Clostridium tetani is a phospholipase (Lecithinase)-producing bacterium [1]

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Letters to the Editor

Clostridium tetani Is a Phospholipase (Lecithinase)-Producing Bacterium

Many *Clostridium* spp. produce phospholipases (lecithinases). The phospholipase of *Clostridium perfringens* is known as alpha-toxin (Cpa), which is the best characterized of all clostridial phospholipases. *C. tetani*, the causative organism of tetanus, has been recognized as a phospholipase-negative clostridium. In the course of our research on clostridial phospholipases (2, 3), a BLAST homology search revealed that the *C. tetani* E88 genome (1) contained a *cpa*-related gene (gene identifier, 1058703; locus tag, CTC00990). Cpa consists of two domains, the N-terminal and C-terminal domains (2). The N-terminal domain has phospholipase activity, while the C-terminal domain is a calcium-dependent putative phospholipid binding domain. According to the E88 genome sequence, the phospholipase gene of *C. tetani* E88 comprising 242 amino acid residues has similarity with the N-terminal domain of Cpa and does not have a domain corresponding to the C-terminal domain. The presence of this gene prompted us to examine phospholipase production by *C. tetani*.

All tested strains—NCTC 279 (the type strain of *C. tetani)*, NCTC 5404, CN 655 (Pasteur Institute strain 105554), Ramon (Pasteur Institute strain 60.28), KZ 1180, KZ 1186, KZ 1189, and KZ 1199—had been identified as *C. tetani*. The strains KZ 1180, KZ 1186, KZ 1189, and KZ 1199 were isolated from soil. In this study we confirmed these strains as *C. tetani* by using a 16S rRNA gene bacterial sequencing kit (Microseq 500; Applied Biosystems). *C. perfringens* KZ 221 (3) and *C. butyricum* NCTC 7423 were used for phospholipase-positive and -negative controls. An anaerobic culture using mixed gases (85% N_2, 10% CO_2, and 5% H_2) was carried out at 37°C for 2 days.

We found that *C. tetani* NCTC 5404 expressed a positive

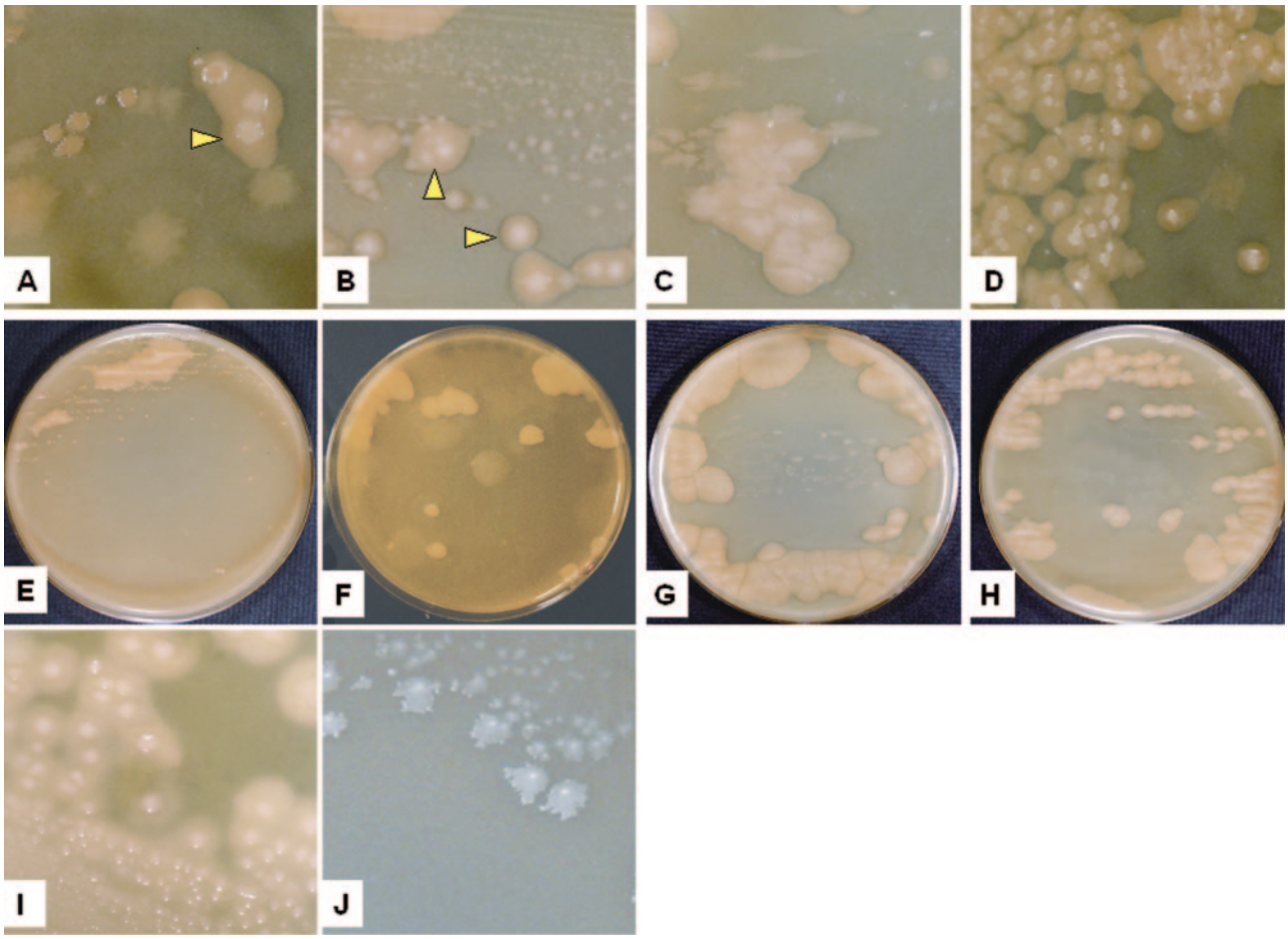

FIG. 1. Phospholipase production by *C. tetani* strains on PRYG agar medium containing 5% egg yolk. (A to D) A phospholipase reaction characterized by opacity around a colony is observed. Yellow arrowheads indicate representative phospholipase-positive colonies. (E to H) A phospholipase reaction mixture is distributed on the medium plates. (A) Ramon; (B) KZ 1189; (C) NCTC 5404; (D) CN 655; (E) NCTC 279; (F) KZ 1180; (G) KZ 1186; (H) KZ 1199; (I) *C. perfringens* KZ 221 (phospholipase-positive control); (J) *C. butyricum* NCTC 7423 (phospholipase-negative control). For *C. butyricum*, 0.5% glucose was added to the medium.

phospholipase reaction, characterized by opacity around a colony, on a medium, the basal formula of which had been used for spore formation of *C. tetani* in our laboratory. We therefore examined medium conditions to enhance phospholipase production. The alterations were based on the knowledge that *C. tetani* possesses an extensive sodium ion bioenergetics and a sodium-glucose or galactose cotransporter (gene identifier, 1059145; locus tag, CTC01237) (1) and that several *C. tetani* strains are also able to utilize glucose (4, 5). Finally, we developed a peptone-rich yeast extract glucose (PRYG) agar medium containing 5% (vol/vol) egg yolk. PRYG agar medium consisted of the following per liter of water: 40 g of Bacto proteose peptone 2 (Difco Laboratories), 10 g of yeast extract (Difco Laboratories), 1 g of glucose, and 20 g of agar (pH 7.4). In brief, PRYG agar medium contained a high concentration of peptone and a small amount of glucose but no sodium chloride. All strains clearly showed the phospholipase reaction on PRYG agar medium containing 5% egg yolk (Fig. 1). It was noted that not all colonies produced phospholipase. Research on the regulation system of the gene expression is awaited. Additionally, we tested a commercial brain heart infusion agar (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) for NCTC 279, NCTC 5404, KZ 1180, KZ 1186, KZ 1189, and KZ 1199. Growth of all strains on brain heart infusion agar containing 5% egg yolk was poor, but the NCTC 5404, KZ 1186, KZ 1189, and KZ 1199 strains exhibited the phospholipase reaction after 4 days of incubation.

These results demonstrated that under the appropriate culture conditions, *C. tetani* is a phospholipase-producing bacterium. *C. tetani* phospholipase may be produced in vivo and contribute indirectly to tetanus by helping nutrient acquisition for cell growth and multiplication.

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