1

Taxonomic relationships among the clostridia

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The class clostridia are a highly diverse group of organisms within the phylum *Bacillota* (formerly *Firmicutes*). Within this class is a large and important group of organisms belonging to the genus *Clostridium* proposed in 1880 by Prazmowski, with *Clostridium butyricum* designated as the type species (Prazmowski, 1880). Due to the rather simple genus criteria, the genus essentially became a repository for all gram-positive-staining, anaerobic, spore-forming, rod-shaped organisms (Rainey et al., 2009). Thus, species of the genus *Clostridium* are extremely heterogeneous, with many species displaying a wide range of phenotypes including psychrophiles, thermophiles, and acidophiles, and organisms that synthesize cytochromes and quinones (Wiegel et al., 2006; Rainey et al., 2009). Although molecular methods and early phylogenetic studies began to provide insights into the diverse nature of the genus (Johnson and Francis, 1975; Tanner et al., 1981; Ludwig et al., 1988; Lawson et al., 1993), it was not until the seminal publication of Collins et al. (1994) that the true diversity and phylogenetic structure of the genus were revealed with the establishment of 19 16S rRNA clusters (Collins et al., 1994). Further support for this enormous diversity followed (Stackebrandt et al., 1999; Gupta and Gao, 2009), and in 2013, Yutin and Galperin (2013) provided a restructuring of the clostridia using genomic analyses to investigate gram-negative-staining spore formers and other misplaced clostridia (Yutin and Galperin, 2013). While potentially useful, these descriptions do not comply with the Bacteriological Code and, therefore, the names could not be validly published in the *International Journal of Systematic and Evolutionary Microbiology* (IJSEM). A major step forward in the taxonomy of the *Clostridium* was achieved when Lawson and Rainey (2016) formally proposed to restrict the genus to the type strain *C. butyricum* and related species (Lawson and Rainey, 2016). The authors recognized that the phylogenetic depth was still too large to represent a single genus, but the genus as it previously stood could not encompass the phylogenetic diversity revealed by 16S rRNA gene sequence analysis. Several species belonging to *Eubacterium* and *Anaerobacter* that fell within *Clostridium sensu stricto* were transferred to the genus *Clostridium*. The authors also proposed the transfer of *Sarcina* to the genus *Clostridium,* but this proved to be controversial with the priority of the names *Clostridium* and *Sarcina* still open to interpretation (Lawson and Rainey, 2016; Tindall, 2016). However, taxonomy should be a practical tool and the transfer of all *Clostridium* to the genus *Sarcina* would cause chaos, especially in the clinical field as well as in the wider scientific community.

A further, as yet unresolved, consideration is with *Clostridium botulinum*. Rather than a single species, this is referred to as the *C. botulinum* complex composed of organisms that produce botulinum neurotoxins (BoNTs). This group of organisms is represented in four separate phylogenetic

Clostridial Diseases of Animals, Second Edition. Edited by Francisco A. Uzal, Bruce A. McClane, Iman Mehdizadeh Gohari, Mauricio A. Navarro, and Michel R. Popoff.

clades (Carter and Peck, 1981; Collins and East, 1998; Smith et al., 2007; Williamson et al., 2016; Smith et al., 2018), with *C. botulinum* type A designated as the type strain located in *C. botulinum* Group 1 (Figure 1.1). Serological methods were first used to distinguish BoNTs more than a century ago with at least seven (A–G) serotypes characterized (Collins and East, 1998; Peck et al., 2017). An

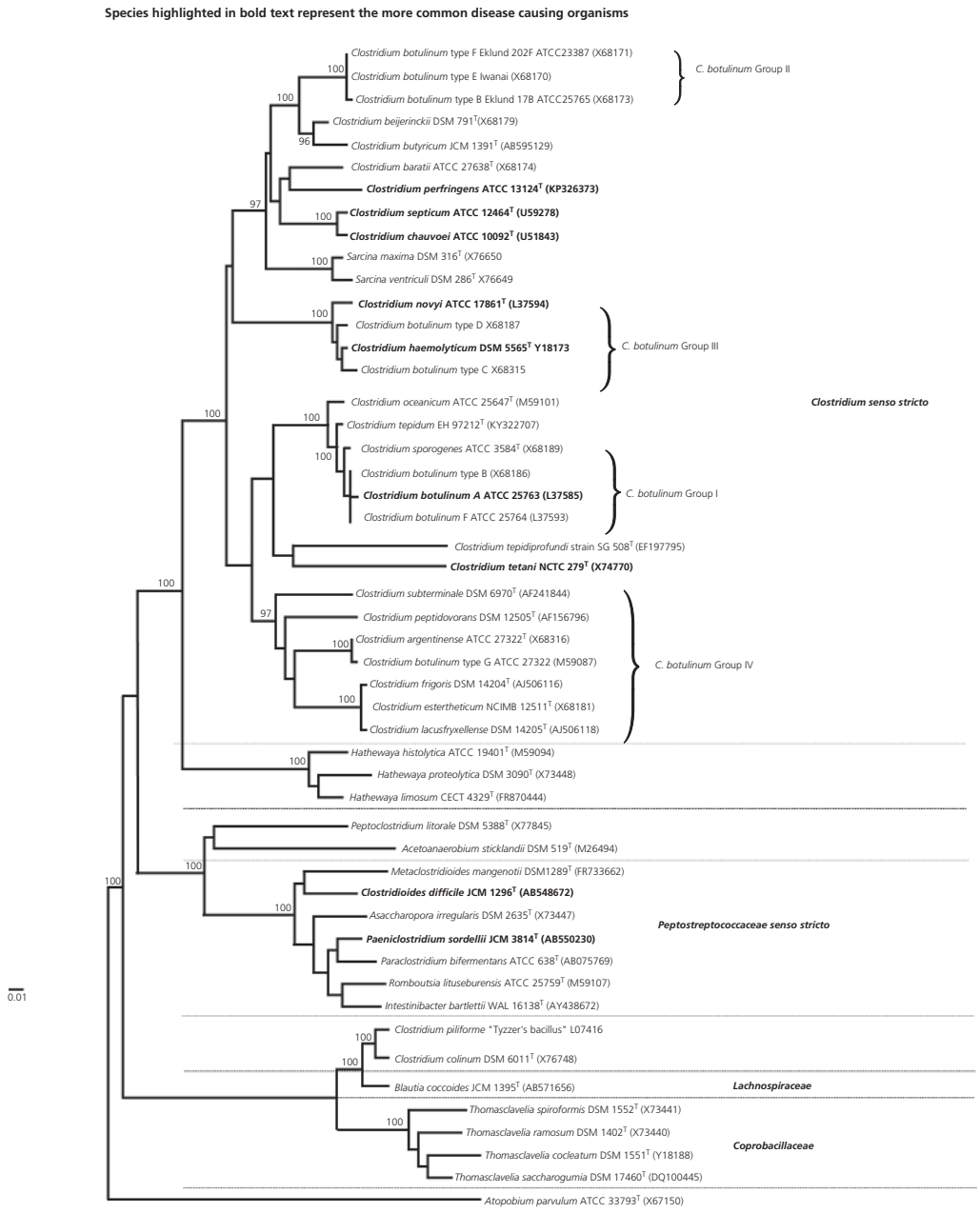

Figure 1.1 Maximum-likelihood phylogenetic tree based on 16S rRNA gene sequences showing the relationships between some *Clostridium* species and relatives. Bootstrap values (%) were obtained with 1000 replicates and are displayed at their branch nodes. *Atopobium parvulum* ATCC 33793^T (X67150) was used as the outgroup. Bar = 1%.

additional BoNT, serotype H, was reported in 2013 but was later designated as a mosaic toxin; it has a hybrid-like structure with regions similar to serotypes A and F and can be neutralized by antibodies against BoNT/A (Barash and Arnon, 2014; Maslanka et al., 2016). An added difficulty is that both *Clostridium baratii* and *C. butyricum* contain strains that produce neurotoxins. It is now clear from a comparative sequence analysis that BoNTs have been laterally transferred between strains, resulting in the four discrete *C. botulinum* groups currently recognized (Collins and East, 1998) (Figure 1.1). Strains comprising *C. botulinum* Groups I and II are primarily responsible for human botulism, *C. botulinum* Group III is responsible for botulism in various animal species, and *C. botulinum* Group IV does not appear to be associated with botulism in humans or animals (Carter and Peck, 1981).

Since the papers of Collins et al. (1994) and Lawson et al. (1993), many former species of *Clostridium* have been reclassified into novel genera, for example, *Blautia* (Liu et al., 2008), *Clostridioides* (Lawson et al., 2016), *Enterocloster* (Haas and Blanchard, 2020), *Hathewaya* (Lawson et al., 2016), *Paraclostridium* (Jyothsna et al., 2016), *Ruminiclostridium* (Zhang et al., 2018), and *Thomasclavelia* (Lawson et al., 2023), to name but a few (Figure 1.1). As the genus currently stands, the List of Prokaryotic Names with Standing in Nomenclature (http://www.bacterio.net) lists 162 validly published names although many more have not been validly published and to date have no standing in the literature.

Although many species of *Clostridium* have been recovered from animal sources (Rainey et al., 2009), only a relatively few may cause disease, including *C. botulinum, Clostridium tetani, Clostridium chauvoei, Clostridium haemolyticum, Clostridium novyi, Clostridium perfringens, Clostridium septicum, Clostridium piliforme, Clostridioides* (formerly *Clostridium*) *difficile, Clostridium spiroforme* (now *Thomasclavelia spiroformis*), and *Paeniclostridium* (formerly *Clostridium*) *sordelli* (Figure 1.1). These diseases will be discussed in detail in subsequent chapters.

The phylogenetic diversity first eluded via 16S rRNA gene sequencing is mirrored with the now routine whole-genome sequencing (WGS); the major branching topologies in phylogenetic/phylogenomic trees are broadly consistent. Indeed, with the genome sequence now required for the description of novel species, two approaches have come to the forefront: digital DNA–DNA hybridization (dDDH) (Meier-Kolthoff et al., 2013) and average nucleotide identity (ANI) (Konstantinidis and Tiedje, 2005). The proposed species boundaries for ANI and dDDH values are 95% and 70%, respectively (Konstantinidis and Tiedje, 2005; Goris et al., 2007; Richter and Rosselló-Móra, 2009; Meier-Kolthoff et al., 2013). A consequence of these more sensitive methods was that the16S rRNA gene similarity threshold value of 97% (Stackebrandt and Goebel, 1994) was raised to 98.2–99% for the delineation of novel species (Konstantinidis and Stackebrandt, 2006; Stackebrandt and Ebers, 2006; Kim et al., 2014). Furthermore, the application of 16S rRNA gene similarities to delineate ranks (Yarza et al., 2014) is now augmented by amino acid identity (AAI), with a threshold value of 65% established to demarcate a genus (Goris et al., 2007; Konstantinidis et al., 2017) alongside a 16S rRNA value of approximately 94% (Yarza et al., 2014). Therefore, based on the above criteria and an examination of the phylogenomic trees in genome databases as exemplified by the Genome Taxonomy Database (https://gtdb.ecogenomic.org) (Parks et al., 2022), it is clear that a major revision of the clostridia is required.

In conclusion, although phylogenetically outside *Clostridium sensu stricto*, many species retain the name "*Clostridium*" and await reclassification, and the genus is considered to be in a state of taxonomic flux (Rainey et al., 2009; Parte et al., 2020). It is clear that additional members of the clostridia and the genus *Clostridium* will be identified, but the relatively small number of organisms that may cause disease is well documented.

References

Barash JR, Arnon SS. A novel strain of *Clostridium botulinum* that produces type B and type H botulinum toxins. *J Infect Dis* 2014;209:183–191.

Carter AT, Peck MW. Genomes, neurotoxins and biology of *Clostridium botulinum* Group I and Group II. *Res Microbiol* 1981;166:303–317.

Collins MD, East AK. Phylogeny and taxonomy of the food-borne pathogen *Clostridium botulinum* and its neurotoxins. *J Appl Microbiol* 1998;84:5–17.

Collins MD, Lawson PA, Willems A, Cordoba JJ, Garayzabal JF, Garcia P, Cai J, Hippe H, Farrow JAE. The phylogeny of the genus *Clostridium*: proposal of five new genera and eleven new species combinations. *Int J Syst Evol Microbiol* 1994;44:812–826.

Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P, Tiedje JM. DNA–DNA hybridization values and their relationship to whole-genome sequence similarities. *Int J Syst Evol Microbiol* 2007;57:81–91.

Gupta RS, Gao B. Phylogenomic analyses of clostridia and identification of novel protein signatures that are specific to the genus *Clostridium sensu stricto* (cluster I). *Int J Syst Evol Microbiol* 2009;59:285–294.

Haas KN, Blanchard JL. Reclassification of the *Clostridium clostridioforme* and *Clostridium sphenoides* clades as *Enterocloster* gen. nov. and *Lacrimispora* gen. nov., including reclassification of 15 taxa. *Int J Syst Evol Microbiol* 2020;70:23–34.

Johnson JL, Francis BS. Taxonomy of the clostridia: ribosomal ribonucleic acid homologies among the species. *J Gen Microbiol* 1975;88:229–244.

Jyothsna TSS, Tushar L, Sasikala CH, Ramana CHV. *Paraclostridium benzoelyticum* gen. nov., sp. nov., isolated from marine sediment and reclassification of *Clostridium bifermentans* as *Paraclostridium bifermentans* comb. nov. Proposal of a new genus *Paeniclostridium* gen. nov. to accommodate *Clostridium sordellii* and *Clostridium ghonii*. *Int J Syst Evol Microbiol* 2016;66:1268–1274.

Kim M, Oh HS, Park SC, Chun J. Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. *Int J Syst Evol Microbiol* 2014;64:1825–1825.

Konstantinidis KT, Stackebrandt E. Defining taxonomic ranks. In: Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E, eds *The Prokaryotes*. Springer, 2006:229–254.

Konstantinidis KT, Tiedje JM. Genomic insights that advance the species definition for prokaryotes. *Proc Natl Acad Sci USA* 2005;102:2567–2572.

Konstantinidis KT, Rosselló-Móra R, Amann R. Uncultivated microbes in need of their own taxonomy. *ISME J* 2017;11:2399–2406.

Lawson PA, Rainey FA. Proposal to restrict the genus *Clostridium* Prazmowski to *Clostridium butyricum* and related species. *Int J Syst Evol Microbiol* 2016;66:1009–1016.

Lawson PA, Llop-Perez P, Hutson RA, Hippe H, Collins MD. Towards a phylogeny of the clostridia based on 16S rRNA sequences. *FEMS Microbiol Lett* 1993;113:87–92.

Lawson PA, Citron DM, Tyrrell KL, Finegold SM. Reclassification of *Clostridium difficile* as *Clostridioides difficile* (Hall and O'Toole 1935) Prévot 1938. *Anaerobe* 2016;40:95–99.

Lawson PA, Perez LS, Sankaranarayanan K. Reclassification of *Clostridium cocleatum, Clostridium ramosum, Clostridium spiroforme* and *Clostridium saccharogumia* as *Thomasclavelia cocleata* gen. nov., comb. nov., *Thomasclavelia ramosa* comb. nov., gen. nov., *Thomasclavelia spiroformis* comb. nov. and *Thomasclavelia saccharogumia* comb. nov. *Int J Syst Evol Microbiol* 2023:73.

Liu C, Finegold SM, Song Y, Lawson PA. Reclassification of *Clostridium coccoides, Ruminococcus hansenii, Ruminococcus hydrogenotrophicus, Ruminococcus luti, Ruminococcus productus* and *Ruminococcus schinkii* as *Blautia coccoides* gen. nov., comb. nov., *Blautia hansenii* comb. nov., *Blautia*

hydrogenotrophica comb. nov., *Blautia luti* comb. nov., *Blautia producta* comb. nov., *Blautia schinkii* comb. nov. and description of *Blautia wexlerae* sp. nov., isolated from human faeces. *Int J Syst Evol Microbiol* 2008;58:1896–1902.

Ludwig W, Weizenegger M, Kilpper-Balz R, Schleifer KH. Phylogenetic relationships of anaerobic Streptococci. *Int J Syst Evol Microbiol* 1988;38:15–18.

Maslanka SE, Lúquez C, Dykes JK, Tepp WH, Pier CL, Pellett S, Raphael BH, Kalb SR, Barr JR, Rao A, et al. A Novel botulinum neurotoxin, previously reported as serotype H, has a hybrid-like structure with regions of similarity to the structures of serotypes A and F and is neutralized with serotype A antitoxin. *J Infect Dis* 2016;213:379–385.

Meier-Kolthoff JP, Auch AF, Klenk HP, Göker M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinf* 2013;14:60.

Parks DH, Chuvochina M, Rink C, Mussig AJ, Chaumeil P-A, Hugenholtz P. GTDB: an ongoing census of bacterial and archaeal diversity through a phylogenetically consistent, rank normalized and complete genome-based taxonomy. *Nucleic Acids Res* 2022;50:D785–D794.

Parte AC, Carbasse JS, Meier-Kolthoff JP, Reimer LC, Göker M. List of Prokaryotic names with Standing in Nomenclature (LPSN) moves to the DSMZ. *Int J Syst Evol Microbiol* 2020;70:5607–5612.

Peck MW, Smith TJ, Anniballi F, Austin JW, Bano L, Bradshaw M, Cuervo P, Cheng LW, Derman Y, Dorner BG, et al. Historical perspectives and guidelines for botulinum neurotoxin subtype nomenclature. *Toxins* 2017;9:38.

Prazmowski A. Untersuchung über die entwickelungsgeschichte und fermentwirking einiger bacterien-arten. Inaugural Dissertation. 1880, Hugo Voigt, Leipzig, Germany.

Rainey F, Hollen BJ, Small A. Genus I. *Clostridium*. In: Vos PD, Garrity GM, Jones D, Krieg NR, Ludwig W, Rainey FA, Schleifer KH, Whitman WB, eds *Bergey's Manual of Systematic Bacteriology*. 2nd ed. New York: Springer, 2009:738–828.

Richter M, Rosselló-Móra R. Shifting the genomic gold standard for the prokaryotic species definition. *Proc Natl Acad Sci USA* 2009;106:19126–19131.

Smith TJ, Hill KK, Foley BT, Detter JC, Munk AC, Bruce DC, Doggett NA, Smith LA, Marks JD, Xie G, et al. Analysis of the neurotoxin complex genes in *Clostridium botulinum* A1-A4 and B1 strains: BoNT/A3, /Ba4 and/B1 clusters are located within plasmids. *PLoS One* 2007;2:e1271.

Smith T, Williamson CHD, Hill K, Sahl J, Keim P. Botulinum neurotoxin-producing bacteria. isn't it time that we called a species a species? *MBio* 2018;9:e01469–e01418.

Stackebrandt E, Ebers J. Taxonomic parameters revisited: tarnished gold standards. *Microbiol Today* 2006;33:152–155.

Stackebrandt E, Goebel BM. Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Evol Microbiol* 1994;44:846–849.

Stackebrandt E, Kramer I, Swiderski J, Hippe H. Phylogenetic basis for a taxonomic dissection of the genus *Clostridium*. *FEMS Immunol Med Microbiol* 1999;24:253–258.

Tanner RS, Stackebrandt E, Fox GE, Woese CR. A phylogenetic analysis of *Acetobacterium woodii*, *Clostridium barkeri*, *Clostridium butyricum*, *Clostridium lituseburense*, *Uubacterium limosum*, and *Eubacterium tenue*. *Curr Microbiol* 1981;5:35–38.

Tindall BJ. Priority of the genus name *Clostridium* Prazmowski 1880 (Approved Lists 1980) vs *Sarcina* Goodsir 1842 (Approved Lists 1980) and the creation of the illegitimate combinations *Clostridium maximum* (Lindner 1888) Lawson and Rainey 2016 and *Clostridium ventriculi* (Goodsir 1842) Lawson and Rainey 2016 that may not be used. *Int J Syst Evol Microbiol* 2016;66:4890–4894.

Wiegel J, Tanner R, Rainey FA. *The* Prokaryotes*, Volume 4: Bacteria:* Firmicutes, Cyanobacteria. Springer US, 2006.

Williamson CHD, Sahl JW, Smith TJ, Xie G, Foley BT, Smith LA, Fernández RA, Lindström M, Korkeala H, Keim P, et al. Comparative genomic analyses reveal broad diversity in botulinum-toxin-producing clostridia. *BMC Genomics* 2016;17:180.

Yarza P, Yilmaz P, Pruesse E, Glöckner FO, Ludwig W, Schleifer KH, Whitman WB, Euzéby J, Amann R, Rosselló-Móra R. Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences. *Nat Rev Microbiol* 2014;12:635–645.

Yutin N, Galperin MY. A genomic update on clostridial phylogeny: gram-negative spore formers and other misplaced clostridia. *Environ Microbiol* 2013;15:2631–2641.

Zhang X, Tu B, Dai L, Lawson PA, Zheng Z, Liu LY, Deng Y, Zhang H, Cheng L. *Petroclostridium xylanilyticum* gen. nov., sp. nov., a xylan-degrading bacterium isolated from an oilfield, and reclassification of clostridial cluster III members into four novel genera in a new *Hungateiclostridiaceae* fam. nov. *Int J Syst Evol Microbiol* 2018;68:3197–3211.