International Journal of Systematic and Evolutionary Microbiology (2000), **50**, 1595–1599 Printed in Great Britain

NOTE

Catenibacterium mitsuokai gen. nov., sp. nov., a Gram-positive anaerobic bacterium isolated from human faeces

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Six strains of *Eubacterium*-like strains from human faeces were characterized by biochemical tests and analysis of cell wall peptidoglycan type and 16S rRNA. They were members of the *Clostridium* subphylum and have a specific phylogenetic association with *Lactobacillus catenaformis* and *Lactobacillus vitulinus*. These organisms resembled *L. vitulinus* in possessing the same A1γ type of murein, but they showed different fermentation end-products. On the basis of a 16S rDNA sequence divergence of greater than 8% from *L. vitulinus* as well as phenotypic characteristics, a new genus, *Catenibacterium*, with one species (*Catenibacterium mitsuokai*), is proposed for six strains. The type strain of *C. mitsuokai* is JCM 10609^T.

Keywords: *Catenibacterium mitsuokai*, 16S rDNA, cell wall

Intestinal microflora in humans and animals consist of a variety of micro-organisms, including anaerobes and aerobes. Species of the genus *Eubacterium*, which are anaerobic, Gram-positive, non-sporing rods, are among the predominant micro-organisms of intestinal microflora.

The definition of the genus *Eubacterium* was unclear and all species that were anaerobic, Gram-positive, non-sporulating, rod-shaped bacteria and which were not members of the genera *Propionibacterium* or *Bifidobacterium* were included in the genus *Eubacterium* (Lewis & Sutter, 1981; Moore & Holdeman-Moore, 1986). The genus is defined by default, so it has, over the years, acted as a repository for a large number of phenotypically diverse species (Andreesen, 1992). Moreover, this genus is probably not phylogenetically homogeneous and contains species from many different phylogenetic groups. In particular, the wide range of genomic DNA base ratios indicates that the genus includes organisms that are phylogenetically unrelated. Recently, some of the species originally assigned to the genus *Eubacterium* have been transferred to other genera or new genera (Kageyama *et al*., 1999a, b; Wade *et al*., 1999; Ludwig *et al*., 1992; Willems & Collins, 1996).

The six strains [RCA14-19 (JCM 10606), RCA14-21 (JCM 10607), RCA14-33 (JCM 10608), RCA14-39^T (JCM 10609^T), RCA14-45 (JCM 10610) and RCA14-50 (JCM 10611)], used in this study were isolated from Papua New Guinea highlanders' faeces in 1982. All bacterial strains were cultivated for 2 d at 37 °C on EG agar (pre-mixed EG agar, pH 7·7, containing 5% horse blood, 3 g beef extract, 5 g yeast extract, 10 g peptone, 1·5 g glucose, 0·5 g L-cysteine.HCl, 0·2 g L-cystine, 4 g Na_2HPO_4, 0·5 g soluble starch, 0·5 g Tween 80, 0·5 g silicone and 15 g agar in 1000 ml; Eiken Chemical) in an anaerobic jar with 100% CO_2.

Carbohydrate fermentation, enzymic reactions and analysis of end-products in 1% glucose broth (PYFG broth) were examined (Holdeman *et al*., 1977; Kaneuchi *et al*., 1976). PYFG broth contained 10 g Trypticase (BBL), 10 g yeast extract (Difco), 0·5 g L-cysteine.HCl (Sigma) and 40 ml salt solution (pH 7·6; containing, per litre, 0·2 g $CaCl_2$, 0·2 g $MgSO_4$, 1 g K_2HPO_4, 1 g KH_2PO_4, 10 g $NaHCO_3$ and 2 g NaCl). The Gram stain followed standard procedures. Motility was tested on PYFG with 0·2% agar slants. After inoculation, cultures were incubated at 37 °C for 1 week. Sensitivity to bile was tested in PYFG broth plus 20% bile for 1 week at 37 °C. The absence of spores was determined using the alcohol treatment method (Koransky *et al*., 1978).

Cell wall peptidoglycan was prepared and hydrolysed using the methods of Kawamoto *et al*. (1981) and the

The GenBank/EMBL/DDBJ accession numbers for the 16S rDNA sequences of *Catenibacterium mitsuokai* JCM 10606–10611 are AB030221–AB030226.

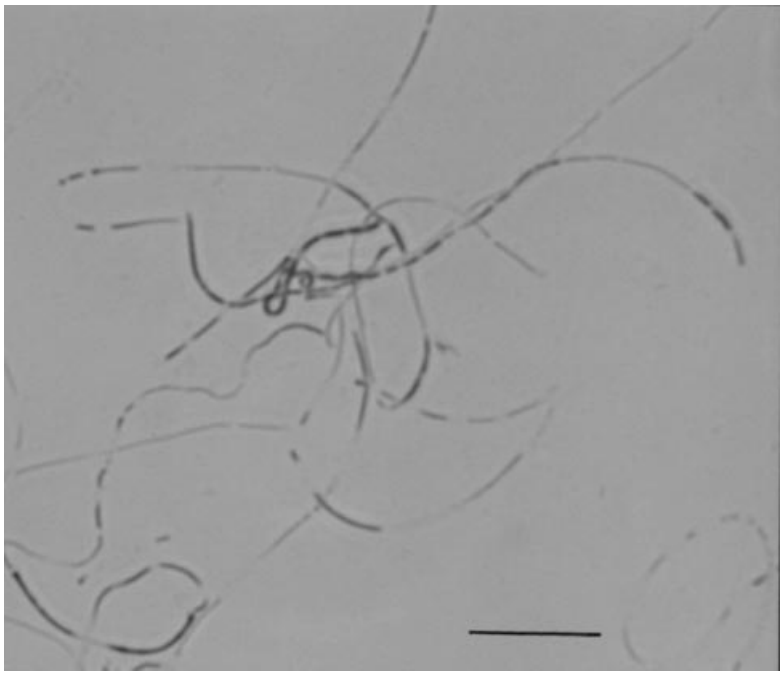

Fig. 1. Cellular morphology of *Catenibacterium mitsuokai*. Bar, 10 μm.

amino acid composition was analysed with an automatic amino acid analyser (model 835; Hitachi). The neutral amino acid fraction was reacted with a chiral reagent [(+)-1-(9-fluorenyl) ethyl chloroformate] and subjected to HPLC as described by Einarsson & Josefsson (1987).

DNA was isolated as described by Saito & Miura (1963). DNA base composition was estimated using HPLC (Tamaoka & Komagata, 1984). Levels of DNA–DNA relatedness were determined by the method of Ezaki *et al*. (1989), using photobiotin and microplates.

Almost complete 16S rRNA gene sequences of strains RCA14-19, RCA14-21, RCA14-33, RCA14-39^T, RCA14-45 and RCA14-50 were determined. The 16S rRNA gene was amplified using the PCR method and prokaryotic 16S rDNA universal primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′). The PCR was performed with a DNA thermal cycler (Perkin-Elmer Cetus) using 30 cycles consisting of denaturation at 94 °C for 60 s, primer annealing at 55 °C for 150 s and primer extension at 72 °C for 150 s (with 30 s per cycle added). Sequencing was performed using the ALFred AutoCycle Sequencing Kit (Pharmacia Biotech) with an ALFexpress DNA sequencer (Pharmacia Biotech).

Species closely related to the new isolates were identified by a sequence database search using FASTA. The sequence data of related species were retrieved from GenBank. Nucleotide substitution rates (K_{nuc} values) were calculated (Kimura & Ohta, 1972) and phylogenetic trees were constructed by the neighbour-joining method (Saitou & Nei, 1987). The topology of the trees was evaluated by a bootstrap analysis of the sequence data with CLUSTAL W software (Thompson *et al*., 1994). The sequence determined in this study has been deposited in the DDBJ database.

Strains were Gram-positive, non-spore-forming rods. The cell size was 0·4 μm × 1·2–2·0 μm. They often occurred in long tangled chains (Fig. 1). The isolates were obligate anaerobes. Spores were absent. All strains were non-motile.

All strains produced acid from glucose, mannose, galactose, fructose, sucrose, maltose, cellobiose, lactose and salicin. No strains produced acid from arabinose, xylose, rhamnose, ribose, trehalose, raffinose, melezitose, starch, glycogen, mannitol, sorbitol, inositol, erythritol, aesculin and amygdalin. All strains hydrolysed starch and none of the strains hydrolysed aesculin. Gas formation, indole production, nitrate reduction, gelatin liquefaction and H_2S production were all negative and all strains failed to grow in medium containing 20% bile. The six strains possessed almost the same biochemical and physiological characteristics. On PYFG broth, the isolates produced large amounts of acetic and lactic acids and small amounts of iso-butyric acid. Strains produced either large or small amounts of butyric acid.

The structure of the cell wall peptidoglycan of RCA14-19 and RCA14-39^T was determined. Both strains possessed the same peptidoglycan type, which contained, in addition to muramic acid and glucosamine, the amino acids glutamic acid, diaminopimelic acid and alanine at a molar ratio of 1:1:2. Diaminopimelic acid occurred in the *meso* configuration. Thus, the structural type was A1γ, (L-Ala)-D-Glu-*m*-Dpm.

The G+C content of the DNA of four strains, RCA14-19, RCA14-21, RCA14-39^T and RCA14-45 ranged

Table 1. DNA base composition and levels of DNA–DNA relatedness among isolated strains

Strain	G+C content (mol%)	DNA–DNA reassociation (%) with:			
		RCA14-19	RCA14-21	RCA14-39^T	RCA14-45
RCA14-19	37·2	100	86·8	98·9	92·7
RCA14-21	38·4	97·8	100	105	103
RCA14-39^T	36·6	96·5	93·4	100	97·0
RCA14-45	36·2	93·2	86·2	93·4	100

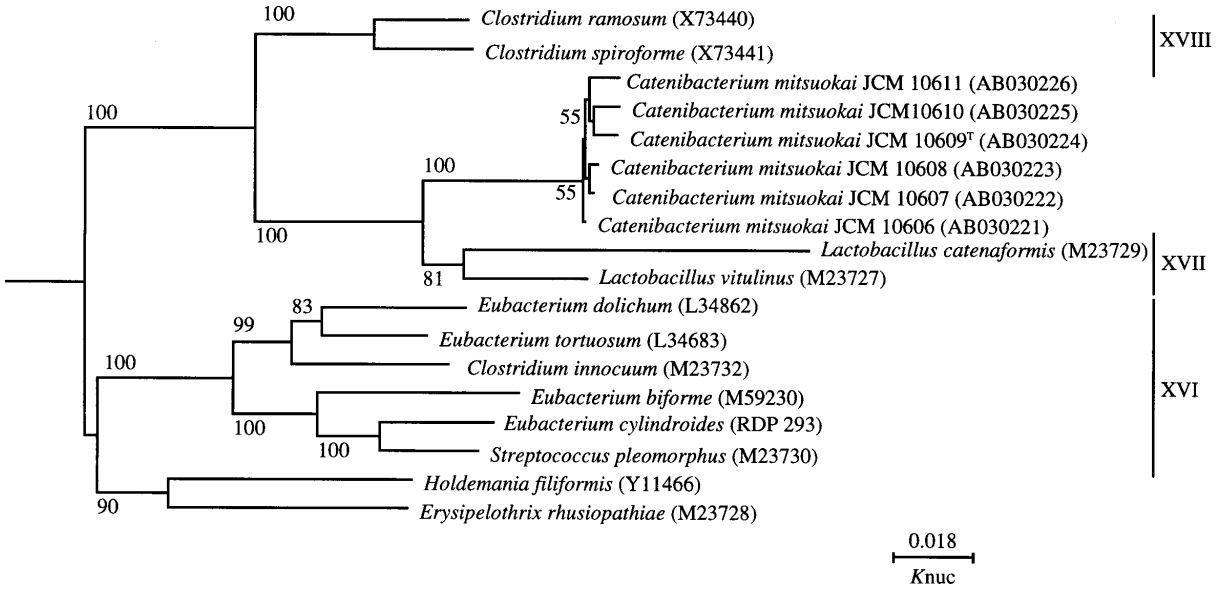

Fig. 2. Phylogenetic tree derived from 16S rDNA sequences. The tree was created using the neighbour-joining method and K_{nuc} values. Numbers on the tree indicate bootstrap values for the branch points. Only values above 50% are indicated. The sequence data for species other than RCA14-19, RCA14-21, RCA14-33, RCA14-39^T, RCA14-45 and RCA14-50 were obtained from the GenBank database. The respective numbers of the *Clostridium* clusters are shown on the right-hand side.

from 36·2 to 38·4 mol% (Table 1). The levels of DNA–DNA relatedness among the four isolated strains ranged from 86·2 to 105% (Table 1). The results showed that these four strains represented the same species.

More than 1400 bases of the 16S rDNA sequences were determined and these sequences have been deposited in the DDBJ database. A database search revealed that these strains belonged to the *Clostridium* subphylum cluster XVII (Collins *et al.*, 1994). A phylogenetic tree including all taxa from cluster XVII and related clusters was constructed (Fig. 2). For the phylogenetic analysis, 1365 bp (positions 99–1488; *Escherichia coli* numbering system) sequences of each species were used. The levels of similarity among the six isolated strains were 97·5–99·3%. The levels of similarity of *Lactobacillus catenaformis* and *Lactobacillus vitulinus* to the isolate were 86·0–87·0% and 89·4–92·0%, respectively.

The six strains of *Eubacterium*-like organisms isolated from the faeces of Papua New Guinea highlanders were characterized. These isolates were Gram-positive, non-spore-forming, rod-shaped organisms. Rods occurring in tangled chains are very unusual. On the basis of the results of phenotypic characterization, sugar fermentation and short fatty acid production from glucose, the strains were consistent with the properties of the genus *Eubacterium*. The sugar-fermentation patterns were the same as those of *Collinsella aerofaciens* (I-B; cells produce acid from sucrose, cellobiose and salicin, but not from aesculin or amygdalin) (Kageyama *et al.*, 1999a), but the cell morphology, the DNA G+C content (mol%) and the cell wall murein type were different.

The type species of genus *Eubacterium* is *Eubacterium limosum*. It was evident from recent studies that *Eubacterium limosum*, *Eubacterium barkeri* and *Eubacterium callanderi* (Mountfort *et al.*, 1988) can

form the nucleus of a redefined genus of *Eubacterium*. On the basis of the characteristics of this group, a preliminary working definition of *Eubacterium sensu stricto* was determined (Willems & Collins, 1996). The remaining species currently classified within the genus *Eubacterium* were heterogeneous according to the criteria of a wide range of DNA G+C content and low 16S rDNA sequence similarity (Nakazawa & Hoshino, 1994). Recently, some species belonging to this genus were transferred to new genera. Examples include *Pseudoramibacter alactolyticus* (Willems & Collins, 1996), *Actinomyces suis* (Ludwig *et al*., 1992), *Eggerthella lenta* (Wade *et al*., 1999), *C. aerofaciens* (Kageyama *et al*., 1999a) and *Atopobium fossor* (Kageyama *et al*., 1999b). A new genus, *Holdemania*, which resembles the genus *Eubacterium* in terms of phenotypic characteristics but differs with respect to 16S rDNA and chemotaxonomic characters, was also established (Willems *et al*., 1997).

In this study, the six isolated strains could be assigned to the genus *Eubacterium* on the basis of many of their phenotypic characteristics. On the basis of 16S rDNA sequence analysis and chemotaxonomic characteristics, the six strains belonged to *Clostridium* cluster XVII. However, the 16S rDNA sequence similarity of the isolates to the closest previously described species, *L. catenaformis* and *L. vitulinus*, was $< 92{\cdot}0\,\%$, suggesting that the new strains should be classified in a novel genus. This conclusion was supported by phenotypic differences. While the structure of the cell wall murein of *L. catenaformis* was L-Lys-L-Ala_3 (A3α), the structure of the cell wall murein of *L. vitulinus* was the same as that of the isolates (Sharpe *et al*., 1973). However, these isolates and *L. vitulinus* had different fermentation products; *L. vitulinus* produced only D(−)-lactic acid (Sharpe *et al*., 1973).

The unknown isolates from human faeces clearly belonged to a hitherto unrecognized, Gram-positive species within the *Clostridium* subphylum. From 16S rDNA gene sequence comparisons, it was evident that the bacterium has a close phylogenetic relationship with *L. catenaformis* and *L. vitulinus*. Although the association between these taxa was significant, a sequence divergence of $> 8\,\%$ suggested that this relationship is one of phylogenetically closely related, but different, genera. On the basis of 16S rDNA sequence considerations and other phenotypic characteristics, we believe that this bacterium merits classification as a new genus, for which the name *Catenibacterium mitsuokai* gen. nov., sp. nov. is proposed.

Description of *Catenibacterium* gen. nov.

Catenibacterium (Ca.te.ni.bac.te′ri.um. L. fem. n. *catena* chain; Gr. dim. n. *bakterion* a small rod; M.L. neut. n. *Catenibacterium* chain rodlet).

Cells occur in tangled chains. Gram-positive and obligatory anaerobic. Spores are absent. Fermentation products of glucose are acetic, lactic, butyric and isobutyric acids. Cell wall contains an A1γ-type peptidoglycan with an (L-Ala)-D-Glu-m-Dpm peptide subunit. The G+C content of the DNA is 36–39 mol%. The type species is *Catenibacterium mitsuokai*. The genus *Catenibacterium* is a member of the *Clostridium* subphylum of Gram-positive bacteria and exhibits a close phylogenetic association with *Lactobacillus catenaformis* and *Lactobacillus vitulinus*.

Description of *Catenibacterium mitsuokai* sp. nov.

Catenibacterium mitsuokai (mit.su.o′kai. N.L. gen. n. *mitsuokai* of Mitsuoka, named after K. Mitsuoka, a Japanese microbiologist).

This description is based on a study of six strains isolated from human faeces. Cells are $0{\cdot}4\ \mu m \times 1{\cdot}2$–$2{\cdot}0\ \mu m$ long and occur in tangled chains. Non-motile. Gram-positive and obligatory anaerobic. Spores are absent. Can be cultivated in 2 d at 37 °C on EG agar in an anaerobic jar with 100% CO_2. Cells produce acid from glucose, mannose, galactose, fructose, sucrose, maltose, cellobiose, lactose and salicin but not from arabinose, xylose, rhamnose, ribose, trehalose, raffinose, melezitose, starch, glycogen, mannitol, sorbitol, inositol, erythritol, aesculin or amygdalin. Hydrolysis of starch is positive and that of aesculin is negative. Gas formation, indole production, nitrate reduction, gelatin liquefaction and H_2S production are all negative. Cell wall contains A1γ-type peptidoglycan with an (L-Ala)-D-Glu-*m*-Dpm peptide subunit. The G+C content of the DNA is 36–39 mol%. The type strain of *Catenibacterium mitsuoka* is strain JCM 10609^T. Isolated from human faeces.

Acknowledgements

We thank M. Chijimatsu for analysis of the cell wall.

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