

## *Clostridium hastiforme* is a later synonym of *Tissierella praeacuta*

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The previously proposed species *Clostridium hastiforme* and *Tissierella praeacuta* appear to be similar from their published descriptions. Accordingly, the aim of the current study was to perform phenotypic and genetic analyses of the type strains of both species, in order to clarify their taxonomic positions. The type strains of *C. hastiforme* (DSM 5675<sup>T</sup>) and *T. praeacuta* (NCTC 11158<sup>T</sup>) exhibited identical biochemical profiles and their 16S rRNA gene sequences displayed 99.9% similarity. DNA–DNA hybridization was also estimated to be 96.5%. Thus, it was concluded that *C. hastiforme* and *T. praeacuta* are synonyms, where *T. praeacuta* has priority. An emended description of the genus *Tissierella* is also given.

*Clostridium hastiforme* MacLennan 1939<sup>AL</sup> (type strain, ATCC 33268<sup>T</sup>=VPI 12193<sup>T</sup>=DSM 5675<sup>T</sup>) was suggested for the organism that was described by Cunningham as bacillus 4a, which is typically a slender, rod-shaped organism with rounded ends, 0.3–0.6 × 2–6 µm in size (MacLennan, 1939). In addition, this terminally spored anaerobe does not ferment carbohydrates and is sluggishly motile, due to numerous peritrichous flagella. It has also been reported that *C. hastiforme* is similar to *Clostridium subterminale*, except that the spores are terminal and no hydrogen is produced (Cato *et al.*, 1982; Holdeman *et al.*, 1991). Cato *et al.* (1982) used protein electrophoresis as a tool to distinguish these two species. Suen *et al.* (1988) reported the DNA–DNA relatedness of strains that were identified initially as *C. hastiforme* and divided several strains into *Clostridium argentinense*, *Clostridium botulinum*, *Clostridium sporogenes*, *C. subterminale* and *C. hastiforme*. A corresponding phenotypic differentiation study between *C. hastiforme* and *C. subterminale* was also reported by Niel *et al.* (1989), based on analysing the reduced-pressure headspaces from 168 h cultures with GC, which showed that strain ATCC 25772 (=DSM 1786), previously identified as *C. hastiforme*, was neither a *C. hastiforme* strain nor a *C. subterminale* strain. Collins *et al.* (1994) reported that *C. argentinense*, *C. botulinum*, *C. sporogenes* and *C. subterminale* all belong to cluster I of the clostridia, whereas *C. hastiforme* is within group XII, indicating that *C. hastiforme* is not a 'real' *Clostridium* species.

Farrow *et al.* (1995) indicated that the 16S rRNA gene

sequence of Gram-negative, non-spore-forming *Tissierella praeacuta* (NCTC 11158<sup>T</sup>) was almost identical to the sequence of Gram-positive, spore-forming *C. hastiforme*. They also compared the 16S rDNA sequences of both strains held in other collections to eliminate any possibility of incorrect strain designation or culture contamination. In current bacterial systematics, a bacterial species is defined as a group of strains that exhibit ≥70% DNA–DNA relatedness at an optimal incubation temperature (Wayne *et al.*, 1987), together with phylogenetic inference based on 16S rDNA sequence comparison. Therefore, the aims of the present study were to determine the levels of DNA–DNA relatedness between *C. hastiforme* and *T. praeacuta* and to determine whether the current taxonomic status of each species is correct.

The type strains of *T. praeacuta* (ATCC 25539<sup>T</sup>=NCTC 11158<sup>T</sup>=NCIMB 703038<sup>T</sup>) and *C. hastiforme* (ATCC 33268<sup>T</sup>=NCTC 11832<sup>T</sup>=DSM 5675<sup>T</sup>) were obtained from the NCIMB (National Collections of Industrial Food and Marine Bacteria, London, UK) and DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany), respectively. The strains were cultured as recommended by the respective culture collections. Shape and size of living and stained cells were determined by light microscopy. Gram reaction was determined by using a Gram-stain kit (Difco), according to the manufacturer's recommended protocol. To distinguish false-negative Gram-staining, a KOH test was performed in parallel with the Gram-stain reaction, based on mixing a visible amount of growth from a colony in a loopful of 3% aqueous KOH on a glass slide (Powers, 1995). Enzyme profiles were generated for each strain by using API

ZYM kits (bioMérieux) according to the manufacturer's instructions. Cellular fatty acid composition was determined by using the method of Suzuki & Komagata (1983). Chromosomal DNA was isolated and purified according to a method described previously (Yoon *et al.*, 1996). DNA-DNA hybridization was carried out according to the method of Ezaki *et al.* (1989). 16S rDNA was amplified by PCR using two universal primers, as described previously (Yoon *et al.*, 1998). The PCR product was purified by using a QIAquick PCR purification kit (Qiagen). The purified 16S rDNA was then sequenced by using an ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems), as recommended by the manufacturer. Purified sequencing reaction mixtures were electrophoresed automatically by using an Applied Biosystems model 310 automatic DNA sequencer. 16S rDNA sequence alignment and phylogenetic tree construction were conducted by using CLUSTAL X software (Thompson *et al.*, 1997).

DNA-DNA hybridization was performed to determine genomic relatedness between the type strains of *T. praeacuta* and *C. hastiforme*; as their DNA-DNA relatedness was 96.5%, they seem to be members of the same genomic species (Wayne *et al.*, 1987). *T. praeacuta* and *C. hastiforme* were also found to be indistinguishable based on their biochemical characteristics. Both type strains exhibited approximately the same enzymic activity of esterase (C4), leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase by using an API ZYM test kit. Contrary to a previous report, *T. praeacuta* tested Gram-positive; the KOH test also confirmed that *T. praeacuta* and *C. hastiforme* were both Gram-positive species. As such, it is possible that the previous result for *T. praeacuta* was false-negative Gram-staining that occurred with a long incubation time (Powers, 1995). The major cellular fatty acids for both strains were iso-C<sub>15:0</sub> (22.3–23.4% total cellular fatty acids) and C<sub>16:0</sub> (12.5–12.7% total cellular fatty acids). Thus, it was evident from the polyphasic taxonomic study that the type strains of *T. praeacuta* and *C. hastiforme* belong to the same species.

The species *T. praeacuta* was originally isolated by Tissier (1908). After a long, unsettled taxonomic history (Farrow *et al.*, 1995), Collins & Shah (1986) reclassified *Bacteroides praeacutus* Tissier in a novel genus, *Tissierella*. So far, three species have been assigned to the genus *Tissierella*, i.e. *T. praeacuta*, *Tissierella creatinini* and *Tissierella creatinophila*. On the basis of the phylogenetic tree constructed by Harms *et al.* (1998), *T. praeacuta* and *C. hastiforme* were related most closely to *T. creatinini* and *T. creatinophila*, and all tested Gram-positive. Accordingly, it is proposed that *C. hastiforme* MacLennan 1939<sup>AL</sup> is a later synonym of *T. praeacuta*. Therefore, an emended description of the genus *Tissierella* is given below.

### Emended description of the genus *Tissierella*

The characteristics of the genus are as described by Collins & Shah (1986), except that cells are all Gram-positive and D-ornithine and meso-diaminopimelic acid are present in

the peptidoglycan. Some species possess malate dehydrogenase and glutamate dehydrogenase activity.

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