Gambierdiscus toxicus, a Ciguatera-Causing Dinoflagellate from Hawaii

Yuzuru SHIMIZU,^{*1,*2} Hiroko SHIMIZU,^{*1} Paul J. SCHEUER,^{*1}
Yoshitsugi HOKAMA,^{*3} Mark OYAMA^{*4}, and James T. MIYAHARA^{*4}

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Wild cells of *Gambierdiscus toxicus*, the alleged causative organism of ciguatera, were collected on the Island of Oahu and their toxicity established. The largest population was found in association with the red alga *Spyridia filamentosa*. Two toxic principles seem to exist in the organism: one was ciguatoxin-like, and the other maitotoxin-like, as shown by their action on the guinea pig atrium. These findings are in agreement with those previously reported for *G. toxicus* from French Polynesia.

Ciguatera is the name of a human intoxication, which is caused by the ingestion of certain tropical reef fishes.¹⁾ It had long been suspected that normally non-toxic fish become toxic through their diet. In 1977 YASUMOTO and co-workers discovered a dinoflagellate in the ciguatera-plagued waters of the Gambier Islands in French Polynesia and demonstrated a direct relationship between the organism and the occurrence of ciguateric fish.^{2,3)} The organism subsequently was recognized as a new species, *Gambierdiscus toxicus* ADACHI et FUKUYO.^{4,5)} The same organism was later discovered by BANNER in a water table at the Hawaii Institute of Marine Biology, Coconut Island, Hawaii, and its identity was established by TAYLOR.⁴⁾ Although substantial studies on the toxicity of *G. toxicus* from French Polynesia exist, no data are available for the Hawaiian strain.⁶⁾ This paper provides basic information about the northern hemisphere strain of *G. toxicus*.

Materials and Methods

Materials

Wild cells of *Gambierdiscus toxicus* (Fig. 1)

were collected at the boat dock of the Hawaii Institute of Marine Biology (21°26.1'N, 157°47.2'W). The water temperature at the time of collection (April 15-May 6, 1981) was 26°C. For large scale collection, the thalli of a red alga *Spyridia filamentosa* were shaken in seawater, and the rinse water was filtered through a 200 μ m sieve. The filtrate was then screened through 75 μ m or 35 μ m sieves. Microscopic examination showed that the residue from both filtrations contained an almost equal number of cells, which were combined. The cells were counted, centrifuged, and immediately extracted with methanol. The major contaminant in this preparation was the broken thalli of *S. filamentosa*.

Cell Counting

Cells were counted microscopically in duplicate on cell suspensions which had been adjusted to appropriate densities. The population of cells attached to the host algae was determined by shaking several grams of wet thalli with 5 ml of seawater in a small container, followed by counting the total number of cells in aliquots of known volumes on plankton counting slides.

Correspondence should be addressed to Professor Paul J. SCHEUER, Department of Chemistry of Hawaii at Manoa, Honolulu, Hawaii 96822, U.S.A.

^{*1} Department of Chemistry, University of Hawaii at Manoa, Honolulu, Hawaii 96822, U.S.A.

^{*2} On leave from Department of Pharmacognosy and Environmental Health Sciences, University of Rhode Island, Kingston, R. I. 02881, U.S.A.

^{*3} Department of Pathology, John A. Burns School of Medicine, University of Hawaii, Honolulu, Hawaii 96822, U.S.A.

^{*4} Department of Pharmacology, John A. Burns School of Medicine, University of Hawaii, Honolulu, Hawaii 96822, U.S.A.

^{*5} A previous study [N. W. WHITHERS, R. H. YORK, and A. H. BANNER, *Am. Zool.* 20, 675 (1980)] on the toxins in cultured *G. toxicus* may have suffered from contamination of the cultures by *Ostreopsis* sp. (T. YASUMOTO, K. FUJIMOTO, Y. OSHIMA, A. INOUE, T. OCHI, R. ADACHI, Y. FUKUYO, and R. BAGNIS: "Ecological and Distributional Studies on a Toxic Dinoflagellate Responsible for Ciguatera," Report to the Ministry of Education, Japan, 1980, pp. 1-50).

(A)

(B)

Fig. 1. Lateral (A) and apical (B) view of wild Hawaiian *Gambierdiscus toxicus* used for toxicity determination.

Mouse Assay

A bioassay was performed by injecting intraperitoneally 1 ml of test solution in 1% of Tween 80 into Swiss Webster mice, weighing 18–25 g. The mice were observed over a period of three days. At least five mice were used at each dilution. One mouse unit is defined as the minimum amount of toxin that causes death in a 20 g mouse.⁶⁾

Fractionation of Extracts

Typically, the centrifuged packed cells (8×10^8) were extracted with methanol (4×100 ml) at room temperature. The extract was evaporated to dryness. The residue was redissolved in 80% methanol (100 ml) and extracted with *n*-hexane (4×100 ml). The methanol layer was evaporated to dryness. To the residue was added 25 ml of

water, and the mixture was extracted with ether four times (1×100 ml and 3×50 ml). The ether layer was backwashed with water (2×25 ml), dried over Na_2SO_4 , and evaporated. The residue thus obtained was designated the ether-soluble fraction, and the aqueous layer the water-soluble fraction.

Results and Discussion

A very dense population of *Gambierdiscus toxicus* was discovered at the boat dock facing the main building of the Hawaii Institute of Marine Biology. The organism was epiphytic on macroalgae and its preference of certain algae was striking (Table 1). Especially a red alga *Spyridia filamentosa* seemed to be the preferred species, and a remarkable number of cells were found on the thalli, while other algae, even with a similar texture and located in close proximity, were found to carry much fewer cells. *S. filamentosa* was not included in the list of macroalgae which TAYLOR examined in his ecological survey of Oahu coastal waters.⁴⁾ However, it is a common alga in Hawaii, and in a spot check at Kahala beach, Oahu, the alga was found to carry a substantial number of *G. toxicus* cells. The morphology of the collected cells with the characteristic fishhook-shaped apical pore was uniform as determined under the microscope, and indistinguishable from that reported^{4,5)} for *G. toxicus*.

Table 1. Population of *Gambierdiscus toxicus* on macroalgae as observed on Coconut Island, Hawaii

Algal species*	Number of cells attached (Cells/g wet thallus)
<i>Hypnea cervicornis</i>	48
<i>Acanthophora spicifera</i>	27
<i>Spyridia filamentosa</i>	823
<i>Microdictyon</i> sp.	9
<i>Rhizoclonium hierglyphica</i>	28
<i>Bryopsis</i> sp.	2
<i>Melosira</i> sp.	2

* All algal specimens were collected within a 0.6 m radius at the dock site, Hawaii Institute of Marine Biology, Coconut Island, Hawaii.

Table 2. Mouse toxicity of wild *Gambierdiscus toxicus* cells collected on Coconut Island, Hawaii

Toxin fraction	Mouse unit/cells*
Water-soluble	$8.2 \pm 0.8 \times 10^3$
Ether-soluble	$3.2 \pm 0.5 \times 10^4$

* Values based upon three separate collections of the cells.

Fig. 2. Physiograph recordings of the effects of the water-soluble toxin (A) and the ether-soluble toxin (B) from wild Hawaiian *Gambierdiscus toxicus* on the spontaneously-contracting guinea pig right artia.

The outer wall of the viable cells showed an affinity to the fluorescence-labelled eel ciguatoxin antibody. The sheep anticiguatoxin was previously prepared and used extensively in a radioimmunoassay.⁷⁾

The wild cell extracts were roughly fractionated into water-soluble and ether-soluble fractions, and their mouse toxicity was established (Table 2). The two fractions gave rise to quite different symptoms in mice. The ether-soluble fraction caused increased excretion at first, while injection of the water-soluble fraction resulted in long-lasting convulsions unaccompanied by diarrhea. Application of the ether-soluble fraction to the isolated guinea pig atria evoked a positive inotropic effect characteristic of ciguatoxin, while the response caused by the water-soluble fraction was different and more like that caused by maitotoxin (Fig. 2).⁸⁾

Above observations are in good agreement with those reported from the wild cells from French Polynesia, except that the Hawaiian cells seem to be somewhat less toxic. To determine if this difference is inherent in the strain and thus related to the fewer incidents of ciguatera in Hawaii, or just due to environmental conditions, a comparative study under standardized conditions may be necessary.

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