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Multilocus Enzyme Electrophoresis of *Clostridium argentinense* (*Clostridium botulinum* Toxin Type G) and Phenotypically Similar Asaccharolytic Clostridia

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Twenty-three strains of *Clostridium argentinense*, *C. subterminale*, *C. hastiforme*, and other phenotypically similar asaccharolytic clostridia recently placed in seven DNA hybridization groups were compared by multilocus enzyme electrophoresis. The three nontoxigenic strains of *C. argentinense* were most closely related to the toxigenic strains of this species. All nine toxigenic strains of *C. argentinense* belonging to a single DNA hybridization group had identical enzyme types on the basis of nine enzymes. All other strains except for two derived from the type strain of *C. subterminale* were differentiable. Overall, there was excellent agreement between DNA relatedness and multilocus enzyme electrophoresis results.

Clostridium botulinum, as described in *Bergey's Manual of Systematic Bacteriology* (1), includes strains of clostridia that are capable of producing any one of the seven immunologically distinct types of botulinal neurotoxin (types A to G). However, phenotypic as well as genetic studies show that *C. botulinum* is not a homogeneous species (1, 6). In addition, each of the four culturally distinct groups of *C. botulinum* (I to IV) is related to a nontoxigenic *Clostridium* species that may be known by a distinct species name (6). Furthermore, strains of *C. baratii* and *C. butyricum* that are capable of producing botulinal neurotoxin have recently been isolated (9).

Suen et al. (8) reported a detailed biochemical and genetic analysis of *C. botulinum* toxin type G, the only toxin type occurring in group IV, and its nontoxigenic but phenotypically similar counterparts, organisms identified as *C. subterminale* or *C. hastiforme* (8). They found that these organisms comprise seven distinct DNA hybridization groups that are separable by a few phenotypic characteristics. DNA relatedness group 1 contains all strains of *C. botulinum* toxin type G as well as three nontoxigenic strains that had been incorrectly identified as *C. subterminale* (two strains) and *C. hastiforme* (one strain). These strains were previously shown to cross-react in serologic tests (3, 4). The name *Clostridium argentinense* has been proposed to include both toxigenic and nontoxigenic strains in DNA relatedness group 1 (8).

We used multilocus enzyme electrophoresis (5) to characterize the organisms studied by Suen et al. (8). These include 12 strains of *C. argentinense* (9 toxigenic and 3 nontoxigenic), 3 strains of *C. subterminale*, 1 strain of *C. hastiforme*, and 7 strains phenotypically similar to these species but belonging to four other DNA hybridization groups (Table 1). Strains were grown on three egg yolk agar plates for 48 h at 37°C in an anaerobic chamber. Enzyme extracts were prepared as previously described (5), filter sterilized, and stored in aliquots at −70°C. All starch gels were run as described elsewhere (5) at a constant 135 V in buffer system A (Tris citrate, pH 8.0) until the tracking dye (0.05% bromphenol blue in Tris hydrochloride, pH 8.0, and 80% glycerol) had migrated 6 cm. Nine enzymes were found in the majority of strains (Table 1). Two bands of nucleoside phosphorylase were consistently found for some strains. They were treated as two independent enzymes (Table 1, NSP1 and NSP2). On gels stained for NAD-dependent glutamate dehydrogenase-2, most strains showed two bands, one of which was identical to glutamate dehydrogenase 1 and therefore not considered again.

All nine toxigenic *C. argentinense* strains had identical enzyme profiles (Table 1). This is surprising in view of the diverse origins of these strains, which were originally identified as *C. botulinum* type G (2, 7). Three were derived from two soil isolates in different provinces in Argentina, and the remaining six were each from different human specimens in Switzerland. Of the remaining strains, only two, AL1453 and BL4856, were not distinguishable by enzyme typing. This should be expected since these two cultures both represent the type strain of *C. subterminale* (ATCC 25744^T) received at different times (8). This finding shows that the technique is reproducible and the enzyme patterns are stable.

To analyze the genetic relatedness among all 23 strains, we calculated a matrix of pairwise unweighted similarity coefficients and produced a dendrogram (Fig. 1) by the average linkage method of clustering (5). There is excellent agreement between DNA hybridization data and multilocus enzyme electrophoresis, although of 27 enzymes tested, only 9 were useful. In addition, the number of null alleles (number of strains showing no activity for a particular enzyme) with the same weight as any other allele was high (30 of 207). The only strain that did not fit as expected was AL14431. However, one can argue that this strain does not belong to DNA hybridization group 2 since its relatedness to the type strain of *C. subterminale* (AL1453 and BL4856) was only 69 to 71% at 50°C and 61 to 82% at 65°C and the divergence in related sequences is 4.5 to 5.5% (8).

Our results confirm that of the strains designated *C. argentinense*, the three nontoxigenic strains, two previously identified as *C. subterminale* and one as *C. hastiforme*, are more closely related to the nine toxigenic strains than to any other strain. However, they differ from the toxigenic strains by at least two alleles, and therefore, multilocus enzyme electrophoresis may allow differentiation between toxigenic

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TABLE 1. Electrophoretic characteristics of nine enzymes for differentiating *C. argentinense*, *C. subterminale*, *C. hastiforme*, and other phenotypically similar clostridia

Strain	Organism	DNA group[a]	Enzyme type	Allele designations for the following nine enzymes[b]:								
				NSP1	NSP2	ADK	ME	GD1	GD2	GOT	IPO	THD
BL714	*C. argentinense* toxigenic[c]	1	1	3	1	5	2	2	4	3	3	3
BL1353		1	1	3	1	5	2	2	4	3	3	3
BL2738		1	1	3	1	5	2	2	4	3	3	3
BL2739		1	1	3	1	5	2	2	4	3	3	3
BL2740		1	1	3	1	5	2	2	4	3	3	3
BL2741		1	1	3	1	5	2	2	4	3	3	3
BL2742		1	1	3	1	5	2	2	4	3	3	3
BL3659		1	1	3	1	5	2	2	4	3	3	3
BL4853^T		1	1	3	1	5	2	2	4	3	3	3
AL4422	*C. argentinense* nontoxigenic	1	2	3	1	5	0	2	2	3	3	3
AL16525		1	3	3	1	4	2	1	0	3	3	4
AL12677		1	4	4	2	6	2	3	1	3	3	3
AL1453^T	*C. subterminale*	2	10	8	0	1	0	4	0	4	1	3
AL14431		2	8	6	0	2	0	3	0	3	3	5
BL4856^T		2	10	8	0	1	0	4	0	4	1	3
NH137	*Clostridium* species	3	13	4	0	5	1	5	3	2	4	1
NH138		3	14	4	0	5	1	5	3	2	4	3
NH134		4	6	5	0	3	0	3	0	6	2	1
NH239		4	5	5	0	3	0	3	0	5	2	2
AL14764		5[d]	12	1	0	8	0	7	5	1	2	1
AL750		5	11	1	0	7	0	7	5	1	2	3
BL4858^T	*C. hastiforme*	6	9	7	0	3	0	6	0	2	1	6
AL1449	*Clostridium* sp.	7	7	2	0	4	0	3	0	5	0	2

[a] From reference 8.
[b] NSP, Nucleoside phosphorylase; ADK, andenylate kinase; ME, malic enzyme; GD, NAD-dependent glutamate dehydrogenase; GOT, glutamic oxaloacetic transaminase; IPO, indophenol oxidase; THD, threonine dehydrogenase.
[c] Toxigenic strains are those which were formerly known as *C. botulinum* toxin type G.
[d] Based on phenotypic studies only.

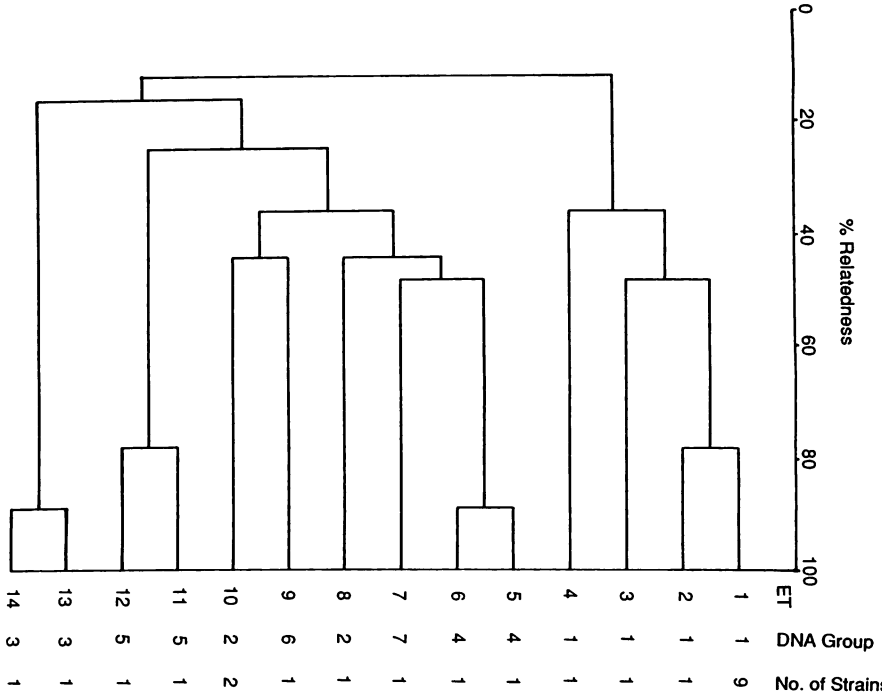

FIG. 1. Dendrogram depicting relationships among *C. argentinense* and phenotypically similar asaccharolytic clostridia based on multilocus enzyme electrophoresis. ET, Enzyme type

and nontoxigenic strains without a toxin assay. Isolates of a cytotoxigenic, enteropathogenic strain of *Escherichia coli* distinguishable as serotype O157:H7 were found to fall into a well-defined group of clonal genotypes based on multilocus enzyme electrophoresis, even though they were obtained from geographically separated cases (10).

It will be especially interesting to see whether the same methods might be useful for differentiating the phenotypic groups I, II, and III of *C. botulinum*, toxin types, toxigenic and nontoxigenic strains, and toxigenic strains within toxin type and phenotypic groups. This approach may be useful in epidemiologic studies.

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