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Evaluation of the sensitivity of marine microalgal strains to the heavy metals, Cu, As, Sb, Pb and Cd

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Abstract

The sensitivity of nine marine microalgal species (consisting of five divisions and seven genera) to the five heavy metals, Cu(II), As(V), Sb(III), Pb(II) and Cd(II) was studied by using a fluorometric growth-inhibition assay with 96-well microplates. The algal strains studied were *Cylindrotheca* sp. and the LPP group that respectively characterize aggregating and filamentous types, and *Chlorococcum littorale*, *Chlorococcum* sp., *Isochrysis galbana*, *Tetraselmis tetrathele*, *Heterocapsa* sp., *Synechococcus* sp. and *Prasinococcus* sp. for types that occur as single cells. A good linear relationship was observed between the chlorophyll *a* concentration and intensity of chlorophyll fluorescence (485-nm excitation filter and 645-nm emission filter) when the chlorophyll *a* concentration was within the range of 0.10–5.0 μg ml^{-1}. A starting cell concentration of 0.10 or 0.25 μg Chl *a* ml^{-1} was therefore selected. In accordance with OECD 201 standard procedures, the IC_{50} value (concentration of a metal producing 50% growth inhibition relative to the control) was determined 72 h after adding a heavy metal by using the biomass integral. The microplate toxicity test used in this study is considered to be applicable to diverse algae, not only enumerating species but also hardly enumerating ones.

Keywords: Chlorophyll fluorescence; Growth-inhibition assay; Heavy metal toxicity; Marine microalgae; Microplate

1. Introduction

Phytoplanktons (microalgae), the primary producers at the base of the aquatic food chain, are the first targets to be affected by heavy metal pollution. The results from heavy metal toxicity tests employing microalgae therefore provide important information for estimating the ecotoxic concentration and for predicting the environmental impact of heavy metal pollution. In the case of freshwater algae, test strains and protocols have been established in several national and international standard test procedures such as those prescribed by Organization for Economic Development (OECD, 1984), European Economic Community (EEC, 1987), United States Environmental Protection Agency (USEPA, 1985), International Organization for Standardization (ISO, 1987) and American Society for Testing and Materials (ASTM, 1993), as summarized by Janssen and Heijerick (2003). The standard methods recommend the use of an Erlenmeyer flask as the test vessel, direct cell counting to evaluate algal growth and 3–4 days' exposure to a heavy metal. However, such an assay requires a large volume of the culture medium, and cell counting is time consuming. There have been several approaches to develop a small-scale microplate toxicity test using freshwater algae, together with such ways of quantifying algal growth as cell counting, optical density (O.D.) measurement, ATP quantification and fluorescence measurement (see Blaise et al., 1997 for a review).

Unlike freshwater environments, there are few tests that can be described as standard for marine microalgae, although guidelines have been published and some marine species have been recommended as test strains (Walsh, 1988, 1994). Information about microplate toxicity tests for marine microalgae is also insufficient (Blaise et al., 1997). Galgani et al. (1992) and Gilbert et al. (1992) have reported a

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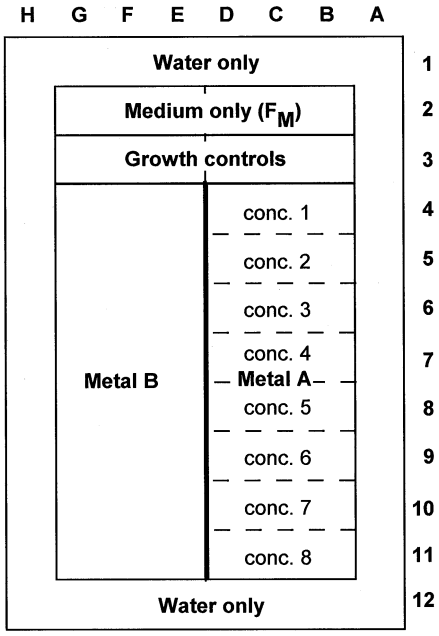

Fig. 1. Experimental disposition of the 96-well microplate for the algal growth-inhibition assay. Peripheral wells were filled with 300 μl of water. 2B–2G, six replicated wells filled with 300 μl of the medium for measuring the background fluorescence; 3B–3C, six replicated wells filled with 300 μl of the cell suspension to obtain a growth control; 4B–11G, three replicated wells per concentration per metal filled with 300 μl of the cell suspension and heavy metal solution from the lowest (conc. 1) to highest concentration (conc. 8).

microplate assay in which the metabolic status of cells was evaluated by measuring the esterase activity 5 h after exposing three unicellular marine algae, *Tetraselmis suecica*, *S. costatum* and *Prorocentrum lima*, to a toxicant. Ismail et al. (2002) used a 24-well plate and 250-ml Erlenmeyer flask to evaluate the IC_{50} value (concentration of a metal producing 50% growth inhibition relative to the control) for Cd, Cu, Mn and As after 96 h, based on O.D. readings and cell counting for *Chaetoceros calcitrans*, *Isochrysis galbana*, *Tetraselmis tetrathele* and *Tetraselmis* species. The measurement of chlorophyll fluorescence is thought to be the best method for estimating microalgal biomass in a microplate toxicity test (Blank and Bjornsater, 1988; Walsh, 1994). This would be particularly true for those hardly enumerating algal species that characterize filamentous, aggregating and autospore forming types.

We therefore investigate in the present study the sensitivity of several marine microalgal strains, including enumerating and hardly enumerating algal species, to the heavy metals, Cu, As, Sb, Pb and Cd, by using a microplate toxicity test with fluorometric growth quantification.

2. Materials and methods

2.1. Algal strains and seed culture

Chlorococcum littorale (MBIC10280), *Chlorococcum* sp. (MBIC10044), *I. galbana* (MBIC10554), *Heterocapsa* sp. (MBIC10793), *Prasinococcus* sp. (MBIC10016), *Cylindrotheca* sp. (MBIC11203), *Synechococcus* sp. (MBIC10073) and LPP group (MBIC10087) were obtained from the Marine Biotechnology Institute Culture Collection (MBIC). Such information about these MBIC algal strains as their morphorogical features and site of collection are available on the MBIC web site (http://www.mbio.co.jp/mbic/). *T. tetrathele* was provided by Dr. Masaharu Hagiwara (Yokosuka City Museum, Japan). These algal cells were maintained in a DAIGO IMK medium (pH 8.5) prepared in DAIGO artificial sea-water (Nihon Pharmaceutical Co. Ltd., Japan) under 3%

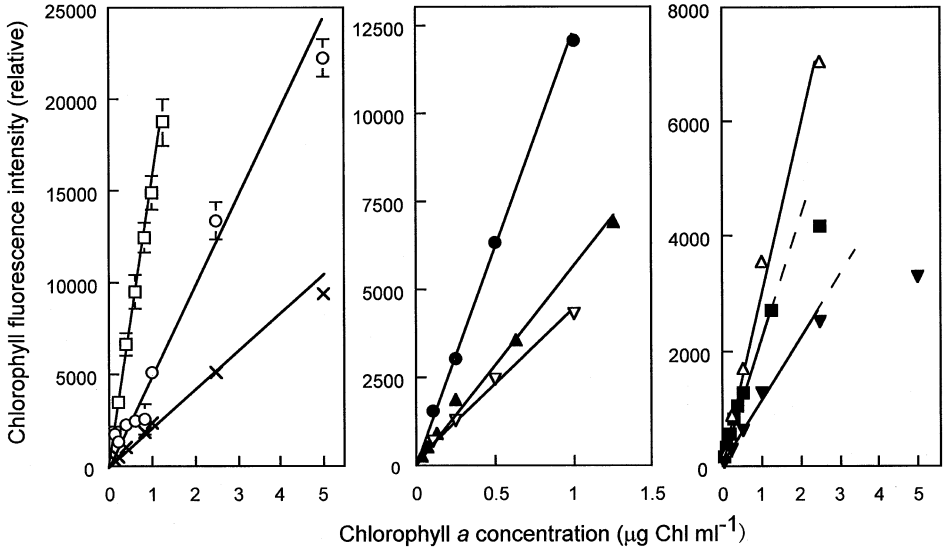

Fig. 2. Relationship between the chlorophyll *a* concentration and chlorophyll fluorescence intensity obtained by an automated fluorometric assay with a 96-well microplate. ○, □, ●, ×, ■, △, ▲, ▽ and ▼: *Chlorococcum littorale*, *Chlorococcum* sp., *Tetraselmis tetrathele*, *Isochrysis galbana*, *Synechococcus* sp., *Prasinococcus* sp., *Heterocapsa* sp., *Cylindrotheca* sp. and LPP group, respectively.