# Mutation and Diversity of Diphtheria Toxin in *Corynebacterium ulcerans*

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*Corynebacterium ulcerans* infection is emerging in humans. We conducted phylogenetic analyses of *C. ulcerans* and *C. diptheriae*, which revealed diverse diphtheria toxin in *C. ulcerans*. Diphtheria toxin diversification could decrease effectiveness of diphtheria toxoid vaccine and diphtheria antitoxin for preventing and treating illnesses caused by this bacterium.

*Corynebacterium ulcerans* is a rod-shaped, aerobic, gram-positive bacterium closely related to *C. diphtheria*. Some strains of *C. ulcerans* can produce diphtheria toxin, which causes respiratory diphtheria in humans and animals. Reports of human infections with *C. ulcerans* have increased during the past 20 years, and *C. ulcerans* is a recognized emerging human pathogen (*1*). Humans can contract toxin-producing *C. ulcerans* from companion animals (*2*,*3*). Human death can occur if appropriate treatment is delayed (*4*). Non–toxin-producing *C. diphtheriae* and *C. ulcerans* can convert to toxin-producing strains through a process of lysogeny with diphtheria toxin gene–carrying corynebacteriophages (*5*–*7*). Although increased coverage of the diphtheria toxoid vaccine has reduced the frequency of *C. diphtheriae* infections, reports of *C. ulcerans* infections in humans are increasing.

A report evaluating the differences in the amino acid sequences of the diphtheria toxins in *C. diphtheriae* and *C. ulcerans* used only limited data, comparing 1 strain of *C. diphtheriae* against 2 strains of *C. ulcerans* (*8*), leaving the differences among the toxins of these 2 species unclear. Others have conducted bacterial genome analyses and deposited several genomic sequences of *C. diphtheriae* and *C. ulcerans* strains into a public database. We collected amino acid sequences of the diphtheria toxin and the nucleic acid sequences of the 16S rRNA gene of 6 *C. diphtheriae* strains and 6 *C. ulcerans* strains from the National Center for Biotechnology Information genome database (https://www.ncbi.nlm.nih.gov/genome). Then, we performed phylogenetic analyses by using MEGA 7.0 (https://www.megasoftware.net).

We found that the 16S rRNA gene sequences divided into separate *C. diphtheriae* and *C. ulcerans* strains with some sequence variability among the strains in each species (Figure, panel A). The amino acid sequences of the toxins also divided into separate clades for each species. However, we noted that *C. diphtheriae* strains were identical, but *C. ulcerans* strains were diverse (Figure, panel B), suggesting that *C. ulcerans* tends to acquire mutations more frequently than *C. diphtheriae.* Two possible explanations for this phenomenon are that *C. ulcerans* is maintained by various animals, increasing its diversity compared with *C. diphtheria*, which is believed to infect only humans; or that

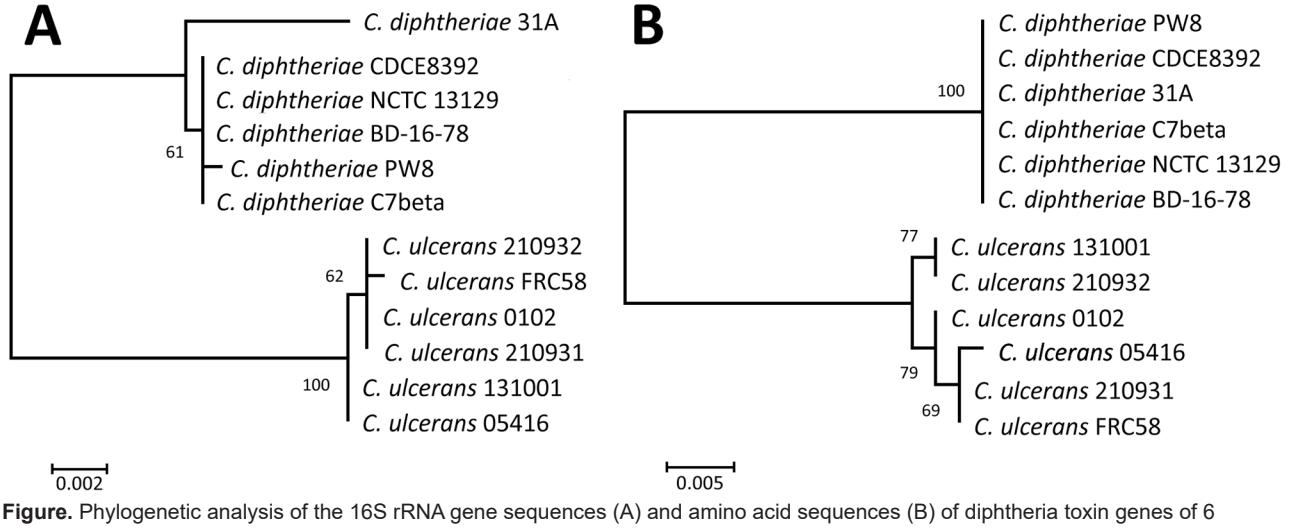


**Figure.** Phylogenetic analysis of the 16S rRNA gene sequences (A) and amino acid sequences (B) of diphtheria toxin genes of 6 *Coynebacterium ulcerans* strains and 6 *C. ulcerans* strains. All strains had the diphtheria toxin gene; whole-genome analysis data are available from the National Center for Biotechnology Information database (https://www.ncbi.nlm.nih.gov/genome). We generated phylogenetic trees by using the maximum-likelihood method in MEGA 7.0 (https://www.megasoftware.net). 16S rRNA gene sequences were analyzed by the Hasegawa-Kishino-Yano model with 1,000 bootstrap replications; amino acid sequences were analyzed by the Whelan and Goldman model with 100 bootstrap replications. Scale bars indicate substitutions per site.

*C. ulcerans* has a phage-independent pathway to acquire the diphtheria toxin–encoding gene, as reported (*9*).

Most severe human cases of disease caused by toxigenic *C. ulcerans* have occurred in unvaccinated or inadequately vaccinated persons. However, a fatal case was reported in a person who received a diphtheria vaccination booster ≈10 years before disease onset (*10*). Diversification of the *C. ulcerans* diphtheria toxin gene is of note because accumulation of these gene mutations potentially could lead to decreased effectiveness of the diphtheria toxoid vaccine for prevention and diphtheria antitoxin for treatment of toxigenic *C. ulcerans* disease.

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### About the Author

Dr. Otsuji is an assistant professor of intensive care medicine at the University of Occupational and Environmental Health Japan, Kitakyushu, Japan. His research interests are critical care and microbiology, including zoonotic infections and microbiota.

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# Ophthalmomyiasis Caused by *Chrysomya bezziana* after Periocular Carcinoma

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We treated a homeless man in Iran with a history of squamous cell carcinoma who had ophthalmomyiasis caused by *Chrysomya bezziana* parasites. This case highlights a much-neglected condition and describes measures to prevent it.

Ophthalmomyiasis is principally manifested as orbital myiasis, ophthalmomyiasis external, and ophthalmomyiasis interna (*1,2*). *Chrysomya bezziana* (screwworm) has been implicated in cancer-associated myiasis of the skin, larynx, face, and breast (*3–5*). Ophthalmomyiasis is uncommon but becomes significant in debilitated and compromised patients.

In December 2017, a 75-year-old homeless man sought care at the University Hospital of Nikookari Eye Center (Tabriz, northwestern Iran). His medical history included a surgery for left-side periocular squamous cell carcinoma ≈15 years earlier. After 2 years, the patient observed recurrence of the squamous cell carcinoma, but he did not seek further evaluation and treatment. He reported loss of sight since 2015 because of the tumor extension into the orbit and globe of his eye. He had intermittent pain.

On examination, his systemic findings were unremarkable. Orbital and periocular examination revealed extensive