Short Paper

**Identification of a Tetrodotoxin-
producing Bacterium Isolated
from the Xanthid Crab
*Atergatis floridus***

Haruo Sugita,*¹ Ryutaro Ueda,*¹ Tamao Noguchi,*²
Osamu Arakawa,*² Kanehisa Hashimoto,*² and
Yoshiaki Deguchi*¹

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Recently we have demonstrated that a bacterium which was isolated from a xanthid crab *Atergatis floridus* and tentatively identified as *Vibrio* sp., produced tetrodotoxin (TTX) and anhydrotetrodotoxin (anhydroTTX), and suggested that bacteria are at least one of the origins of this toxin.¹⁾ Since then, TTX-producing bacteria have also been isolated from a puffer *Fugu vermicularis vermicularis*²⁾ and a starfish *Astropecten polyacanthus*³⁾ and were both identified as *Vibrio alginolyticus*. Another TTX-producer was isolated from a calcareous alga *Jania* sp. and tentatively identified as *Pseudomonas* sp.⁴⁾ In this situation, the present study was undertaken to identify *Vibrio* sp. S-173, the main strain of the TTX-producing bacteria from the intestines of xanthid crab *A. floridus*.¹⁾

Strain S-173 was essentially characterized by techniques described in the Manual of Methods for General Microbiology.⁵⁾ Incubation was performed on PYBG medium⁶⁾ at 25°C, unless otherwise specified.

The strain was a facultatively anaerobic, chemo-organotrophic, and non-sporeforming Gram-negative bacterium.

The cells were rod-shaped in the logarithmic phase of growth, the sizes being about 0.9–1.1 by 1.7–4.1 μm . They occurred singly or in chains, and were motile by means of flagella. The cells were not homogeneous in respect of morphology of flagella: Most cells with a bunch of 2–12 flagella at one pole, some cells with a single flagellum at one pole (monotrichate), rather few cells with a single flagellum at the opposite pole (lophotrichate), or with a single flagellum at each pole plus a lateral flagellum.

Surface colonies of strain S-173, grown on PYBG agars at 25°C for 3 days, were circular, entire, smooth, yellow and 1–2 mm in diameter. They emitted no fluorescence and luminescence, and did not swarm on

a PYBG agar. They formed green colonies on a TCBS agar (Eiken).

This strain produced oxidase and catalase; fermented glucose without gas production; was sensitive to the vibrio-static agent (O/129); produced acids from cellobiose, fructose, galactose, glucose, glycerol, maltose, mannitol, mannose and trehalose; did not produce any acid from arabinose, erythritol, inositol, melibiose, rhamnose, ribose, salicin, sorbitol, sucrose and xylose; did not produce arginine dihydrolase and ornithine decarboxylase; produced lysine decarboxylase; reduced nitrate to nitrite; did not produce indole, and acetoin and/or diacetyl; did not utilize L-tyrosine, β -alanine, L- α -alanine, D- α -alanine, L-arginine, L-aspartate, L-citrulline, glycine, L-proline, L-serine, L-leucine, L-glutamate, L-threonine, L-ornithine, ethanol, propanol, D-gluconate, propionate, heptanoate, DL-malate, pyruvate, *p*-hydroxybenzoate, acetate, butyrate, isobutyrate, isovalerate, caproate, caprylate, pelargonate, succinate, fumarate, L-tartrate, aconitate, N-acetylglucosamine, malonate, benzoate and hippurate; did not hydrolyze starch, gelatin and chitin but hydrolyzed Tween 80 and lecithin; grew in broths containing 0.5 to 7% NaCl; and grew at 4 to 30°C.

As described above, strain S-173 agreed well with *Vibrio fischeri* in many properties, except that it did not produce any acid from ribose, grew at 4°C, did not utilize L-proline, succinate, fumarate and N-acetylglucosamine.⁷⁾ The possibility can not be excluded that this strain is a new species of *Vibrio*. At present, however, it seems reasonable to identify the microorganism as a *Vibrio fischeri*-like bacterium.

References

- 1) T. Noguchi, J. K. Jeon, O. Arakawa, H. Sugita, Y. Deguchi, Y. Shida, and K. Hashimoto: *J. Biochem.*, **99**, 311–314 (1986).
- 2) T. Noguchi, D. F. Hwang, O. Arakawa, H. Sugita, Y. Deguchi, Y. Shida, and K. Hashimoto: *Mar. Biol.*, **94**, 625–630 (1987).
- 3) H. Narita, S. Matsubara, N. Miwa, S. Akahane, M. Murakami, T. Goto, M. Nara, T. Noguchi, T. Saito, Y. Shida, and K. Hashimoto: *Nippon Suisan Gakkaishi*, **53**, 617–621 (1987).
- 4) T. Yasumoto, D. Yasumura, M. Yotsu, T. Michishita, A. Endo, and Y. Kotaki: *Agri. Biol. Chem.*, **50**, 793–795 (1986).
- 5) P. Gerhardt (ed.): *Manual of Methods for General Bacteriology*, Amer. Soc. Microbiol., Washington, D. C., 1981, pp. 409–443.
- 6) H. Sugita, H. Tanaami, T. Kobashi, and Y. Deguchi: *Nippon Suisan Gakkaishi*, **47**, 655–661 (1981).
- 7) N. R. Krieg and J. G. Holt (ed.): *Bergey's Manual of Systematic Bacteriology*, Vol. 1, Williams & Wilkins, Baltimore, 1984, pp. 516–550.

*¹ Department of Fisheries, Nihon University, Shimouma 3, Setagaya, Tokyo 154, Japan (杉田治男, 上田龍太郎, 出口吉昭: 日本大学農獣医学部).

*² Laboratory of Marine Biochemistry, Faculty of Agriculture, University of Tokyo, Bunkyo, Tokyo 113, Japan (野口玉雄, 荒川 修, 橋本周久: 東京大学農学部).