

## Comparison of Nontoxigenic and Neurotoxigenic *Clostridium butyricum* by Molecular Typing Methods

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*C. butyricum* has been considered entirely non-pathogenic. However, recently, neurotoxigenic *C. butyricum* has been identified as occurring in the natural environment (X. Meng *et al.* *J Med Microbiol* 48: 133-7, 1999). Moreover, it has been shown that 13 neurotoxigenic strains are at least divided into three clusters by molecular typing methods (X. Wang *et al.* *Appl Environ Microbiol* in press).

In this study, we comparatively analyzed eight nontoxigenic (MIYAIRI 588, MIYAIRI 595, MIYAIRI 630, SI 293-2, RU 063-3, ATCC 19398 and IFO 3315) and seven neurotoxigenic (BL 5262, BL 6340, LCL 155, LCL 063, LCL 095, KZ 1886 and KZ 1887) *C. butyricum* strains by using PCR assay and Southern blot hybridization for the type E botulinum toxin gene (*bont/E*), random amplified polymorphic DNA (RAPD) assay, and pulsed-field gel electrophoresis (PFGE). With the PCR

assay and Southern blot hybridization, the *bont/E* gene was detected in all seven strains of neurotoxigenic *C. butyricum*, but not in nontoxigenic strains, indicating that there were no partial *bont/E* gene fragments in the nontoxigenic *C. butyricum* strains. All strains were successfully analyzed by RAPD assay. In contrast to the RAPD assay, two strains of nontoxigenic *C. butyricum* could not be analyzed by PFGE, probably due to the DNase activity. Nontoxigenic strains SI 293-2 and GU-2 shared an identical RAPD or PFGE pattern. The other nontoxigenic strains showed unique RAPD and PFGE patterns, and these patterns differed from those of all neurotoxigenic *C. butyricum* strains.

The present results suggest that nontoxigenic and neurotoxigenic *C. butyricum* may possess the distinct genetic characters despite the same species.