Short Paper

Separation of Neurotoxins from
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The phytoflagellate *Chattonella marina* has frequently formed red tides in Kagoshima Bay, causing massive fish kills and smashing other marine life. Ichthyolethality possibly occurred on exposure to the neurotoxins released during the bloom of this red-tide organism, although some evidence was found that hemagglutinating and hemolytic components might also play a part in the observed disasters.¹⁾ The former fraction was more potent than the latter two.

In the present study neurotoxins were separated from unialgal cultures of *C. marina* by use of the brevetoxin-preparation technique of Baden *et al.*,²⁾ and compared with the standard brevetoxins on TLC.

Freeze-dried *C. marina* cells (12.3 g, 1×10^8 cells/g) were shaken with chloroform: methanol (2: 1). The chloroform extract was dissolved in 90% methanol and shaken with petroleum ether. The methanol layer was evaporated and reextracted with acetone. The acetone extract (80 mg) was applied to a Wakogel C column (1×20 cm) and eluted with chloroform: methanol: acetic acid (100: 10: 1). A 5-ml fraction was collected. The fractions lethal to juvenile red seabream (yield, 1.4 mg/ 10^8 cells; 24-hour LC_{50} , 4.5 mg/l) were recrystallized in methanol. A portion of the crystalline substance (LC_{50} in fish, 4.3 mg/l) was dissolved in petroleum ether and applied to a fluorescent TLC plate (Wako) with a solvent system of acetone: petroleum ether (30: 70).

On TLC analysis the crystalline substance was resolved into three components; one major (R_f value 0.72) and two minors (0.65 and 0.78). They were visible on the fluorescent plate under a UV lamp, or as dark spots when sprayed with sulfuric acid. R_f values of these three substances were compared with those of authentic brevetoxin standards, PbTx-1, PbTx-2 and oxidized PbTx-2, which were cordially provided by Dr. D. G. Baden, University of Miami, Rosenstiel School of Marine and Atmospheric Science. See Fig. 1 for their structural details.³⁾

The major *Chattonella* toxin coincided well in its R_f value (0.72) with the oxidized form of PbTx-2, al-

Fig. 1. Structures of brevetoxins.

though the other two gave no comparable R_f 's. Ichthyolethality of the separated *Chattonella* toxin seemed not to be strong, but Baden *et al.*⁴⁾ mentioned that the toxicity of PbTx-2 was greatly attenuated on conversion to the oxidized form.

Similar toxins have also been separated from the dinoflagellate *Gymnodinium* type '84K.¹⁾ Their structural details are now under investigation, and will be reported elsewhere. This work was partly supported by a research fund from the Ministry of Agriculture, Forestry and Fisheries of Japan.

References

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