CAN NON-TOXIGENIC *CLOSTRIDIUM BOTULINUM*-LIKE ISOLATES BE RENAMED?

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Background

Clostridium botulinum produces an extremely potent neurotoxin which, when ingested, causes botulism, a severe and often deadly illness. Historically, low incidence of botulism in New Zealand (Dodds, 1993) has appeared to reflect the low environmental incidence of spores (Gill and Penney, 1982). Surprisingly, between 1993 and 1996 a number of psychrotolerant *Cl. botulinum*-like strains have been isolated from spoiled vacuum-packed meat, as well as from various environmental sources. Classical biochemical characterisation and restriction fragment length polymorphism (RFLP) patterns of the 16S rRNA gene have suggested that some of these New Zealand meat isolates may belong to the species *Cl. botulinum* (Broda *et al.*, 2000). However, when some *Cl. botulinum*-like isolates of NZ clostridia were tested using standard mouse bioassay, they were found to be non-toxic to mice. Subsequent study has revealed that none of these isolates carried gene(s) encoding botulinal neurotoxin production (Broda *et al.*, 1998). Consequently, these isolates were genetically incapable of producing botulinal toxin and are, therefore, unable to cause botulism.

In classical medical/veterinary bacteriology the species epithet "botulinum" was reserved for organisms with a confirmed potential to produce one of the several botulinal neurotoxins and cause botulism. The presence of NZ *Cl. botulinum*-like isolates in vacuum-packed meat would not constitute a real food safety hazard. However, the use of "botulinum" epithet with these isolates may introduce to a customer a perceived safety concern and, therefore, there is a strong expediency argument against its continued use. In the present study, chemotaxonomic markers were used along with 16S rRNA gene sequencing to find out whether non-toxigenic NZ *Cl. botulinum*-like isolates differ sufficiently from reference strains of toxigenic non-proteolytic *Cl. botulinum* to warrant a valid proposal of an alternative species epithet for these isolates.

Objective

To reduce perception of the food safety hazard associated with the non-toxigenic psychrotolerant *Clostridium botulinum*-like microorganisms by establishing their detailed taxonomic position and, if appropriate, by suggesting an alternative species epithet.

Methods

A total of 25 local meat strains were selected for this study from a group of 244 non-toxigenic *Clostridium botulinum*-like isolates (Broda *et al.*, 1998). These strains were selected to represent every colony morphotype encountered among isolates from different meat species. Reference strains used were psychrotolerant non-proteolytic *Cl. botulinum* types B (17B), E (Beluga) and F (202F). Full forward sequences of 16S rRNA genes from all meat and reference strains were obtained and analysed as described previously (Broda *et al.*, 2000). Cellular fatty acid (CFA) analysis and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of whole-cell proteins were conducted for all meat and reference strains, as described previously (Broda *et al.*, 1999).

Results and discussion

The near-complete 16S rRNA gene sequences, each consisting of between 1483 and 1492 bp, were obtained from all strains. Aligned sequences of meat strains were nearly identical and differed from each other by less than 0.1%. Full forward 16S rRNA gene sequences of meat strains had between 99.6 and 99.7% similarity to the sequences of reference strains of non-proteolytic *Cl. botulinum* types B (17B), E (Beluga) or F (202F). The major CFA identified in cell extracts of meat and reference strains were myristic (C14:0), palmitic (C16:0) and oleic (C18:1). A number of minor fatty acids were also detected. There were distinct differences in the presence and proportions of some major (e.g. C18:1) and minor (e.g. C12:0 and C18:1 *cis* 7) fatty acids, between profiles of majority of meat strains and those of reference strains of *Cl. botulinum*. However, very few differences were present between SDS-PAGE profiles of NZ *botulinum*-like isolates and reference strains of non-proteolytic *Cl. botulinum* types B (17B), E (Beluga) and F (202F). Gel electrophoresis of soluble cell proteins demonstrated that meat and reference strains had almost indistinguishable profiles.

In the present study, the use of 16S rRNA gene sequencing has resulted in a number of non-toxigenic isolates associated with blown pack spoilage being "identified" as strains of *Cl. botulinum*. However, it is possible for two different species to have over 99% 16S rRNA gene sequence homology (Stackebrandt and Goebel, 1994). When this happens, supplementary chemotaxonomic methods and DNA-DNA hybridisation are used to demonstrate differences between bacterial strains and their nearest taxonomic neighbours (Cato and Stackebrandt, 1989). In the present study, distinct differences between profiles of non-toxigenic meat strains and toxigenic reference strains were demonstrated with CFA analysis but not with SDS-PAGE analysis of whole-cell proteins. Consequently, analyses using additional chemotaxonomic markers could not resolve the detailed taxonomic position of meat strains. It is postulated that DNA-DNA homology studies will establish in a definitive manner whether, in accordance with current requirements for bacterial nomenclature, an alternative species epithet can be validly suggested for local non-toxigenic *Cl. botulinum*-like isolates.

Renaming of non-toxigenic meat strains may still be possible, should DNA-DNA hybridisation data confirm genomic homogeneity of NZ non-toxigenic *Cl. botulinum*-like isolates and toxigenic reference strains of non-proteolytic *Cl. botulinum* types B (17B), E (Beluga) or F (202F). A precedent for maintaining separate names for non-toxigenic and toxigenic, otherwise phenotypically and genomically similar microorganisms, has been set recently (Judicial Commission of the International Committee on Systematic Bacteriology, 1999). To avoid problems in communication in medical microbiology and diagnosis of botulism the

International Committee on Systematic Bacteriology conserved species epithets of *Cl. sporogenes* for non-toxigenic, and *Cl. botulinum* type A for toxigenic, genomically highly similar, clostridial strains.

Conclusions

For taxonomy to be viable in practical situations, such as food-borne disease diagnosis, nomenclature should reflect the connection between a pathogen and a disease. Maintaining the species epithet *Cl. botulinum* for NZ isolates would not reflect their non-toxigenic status. Therefore, practical taxonomy should override academic taxonomy, such as that based on the 16S rRNA gene sequence. We suggest that these isolates are renamed.

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