Applicability of Luminescent Assay Using Fresh Cells of *Vibrio fischeri* for Toxicity Evaluation

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Toxicities of antifouling chemicals and natural marine samples were evaluated by three assays, among which bioluminescence assay using freshly incubated *Vibrio fischeri* (*V. fischeri*) cells (NZ assay) and MicroTox were regarded as short-term assays, and growth inhibition assay was conducted as long-term assay. Short-term toxicity levels evaluated by NZ assay were in good agreement with those by MicroTox assay for all of the samples examined. Based on the EC_{50} values of each chemical by respective assay, NZ assay showed prior reproducibility and similar levels of sensitivity when compared with those of MicroTox assay. On the other hand, growth inhibition assay showed lower sensitivity and reproducibility than NZ and MicroTox assays. Four kinds of antifouling chemicals, Irgarol 1051, Diuron, thiabendazole (TBDZ), and *N*-dichlorofluoromethylthio-*N*′,*N*′-dimethyl-*N*-phenylsulfamide (DCF), were detected to possess delayed toxicity from the judgments on the difference of short-term and long-term toxicities. Four out of 16 seawater samples collected in Japan showed remarkable toxicity in NZ assay, suggesting that they were contaminated by several types of antifouling chemicals. Considering time consumed, facility for operation, cost, and requirements, NZ assay was proved to be efficient for toxicity evaluations for artificial and natural samples.

Key words —— bioluminescent assay, antifoulants, delayed toxicity, *Vibrio fischeri*

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INTRODUCTION

The use of bioluminescent bacteria in the detection of toxic chemicals was proposed as relatively simple and rapid method based on the measurement of decrease in bioluminescence intensity (BLI) due to the contamination of toxic compounds.[1–4] MicroTox assay, as representative one of short-term assay, has been accepted as a standard method by International Standard Organization (ISO) due to its remarkable sensitivity.[2,3] In addition to these short-term methods, various assays with longer incubation time to detect the toxicities of new substances have also been proposed.[5,6] The long-term assay for *Vibrio fischeri* (*V. fischeri*) using growth inhibition was proposed to detect the persistent toxicity by longer incubation time, normally more than 7 hr.[5,6]

Recently Nagata and Zhou proposed a short-term assay system using freshly incubated *V. fischeri* cells,[7] named as NZ assay in this text, which was successfully applied to the toxicity evaluation for some new antifouling chemicals.[8] The benefits of NZ assay, however, were not clearly demonstrated due to the lack of data to compare with standard or traditional assays.

To clarify the NZ assay's sensitivity and reproducibility for toxic chemicals, comparison of toxicity data, which were obtained by NZ, MicroTox, and growth inhibition assays, were conducted for 11 types of typical antifouling chemicals. Total 16 seawater samples, which were collected in seacoast of Japan and possibly contaminated by antifouling chemicals, were evaluated to clarify the sensitivity and feasibility of NZ assay for natural samples.

MATERIALS AND METHODS

Culture Conditions —— A strain used in this study was *V. fischeri* Deutsche Sammulung von Mikroorganismen (DSM) 7151, which was grown in two kinds of media. One was a LB medium which contains 0.5% yeast extract (Difco Laboratories, Detroit, MI, U.S.A.), 1.0% tryptone (Difco), and 0.5 M NaCl. The other was luminescence (LM) medium, in which 0.5% yeast extract (Difco), 0.5% tryptone (Difco), 0.1% $CaCO_3$, and 0.3% glycerol were involved in artificial seawater (ASW, JIS K-2510). The pH of both media was adjusted to 7.0 with NaOH. Another two strains, *Brevibacterium* sp. JCM 6894 and *Escherichia coli* (*E. coli*) K-12 IFO 3301, were incubated in LB medium with and without 0.5 M