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Environmental microbiology

A tetrodotoxin-producing marine pathogen

Disease-related mortalities of sea urchin populations have occurred globally over the past 20 years, although the causative agents have rarely been identified¹. We have discovered a potent new marine pathogen that caused a sudden die-off of the sea urchin *Meoma ventricosa* in Curaçao, Netherlands Antilles, in January of 1997 (ref. 2), and which also has implications for human health. This turns out to be a neurotoxin-producing bacterium that is closely related to the deadly *Pseudoalteromonas haloplanktis tetraodonis*, which is responsible for numerous deaths each year in Japan resulting from the consumption of pufferfish^{3–5}.

The most extensive epizootic reported for sea urchins occurred in the early 1980s, with populations of *Diadema antillarum* being affected throughout the Caribbean sea and western Atlantic: some areas lost more than 97% of mature animals^{6,7}. The ecological impact of the *D. antillarum* die-off on coral reefs was profound, resulting in a dramatic increase of algal cover, which in turn reduced the settlement rates of coral larvae^{7,8}.

M. ventricosa inhabits sandy sediment adjacent to and within sea-grass beds and patch reefs throughout the Florida reef tract and the Caribbean. The die-off in Curaçao extended from the Curaçao harbour to an area 3.5 km downcurrent along the coast². Necropsy of infected animals revealed a progressive loss of spines associated with the disease. Microscopic comparison of the base of the spines and the catch apparatus (where the spine is connected to the skeleton) from affected and healthy animals revealed Gram-negative bacteria in diseased tissue. We isolated these bacteria using a non-selective artificial seawater medium⁹: in all 249 bacterial strains were found in various tissues associated with affected and unaffected urchins.

We exposed these isolates to 95 different carbon sources by using a Biolog microplate assay system^{9,10}. Two metabolic groups (VL-1 and PL-1) were found to occur only in affected tissue (Fig. 1a), so we tested these

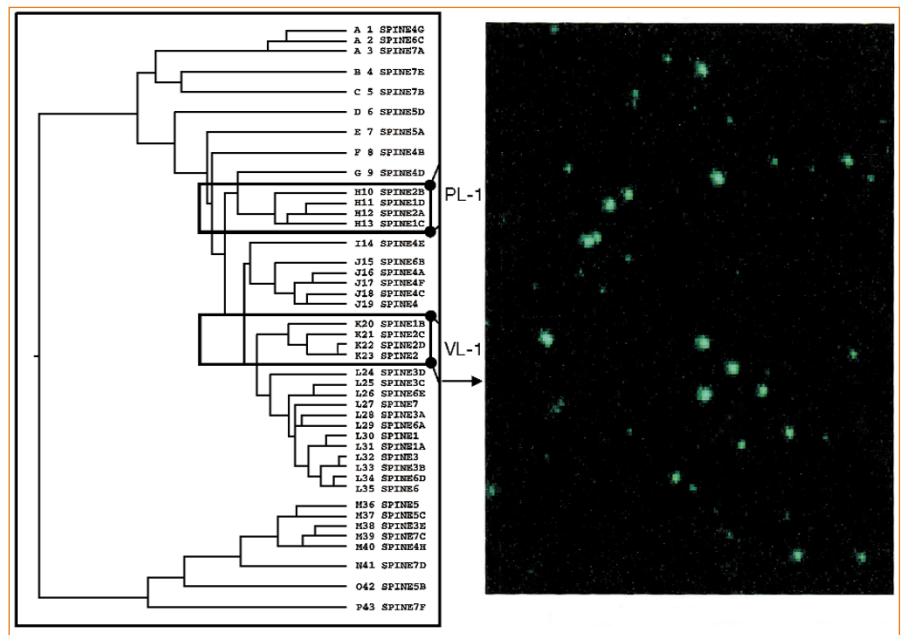


Figure 1 Identification of the new species of tetrodotoxin-producing bacterium. **a**, Dendrogram showing metabolic relationships among bacterial strains isolated from *M. ventricosa* spine catch apparatus. Dendrograms were constructed by clustering hierarchical trees using a nearest-neighbour unweighted pair-group averaging cluster analysis. Isolates PL-1 and VL-1 were indicated as possible pathogens from this grouping. **b**, Immunoassay of the pathogenic isolate VL-1. Fluorescein isothiocyanate-conjugated anti-mouse IgG (green fluorescence) can be seen bound to anti-tetrodotoxin mouse IgG bound to cells producing tetrodotoxin (TTX). Cells were viewed using a confocal laser microscope.

isolates for pathogenicity by infecting the urchin *Lytechinus variegatus*. Aquaria trials with the two isolates showed that urchins inoculated with isolate VL-1 started to shed their spines after two days, whereas control and PL-1-treated urchins remained healthy for over three weeks. We therefore re-isolated VL-1 from the catch apparatus of experimentally infected urchins.

Using DNA-sequence analysis of small ribosomal RNA (16S rDNA), we identified VL-1 as a new bacterium closely related to *Pseudoalteromonas haloplanktis* subsp. *tetraodonis*¹¹, which produces tetrodotoxin² (GenBank accession number, AF154414). Tetrodotoxin has been isolated from various marine organisms, but is best known for its association with pufferfish (order Tetraodontiformes): poisoning by pufferfish (fugu) consumption remains a major health concern, primarily in Japan, where fugu is a delicacy⁴. We tested whether our VL-1 isolate could also produce tetrodotoxin by exposing three-day cultures of VL-1 and PL-1 (as a control) to anti-tetrodotoxin mouse IgG, followed by fluorescein-conjugated anti-mouse IgG¹². We found that a subset of these isolates in the culture could produce tetrodotoxin (Fig. 1b).

To our knowledge, this is the first report of an epizootic affecting *M. ventricosa*. Although the ecological consequences of this urchin die-off are unclear and this epizootic is possibly an isolated incident, it is becoming increasingly important to identify the agents responsible for marine mortality events. In addition, this is the first

time that a tetrodotoxin-producing bacterium has been found to cause disease in a marine animal. And because many urchin species are commercially valuable, our demonstration that this bacterium can cause identical disease symptoms in a different sea urchin species is an important consideration.

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