FIRST EVIDENCE OF TOXIN PRODUCTION BY BACTERIA IN A MARINE ORGANISM

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A Coryneform bacterium, isolated from the digestive gland of the Japanese ivory shell, Babylonias japonica, was shown to produce neosurugatoxin and prosurugatoxin, the causative toxins of a food poisoning outbreak in 1965 following ingestion of the shellfish. This is the first evidence that marine toxins may be produced by bacteria.

KEYWORDS-- surugatoxin; neosurugatoxin; prosurugatoxin; marine bacteria; food poisoning; toxic shellfish; toxin production; ganglionic blocking agent

Outbreaks of food poisoning caused by consumption of toxic shellfish occur frequently throughout the world. In September 1965, the Japanese ivory shell, Babylonias japonica harvested from a certain area of Suruga Bay, caused an outbreak of food poisoning.1) Previously nontoxic surugatoxin2) and recently the causative toxins, designated as neosurugatoxin3,4) 1 and prosurugatoxin5) 2, were isolated from the digestive gland of the shellfish, and their chemical structures were determined by us. This paper deals with the isolation of surugatoxin from a culture medium of a marine bacterium, and a biochemical determination of the existence of neosurugatoxin and prosurugatoxin in the culture medium.

It is generally believed that toxins in marine organisms arise from ingested materials, and it has already been demonstrated that the toxins saxitoxin6) and

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\begin{align*}
1: & \text{neosurugatoxin} \\
& R = -6'-(\text{myoinositol})_5 \beta-1 \text{xylopyranose} \\
2: & \text{prosurugatoxin} \\
& R = -6'-(\text{myoinositol})
\end{align*}
\]
dinophisistoxin \(^7\) originate from plankton. Interestingly, when toxic Japanese ivory shells harvested from the limited area of Suruga Bay were cultivated in another area where no toxic shellfish had ever been found, their toxicity completely disappeared within one month. Further, nontoxic shellfish gained a degree of toxicity after cultivation in the limited area of Suruga Bay for two months. These results indicate that some environmental factor(s) in the limited area might be involved in the generation of toxicity. However, the population of plankton in the area was normal and the shellfish is carnivorous, suggesting that the toxins had not originated from plankton. The shellfish was only toxic from July to September when the temperature of the sea water in the area sometimes reached 25\(^\circ\) C. Further, the area was quite heavily polluted during this season. These facts led us to consider that conditions might be favorable for bacterial growth, and to speculate that bacteria may have produced the toxin and the shellfish may have accumulated it without decomposition.

Therefore, we investigated more than one hundred strains of bacteria which were isolated from seabed slime of the toxic area and from the toxic shellfish. Finally it was found that a bacterium isolated from the digestive gland of the shellfish produced the toxins. The bacterium was a gram-positive, catalase produced, strict aerobe and a facultative halophile. It belongs to the Coryneform group. The bacterium was cultured\(^8\) and the culture filtrate was roughly fractionated on a Sephadex G-25 column to yield a fraction which was estimated from the Kd value to contain the toxins.\(^4,5\) The fraction evoked mydriasis in mice in the same way authentic toxins do. The fraction was further purified by HPLC to obtain two fractions which showed the same retention times as authentic neosurugatoxin and prosurugatoxin. The two fractions both evoked mydriasis in mice and showed anti-nicotinic activity in isolated guinea pig ileum, biochemically confirming that the bacterium produced these toxins.

Based on the biological activity, the yield of the toxins was estimated to be about 20 ng from 1 liter of the cultured medium. This yield was too small to allow direct confirmation of the chemical structures. However, it has been found that the toxins are unstable and prosurugatoxin degrades into nontoxic surugatoxin \(^3\) in solution;\(^5\) thus, surugatoxin probably is accumulated as a decomposition...
product in the culture medium. A crystalline compound isolated from the culture medium (0.6 mg from 3 liters of medium) by successive Sephadex G-25 column chromatography, Bio-Gel P-2 column chromatography and HPLC showed the same Kd value and retention time as authentic surugatoxin. The secondary ionization mass spectrum and \(^1\)H-NMR spectrum of the compound were identical with those of surugatoxin, providing further support for the view that the toxins produced by the bacterium were neosurugatoxin and prosurugatoxin.

The widely held food chain hypothesis is that fish or shellfish gain toxicity by accumulating the toxic substance(s) present in their food. However, the present results provide the first evidence that marine organisms can become toxic by rapidly accumulating toxic metabolite(s) of microorganisms.

Neosurugatoxin and prosurugatoxin are over five thousand times more active as ganglion blocking agents than existing drugs such as mecamylamine or hexamethonium, and specifically block only nicotinic receptors in the ganglion. Therefore the toxins are excellent tools for studying the neurosystem or brain. However, the shellfish of the limited area of Suruga Bay have rapidly decreased in toxicity since 1978 and are no longer available for the production of the toxins. Production of the toxins by culturing the bacterium should now assure the availability of these compounds for research.

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REFERENCES AND NOTES
8) Composition of medium: Trypto soy broth(Eiken) 5 g, yeast extract(Oxoid) 0.5 g, NaBr 0.2 g, sea water 400 ml, distilled water 600 ml. Incubated at 25°C for 5 days under aerobic condition.

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