts from both strains of *A. variabilis* exhibit activity at a lower concentration of $1 \times 10^{-5}$ kg/l. On the contrary, no activity was found in our tests of all the aqueous extracts. In addition, we observed some increase in cell growth in the aqueous extracts of all algae. This is remarkable for the case of *C. brevissima* and might be due to some nutritive substances present in the extracts. Since cytotoxic activities of aqueous extracts of other species of algae have been reported[5,10,11], re-examination of the activity of aqueous extracts by a different extracting scheme for algae will be required in future work. In the course of preceding extraction using chloroform/methanol or ethanol, some active aqueous substances would be extracted from algae.

To determine values of the effective dose ($IC_{50}$) for chloroform and ethanol extracts, supplemental tests were performed. The results of these tests are shown in **Table 2**. The chloroform extract from *A. variabilis* IAM M-2 exhibited the highest activity against leukemia cells with an $IC_{50}$ value of $1.9 \times 10^{-5}$ kg/l. All other chloroform extracts showed activity slightly weaker than that of *A. variabilis* IAM M-2 and had $IC_{50}$ values of $2.5 \times 10^{-5}$ to $3.6 \times 10^{-5}$ kg/l. The ethanol extract from *A. variabilis* IAM M-205 showed comparable activity to that of the chloroform extracts. The values of $IC_{50}$ for other ethanol extracts were in the range of $6.3 \times 10^{-5}$ to $7.5 \times 10^{-5}$ kg/l. Further study is required to identify the active fractions and to examine the role of such activity in nature.

**Table 2.** Values of $IC_{50}$ (the concentration required for 50% inhibition of growth) of chloroform and ethanol extracts

| Algal specimen | Type of crude extract* | Cell growth (T/C %) concentration($10^{-6}$kg/l) 20 | 40 | 70 | $IC_{50}$ [$10^{-6}$kg/l] |
|---|---|---|---|---|---|
| *A. nidulans IAM M-6* | C | 82 | 25 | 3 | 32 |
| | E | 101 | 93 | 51 | 68 |
| *A. variabilis IAM M-2* | C | 52 | 7 | 4 | 19 |
| | E | 107 | 92 | 53 | 75 |
| *A. variabilis IAM M-205* | C | 68 | 19 | 2 | 25 |
| | E | 66 | 31 | 4 | 28 |
| *C. brevissima IAM M-7* | C | 58 | 25 | 3 | 25 |
| | E | 93 | 70 | 45 | 63 |
| *O. neglecta IAM M-82* | C | 91 | 42 | 11 | 36 |
| | E | 108 | 84 | 44 | 65 |

*C: chloroform extract; E: ethanol extract

## Conclusion

Lipophilic crude extracts from all algae studied showed excellent cytotoxicity against mouse leukemia L5178Y cells at relatively low dosages. The chloroform extract of *A. variabilis* IAM M-2 was the most active. This finding points to the fresh-water blue-green algae tested as potential sources of natural cytotoxic compounds.

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