Proposal to unify *Clostridium orbiscindens* Winter *et al.* 1991 and *Eubacterium plautii* (Séguin 1928) Hofstad and Aasjord 1982, with description of *Flavonifractor plautii* gen. nov., comb. nov., and reassignment of *Bacteroides capillosus* to *Pseudoflavonifractor capillosus* gen. nov., comb. nov.

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We isolated several strains from various clinical samples (five samples of blood, four of intra-abdominal pus and one of infected soft tissue) that were anaerobic, motile or non-motile and Gram-positive rods. Some of the strains formed spores. Phylogenetic analysis of the 16S rRNA gene sequence showed that these organisms could be placed within clostridial cluster IV as defined by Collins *et al.* [(1994). *Int J Syst Bacteriol* **44**, 812–826] and shared more than 99 % sequence similarity with *Clostridium orbiscindens* DSM 6740^T and *Eubacterium plautii* DSM 4000^T. Together, they formed a distinct cluster, with *Bacteroides capillosus* ATCC 29799^T branching off from this line of descent with sequence similarities of 97.1–97.4 %. The next nearest neighbours of these organisms were *Clostridium viride*, *Oscillibacter valericigenes*, *Papillibacter cinnamivorans* and *Sporobacter termitidis*, with sequence similarities to the respective type strains of 93.1–93.4, 91.2–91.4, 89.8–90 and 88.7–89.3 %. On the basis of biochemical properties, phylogenetic position, DNA G + C content and DNA–DNA hybridization, it is proposed to unify *Clostridium orbiscindens* and *Eubacterium plautii* in a new genus as *Flavonifractor plautii* gen. nov., comb. nov., with the type strain Prévot S1^T (=ATCC 29863^T =VPI 0310^T =DSM 4000^T), and to reassign *Bacteroides capillosus* to *Pseudoflavonifractor capillosus* gen. nov., comb. nov., with the type strain CCUG 15402A^T (=ATCC 29799^T =VPI R2-29-1^T).

The genus *Clostridium* has undergone extensive revision in recent years and has been subdivided into 19 clusters on the basis of 16S rRNA gene sequence analysis. Cluster I, containing the type species *Clostridium butyricum*, and clusters XI and XIVa include the majority of pathogenic species (Collins *et al.*, 1994). The members of cluster IV

exhibit a combination of *Clostridium*- and non-*Clostridium*-type properties and a broad range of chromosomal DNA G + C content (e.g. *Clostridium sporosphaeroides*, a Gram-positive spore-forming rod, and *Bacteroides capillosus*, a Gram-negative non-sporulating species, with 27 and 60 mol% G + C, respectively). This cluster also includes *Clostridium orbiscindens* and *Eubacterium plautii*, species which are asaccharolytic and produce acetate and butyrate. The name *Clostridium orbiscindens* was proposed for an anaerobic bacillus isolated from human faeces and capable of cleaving the C3–C4 bond of quercetin (Winter *et al.*, 1991) and other flavonoids (Schoefer *et al.*, 2003). This organism is motile, peritrichous, Gram-variable and forms spores.

Eubacterium plautii (type and only known strain Prévot S1^T =DSM 4000^T =ATCC 29863^T =VPI 0310^T) was

†Deceased.

Abbreviation: DPH, 1,6-diphenyl-1,3,5-hexatriene.

The GenBank/EMBL/DBJ accession numbers for the 16S rRNA gene sequences of strains AIP 162.06, AIP 165.06, AIP 028.07, AIP 201.04 and AIP 029.07 are EU541435–EU541439, respectively.

Results of DPH fluorescence-quenching tests and PCR amplification of *spoOA* fragments are available as supplementary material with the online version of this paper.

originally described by Séguin (1928) as *Fusobacterium plauti* (sic), a motile, Gram-negative, non-spore-forming bacillus. On the basis of electron microscope examination, Hofstad & Aasjord (1982) showed that *Fusobacterium plautii* is a non-motile, Gram-positive organism and transferred this species to the genus *Eubacterium*. However, in *Bergey's Manual of Systematic Bacteriology*, this organism was described as motile and Gram-negative with very occasional, weakly Gram-positive areas (Moore & Holdeman Moore, 1986). The ability of *Eubacterium plautii* to cleave quercetin is not known. Thus, the characteristics and taxonomic status of this species need to be clarified.

The isolation from various clinical samples (five samples of blood, four of intra-abdominal pus and one of infected soft tissue) of several strains with 16S rRNA gene sequences that were phylogenetically closely related to those of *Clostridium orbiscindens* DSM 6740^T, *Eubacterium plautii* DSM 4000^T and *Bacteroides capillosus* ATCC 29799^T prompted us to re-evaluate the phylogenetic position of these three species.

Selected isolates AIP 201.04, AIP 162.06 (=LBN 202), AIP 165.06 (=LBN 204), AIP 028.07 (=LBN 205) and AIP 029.07 (=LBN 206) and the three type strains were maintained in trypticase-glucose-yeast extract (TGY) medium (Carlier *et al.*, 2002) under anaerobic conditions at 37 °C for 24 h in an anaerobic jar containing 5 % H₂, 5 % CO₂ and 90 % N₂ (by vol.). 16S rRNA gene sequences were determined for each strain as described previously (Carlier *et al.*, 2004). Alignments were done using CLUSTAL W (Thompson *et al.*, 1994). A distance matrix was calculated by using DNADIST with the Jukes–Cantor parameter (Jukes & Cantor, 1969). Phylogenetic analysis established that these isolates were part of clostridial cluster IV, as defined by Collins *et al.* (1994), and were closely related to *Clostridium orbiscindens* DSM 6740^T and *Eubacterium plautii* DSM 4000^T, with 99.3–100 % sequence similarity. Together, the latter two type strains and the clinical isolates formed a distinct cluster, with *Bacteroides capillosus* ATCC 29799^T branching off from this line of descent with sequence similarities of 97.1–97.4 % (Fig. 1).

The next nearest neighbours of this cluster were *Clostridium viride* DSM 6836^T, *Oscillibacter valericigenes* Sjm18-20^T, *Papillibacter cinnamivorans* CIN1^T and *Sporobacter termitidis* SYR^T, with respective 16S rRNA gene sequence similarities of approximately 93, 91, 90 and 89 %.

The sequences of *Clostridium orbiscindens* DSM 6740^T and *Eubacterium plautii* DSM 4000^T shared 99.7 % similarity. This high similarity suggested that these species may be identical, notwithstanding some physiological differences. To verify this hypothesis, the G + C content of the DNA for each strain was determined by HPLC as described by Mesbah *et al.* (1989) and DNA–DNA hybridization was performed. DNA was extracted using a French pressure cell (Thermo Spectronic) and purified by chromatography on hydroxyapatite as described by Cashion *et al.* (1977). DNA–DNA hybridization was carried out using the method described by De Ley *et al.* (1970), modified according to Huß *et al.* (1983), using a model Cary 100 Bio UV/Vis spectrometer equipped with a Peltier-thermostatted multicell changer and a temperature controller with *in-situ* temperature probe (Varian). The two type strains shared 72.6–76.9 % DNA–DNA reassociation. The DNA base composition for *Eubacterium plautii* DSM 4000^T and *Clostridium orbiscindens* DSM 6740^T was 61.6 and 58.0 mol% G + C, respectively. Thus, according to the established molecular criteria for species-level relatedness, i.e. strains whose DNAs are at least 70 % related under optimal conditions and whose 16S rRNA gene sequences show less than 3 % divergence (Wayne *et al.*, 1987; Stackebrandt *et al.*, 2002), these strains could be regarded as belonging to a single species. Nevertheless, some phenotypic properties were further examined.

Degradation of quercetin

Bacterial degradation of quercetin was checked by the fluorescence-quenching test according to Schoefer *et al.* (2001) and modified as follows. Aliquots (150 µl) of stock solutions containing 1 mM 1,6-diphenyl-1,3,5-hexatriene (DPH) and 20 mM quercetin were added in loosely covered 180 × 8 mm tubes. The tubes contained 5 ml

Fig. 1. Dendrogram indicating the phylogenetic positions of *Eubacterium plautii*, *Clostridium orbiscindens* and *Bacteroides capillosus* and closely related species among the clostridial groups. Evolutionary distances (based on 1400 unambiguous nucleotides) were computed by using the neighbour-joining method and bootstrap values calculated from 100 resamplings (Felsenstein, 1993). The sequence of *Clostridium butyricum* VPI 3266^T was used as an outgroup. Bar, 5 % sequence divergence.

TGY agar and were inoculated with 0.5 ml of an overnight growing culture of each strain and incubated at 37 °C for 48 h. For detection of fluorescence, the tubes were examined under a UV lamp (wavelength 365 nm). Quercetin was degraded by all strains, including *Eubacterium plautii* DSM 4000^T, as demonstrated by the strong fluorescence observed (Supplementary Fig. S1, available in IJSEM Online). On the other hand, *Escherichia coli* K-12, which does not degrade quercetin, and the negative control (without bacteria) did not affect the quenching of DPH fluorescence. Thus, *Eubacterium plautii* DSM 4000^T, like *Clostridium orbiscindens* DSM 6740^T, is able to degrade quercetin.

Detection of the sporulation-specific gene *spo0A*

Spore formation was not observed with some clinical isolates or the type strain of *Eubacterium plautii*, DSM 4000^T. However, the inability to form spores is not an effective criterion for the determination of taxonomic status (Cato & Stackebrandt, 1989). Lack of spore formation may result from defective strains; such asporogenic strains must have the majority of sporulation-specific genes (Onyenwoke *et al.*, 2004). Brill & Wiegel (1997) described a PCR assay to distinguish asporogenic from non-spore-forming bacteria by employing probes directed against three representative and specific sporulation genes. Thus, we chose to investigate the presence of the sporulation-specific gene *spo0A* for the strains for which endospores have not been observed. The PCR assay was used with the following modifications: DNA was extracted using the InstaGene Matrix kit (Bio-Rad) as recommended by the manufacturer. PCRs were performed using 1.5 mM MgCl₂ instead of 0.125–0.375 mM. Our results showed that the isolates for which endospores have not been observed, as well as *Eubacterium plautii* DSM 4000^T, exhibited the sporulation-specific gene *spo0A* (Supplementary Fig. S2).

Cellular fatty acid composition

Cellular fatty acid composition was analysed by gas chromatography according to Veys *et al.* (1989) on a fused-silica capillary column (25 m × 0.25 mm i.d.) coated with 5 % methyl phenyl silicone. The cellular fatty acid compositions of *Eubacterium plautii* DSM 4000^T, *Clostridium orbiscindens* DSM 6740^T and the clinical strains were very similar. All strains contained an unknown compound that eluted between C_{12:0} and C_{13:0} (6.5–8.2 %), C_{14:0} (34–36.9 %), a second unknown compound that eluted between anteiso-C_{15:0} and C_{15:0} (22.6–28.3 %), C_{16:0} (14.4–18.1 %), C_{17:1ω8c} (3.2–5.2 %) and C_{18:0} (3.1–7.3 %) as the major components. Minor fatty acids included iso-C_{15:0} (1.6–2.2 %) and C_{16:1ω9c} (0–3.2 %).

Susceptibility to glycopeptides

Determination of MICs of vancomycin and teicoplanin according to CLSI standards (CLSI, 2007) showed that all

the clinical isolates as well as the type strains of *Eubacterium plautii* and *Clostridium orbiscindens* were susceptible to teicoplanin (MICs 0.25–0.5 µg ml⁻¹) and exhibited reduced susceptibility to vancomycin (MICs 4–8 µg ml⁻¹). Known glycopeptide-resistance genes were not detected using a PCR assay (Domingo *et al.*, 2005; Fines *et al.*, 1999; McKessar *et al.*, 2000; Mory *et al.*, 1998; Perichon *et al.*, 1997; Rippere *et al.*, 1998).

In conclusion, the phenotypic and genotypic characteristics of *Eubacterium plautii* and *Clostridium orbiscindens* were very similar and strongly suggest that the two species represent the same taxon. The only differences observed were the inability of *Eubacterium plautii* DSM 4000^T to produce spores and the motility of *Clostridium orbiscindens* DSM 6740^T. These differences were also observed among the clinical isolates and could simply represent strain variations within the species.

Accordingly, we propose that *Clostridium orbiscindens* Winter *et al.* 1991 and *Eubacterium plautii* (Séguin 1928) Hofstad and Aasjord 1982 be unified and reclassified in a new genus, *Flavonifractor* gen. nov., with *Flavonifractor plautii* comb. nov. as the type species. Since our clinical isolates share identical properties with *Eubacterium plautii* and *Clostridium orbiscindens*, they should be regarded as belonging to the species *Flavonifractor plautii*.

Taxonomic status of *Bacteroides capillosus* and *Clostridium viride*

Bacteroides capillosus (Tissier 1908) Kelly 1957 is a non-motile, Gram-negative, non-spore-forming bacillus. Because the original type strain was lost, Cato *et al.* (1979) selected strain ATCC 29799^T as the neotype strain of the species. However, it is evident from tree branching patterns, cellular fatty acids and 5S rRNA sequence studies (Van den Eynde *et al.*, 1989) that *Bacteroides capillosus* ATCC 29799^T is phenotypically and phylogenetically distinct from the genus *Bacteroides* as defined by Shah & Collins (1989). In addition, the G + C content of the DNA of *Bacteroides capillosus* (60 mol%) differs from those of members of the genus *Bacteroides*, which range between 39 and 48 mol% (Shah & Collins, 1989). Consequently, *Bacteroides capillosus* cannot be maintained in the genus *Bacteroides*, and we propose its placement within clostridial cluster IV. The very high level of sequence similarity (>97 %) together with biochemical and physiological properties and similar DNA base compositions (58 and 61.6 mol% G + C) showed that *Bacteroides capillosus* is closely related to the new genus *Flavonifractor* (Table 1). However, we tested strain CCUG 15402A^T (=ATCC 29799^T) for the presence of the sporulation-specific gene *spo0A* and for its potential to degrade quercetin. Although this strain has the gene *spo0A*, it is unable to degrade quercetin (data not shown). This feature and other major differences such as Gram staining and metabolic end products prevent its placement within this genus. In this context, we propose that *Bacteroides capillosus* be placed in

Table 1. Comparison of biochemical and physiological properties of *Clostridium orbiscindens*, *Eubacterium plautii*, *Bacteroides capillosus* and *Clostridium viride*

Data were obtained from this study (*Clostridium orbiscindens* and *Eubacterium plautii*), Cato *et al.* (1979) and Van den Eynde *et al.* (1989) (*Bacteroides capillosus*) and Buckel *et al.* (1994) (*Clostridium viride*). v, Variable; –, negative; +, positive; NA, no data available. All species are non-fermentative.

Property	<i>C. orbiscindens</i>	<i>E. plautii</i>	<i>B. capillosus</i>	<i>C. viride</i>
Gram staining	v	v	–	+
G + C content (mol%)*	58.0	61.6	60	41.5
Metabolic end products†	A, B	A, B	A, S	A, P, B, V
Major cellular fatty acids	C _{14:0} , C _{16:0}	C _{14:0} , C _{16:0}	C _{14:0} , C _{16:0}	NA

*Data for the type strains.

†A, Acetic acid; B, butyric acid; P, propionic acid; S, succinic acid; V, valeric acid.

a new genus, *Pseudoflavonifractor* gen. nov., as *Pseudoflavonifractor capillosus* comb. nov.

Phylogenetically, *Clostridium viride* (Buckel *et al.*, 1994) clearly belongs to cluster IV. However, with sequence similarities of 93.1–93.4%, it is too remote from *Flavonifractor* or *Pseudoflavonifractor* to be affiliated with either of these genera. This is supported by a high bootstrap value (Fig. 1). Moreover, Gram staining, DNA base composition (41.5 mol% G + C) and metabolic end products support the view that this species represents another genus (Table 1). Further investigations are needed to define this probable new genus.

Description of *Flavonifractor* gen. nov.

Flavonifractor (Fla.vo.ni.frac'tor. N.L. n. *flavonum* flavone; L. masc. n. *fractor* breaker; N.L. masc. n. *Flavonifractor* flavone-breaker).

Strictly anaerobic bacilli. Cells are Gram-variable after staining. Motility is variable. Spores may or may not be produced, but the sporulation-specific gene *spo0A* is present. Asaccharolytic; glucose, fructose and ribose can be fermented weakly. Able to cleave quercetin and other flavonoids. Exhibit reduced susceptibility to vancomycin. Major metabolic end products are acetic and butyric acids in TGY broth. Major cellular fatty acids are C_{14:0} and C_{16:0}. Phylogenetically, the genus represents a distinct lineage in clostridial cluster IV. The type species is *Flavonifractor plautii*.

Description of *Flavonifractor plautii* comb. nov.

Flavonifractor plautii (plau'ti.i. N.L. gen. masc. n. *plautii* of Plaut, named for H. C. Plaut, the bacteriologist who first described this organism).

Basonym: *Fusobacterium plautii* Séguin 1928.

Other synonyms: *Eubacterium plautii* (Séguin 1928) Hofstad and Aasjord 1982; *Clostridium orbiscindens* Winter *et al.* 1991.

The description combines characteristics of *Eubacterium plautii* as determined by Séguin (1928) and emended by Hofstad & Aasjord (1982) and those of *Clostridium orbiscindens* (Winter *et al.*, 1991). Displays the following characteristics in addition to those given in the genus description. Straight or slightly curved rods, 2–10 µm long, and occur singly or in pairs. Some cells are fusiform. Colonies are minute, circular, convex, grey or white, smooth, non-haemolytic on sheep blood agar. Nitrate is not reduced. Production of indole and H₂S is variable. Gelatin and meat are not digested. Lecithinase is not produced. The genomic DNA G + C content is 58–61.6 mol% (as determined by HPLC). The species includes strains previously classified as *Clostridium orbiscindens*.

The type strain is DSM 4000^T (=ATCC 29863^T =VPI 0310^T =Prévot S1^T). Strains have been isolated from the normal faecal flora, blood, intra-abdominal pus and infected soft tissues in humans.

Description of *Pseudoflavonifractor* gen. nov.

Pseudoflavonifractor (Pseu.do fla.vo.ni.frac'tor. Gr. adj. *pseudes* false; N.L. masc. n. *Flavonifractor* a bacterial genus name; N.L. masc. n. *Pseudoflavonifractor* a false *Flavonifractor*).

Strictly anaerobic, Gram-negative, non-motile, non-spore-forming bacilli. Phylogenetically, the genus represents a distinct lineage in clostridial cluster IV. The type species is *Pseudoflavonifractor capillosus*.

Description of *Pseudoflavonifractor capillosus* comb. nov.

Pseudoflavonifractor capillosus (ca.pil.lo'sus. L. masc. adj. *capillosus* full of hair, very hairy).

Basonym: *Bacteroides capillosus* (Tissier 1908) Kelly 1957.

The phenotypic and morphological characters are as described by Cato *et al.* (1979) for *Bacteroides capillosus*. Displays the following properties in addition to those described for the genus. Spores are not formed but the

sporulation-specific gene *spo0A* is present. Straight or curved rods, 1.6–7.1 µm long, predominantly regular in shape, arranged singly, in pairs or in short chains. Filaments with tapered ends are observed. Surface colonies on 48-h-old, anaerobic blood agar are pinpoint to 0.5 mm in diameter, circular, slightly erose, colourless, translucent and slightly peaked. Growth is stimulated by the addition of approx. 0.02 % Tween 80 but is inhibited by 20 % bile. Good growth is observed at 37 and 45 °C. Asaccharolytic; glucose, cellobiose, fructose, galactose, lactose, maltose, mannose, starch and sucrose can be fermented weakly. Aesculin is hydrolysed. Nitrate is not reduced. Indole is not produced. H₂S is usually not produced. Gelatin is usually not digested. Lecithinase and lipase are not produced. Unable to cleave quercetin. Acetic and succinic acids are detected after culture in TGY broth. The major cellular fatty acids are C_{14:0} and C_{16:0}. The G+C content of the DNA of the type strain is 60 mol%.

The type strain ATCC 29799^T (=CCUG 15402A^T =VPI R2-29-1^T) was isolated from human faeces.

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