Moryella indoligenes gen. nov., sp. nov., an anaerobic bacterium isolated from clinical specimens

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Three Gram-positive, anaerobic, non-spore-forming, rod-shaped bacteria with pointed ends were isolated from clinical specimens. The organisms were weakly saccharolytic and produced indole, acetate, butyrate and lactate as major metabolic end products. 16S rRNA gene sequence analysis indicated that the isolates had no known close relatives among recognized bacteria but that they exhibited a phylogenetic association with *Clostridium* rRNA cluster XIVa [as defined by Collins, M. D. *et al.* (1994). *Int J Syst Bacteriol* **44**, 812–826]. The closest recognized relatives were the type strains of *Clostridium clostridioforme*, *Clostridium bolteae* and *Clostridium asparagiforme* (16S rRNA gene sequence similarity values of 90.2–91.4 %). These results suggest that these three clinical isolates represent a novel species of a new genus, for which the name *Moryella indoligenes* gen. nov., sp. nov. is proposed. The type strain of *Moryella indoligenes* is AIP 220.04^T (= CIP 109174^T = CCUG 52648^T).

Anaerobic bacteria constitute an important part of the human microbial community. Although the majority are considered to be commensals, many of them behave as opportunistic pathogens. Notwithstanding intensive investigations by using conventional identification techniques, we still know relatively little about the bacterial diversity of these microbial communities. Indeed, molecular genetic tools have indicated that 60–80 % of organisms in the total human microflora have not been cultivated (Langendijk *et al.*, 1995; Suau *et al.*, 1999) and only 24 % of the molecular species recovered from the human intestinal tract corresponded to recognized species (Suau *et al.*, 1999). Thus, the combination of genetic tools and traditional phenotypic methods of identification should be used whenever possible with the aim of providing greater knowledge of these 'hidden' bacteria. Here we report on the characterization of an indole-producing anaerobic bacterium that was recovered from different clinical specimens. Phylogenetically, the strains described represent a hitherto unknown subline within the *Clostridium coccoides* rRNA group. Based on the data presented, we describe a novel species in a new genus for these strains.

The new isolates were recovered from clinical sources: strain AIP 241.03 from a buttock abscess, strain AIP 220.04^T from

an intra-abdominal abscess (both from France) and strain MDA2477 from a polymicrobial thigh abscess (from Houston, TX, USA). Thus, these strains might all have originated from the intestinal tract. The strains were maintained in trypticase/glucose/yeast extract medium (TGY) consisting of 3 % (w/v) biotrypcase (bioMérieux), 0.5 % glucose (Prolabo), 2 % yeast extract (Difco), 0.05 % L-cysteine hydrochloride (Prolabo) and 5 µg haemin ml⁻¹ (Calbiochem), under anaerobic conditions at 37 °C for 24 h in an anaerobic jar containing 95 % H₂ and 5 % CO₂ (v/v). Colony morphology determinations and presumptive identification tests (Engelkirk *et al.*, 1992) were performed on Wilkins–Chalgren agar plates. Biochemical reactions were examined according to the procedures described by Holdeman *et al.* (1977). Metabolic end products were assayed by quantitative GC as described by Carlier (1985). For electron microscopy, cells were prepared as described by Carlier *et al.* (2004), and electron microphotographs were taken with a JEOL 1010 transmission electron microscope operating at 80 kV.

Colonies appeared on Wilkins–Chalgren blood agar after 24–48 h incubation. They were circular, convex, about 0.5–1 mm in diameter, non-pigmented and non-haemolytic. Cells were elongated, sometimes warped rods with pointed ends, about 0.8–1.7 µm long and 0.5–0.6 µm wide, usually occurring singly, in pairs or occasionally in short chains (Fig. 1a). They were non-motile, Gram-variable after staining but structurally Gram-positive (Fig. 1b). Spore formation was not observed.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains AIP 241.03, AIP 220.04^T and MDA2477 are DQ377946, DQ377947 and AF527773, respectively.

Fig. 1. Transmission electron micrographs of cells of strain AIP 220.04^T. (a) General morphology after negative staining; (b) ultrathin section showing the Gram-positive cell wall (CW) and cytoplasmic membrane (CM). Bars, 5 μ m (a) and 1 μ m (b).

The isolates were strictly anaerobic. They were susceptible to discs containing 1 mg kanamycin, 10 μ g colistin, 5 μ g vancomycin and 4 μ g metronidazole and to bile discs. They were indole-positive. Catalase activity and nitrate and nitrite reduction were not detected. Gelatin was not liquefied and milk was not modified. Glucose, galactose, maltose and ribose fermentation were variable. Acid was not produced from raffinose, sucrose, aesculin, arabinose, cellobiose, fructose, glycerol, inositol, lactose, mannitol, mannose, melezitose, melibiose, rhamnose, salicin, sorbitol, starch, trehalose or xylose. Aesculin was not hydrolysed. The major metabolic end products were acetic acid (6.4–14 mmol l⁻¹), butyric acid (12–28 mmol l⁻¹) and lactic acid (6–25 mmol l⁻¹). Table 1 provides the primary characteristics of these isolates that can be used to differentiate them from several closely related species.

The G+C composition of strain AIP 220.04^T was 50.2 mol%, as determined by HPLC at the Identification Service of the DSMZ (German Collection of Micro-organisms and Cell Cultures). Cellular fatty acid composition was analysed by GC according to Veys *et al.* (1989). Briefly, the three strains were grown anaerobically in 10 ml TGY for 48 h and chromatography of the methyl esters was on a fused-silica capillary column (25 m $\times$ 0.25 mm ID) coated with 5 % methyl phenyl silicone. Fatty acid methyl ester analysis showed that the strains contained an unknown compound that eluted between C_{12:0} and C_{13:0} (14 %), C_{14:0} (47 %) and C_{16:0} (9 %) as the major components. Minor fatty acids included C_{12:0} (3.4 %), iso-C_{14:0} (2.7 %), iso-C_{15:0} (2.4 %), anteiso-C_{15:0} (7 %), a second unknown compound that eluted between C_{14:0} and iso-C_{15:0} (6.2 %), iso-C_{16:0} (1.4 %), C_{16:1} ω 9c (2.6 %) and C_{18:0} (1.4 %). No hydroxy fatty acids were detected.

The 16S rRNA gene sequences were determined for each strain as described by Carlier *et al.* (2004). Alignment was via the CLUSTAL W program (Thompson *et al.*, 1994). Regions showing alignment uncertainties and gaps were excluded and 1375 unambiguous nucleotide positions were used. A distance matrix was calculated by using DNADIST with the Jukes–Cantor parameter (Jukes & Cantor, 1969). A phylogenetic tree (Fig. 2) was constructed according to the neighbour-joining method with 100 bootstrap resamplings

(Felsenstein, 1993). Sequence comparison revealed that the new isolates shared more than 99.9 % 16S rRNA gene sequence similarity with each other and had no known close relatives among recognized bacteria. Indeed, the nearest phylogenetic neighbours were the type strains of *Clostridium clostridioforme*, *Clostridium bolteae* and *Clostridium asparagiforme*, with sequence similarity values of 90.2–91.4 %. The new isolates formed a distinct lineage within the *C. coccoides* rRNA group of organisms, and the monophyly of this lineage was strongly supported by the high bootstrap value of 98 %. Thus, these isolates were considered to represent a novel taxon. Within the lineage, an oral clone MCE9_173 branched off; this clone, described by Munson *et al.* (2002) as '*Lachnospiraceae*', shared 93.4 % sequence similarity with the novel organism. To determine the genus and species status of the novel organism, its relationship with members of the *C. coccoides* rRNA cluster was examined. This cluster encompasses a phenotypically heterogeneous collection of organisms, including spore-forming and non-spore-forming genera and species. Several butyrate-producing members are also widely distributed within this cluster (Barcenilla *et al.*, 2000). Although the novel bacterium was phylogenetically closer to the *C. clostridioforme* group, it differed from these clostridia because it was not a spore-former and produced butyrate. On the other hand, it was phylogenetically too distant from the butyrate-producing members to be assigned to those genera or species. Thus, given the $\sim$ 10 % 16S rRNA gene sequence divergence from their closest relatives and several distinct phenotypic features, the three strains are considered to represent a novel species of a new genus, for which the name *Moryella indoligenes* gen. nov., sp. nov. is proposed.

Description of *Moryella* gen. nov.

Moryella (Mo.ry.el'la. L. fem. dim. ending -ella; N.L. fem. n. *Moryella* named in honour of the French microbiologist Francine Mory, who has contributed to our understanding of anaerobes).

Cells are Gram-variable after staining but structurally Gram-positive, elongated, non-motile rods with pointed ends. Cells may lose their colour easily. No spores are formed. Strictly anaerobic, without growth under

Table 1. Characteristics that differentiate the three new clinical strains from other close relatives

ND, Not determined/no data available; v, strain variation; d, 11–89 % of strains positive.

Characteristic	AIP 220.04 ^T	AIP 241.03	MDA2477	<i>Oribacterium sinus</i>	<i>Lachnospira multipara</i>	<i>Lachnospira pectinoschiza</i>	<i>Butyrivibrio crossotus</i>	<i>Butyrivibrio fibrisolvens</i>	<i>Clostridium clostridioforme</i>	<i>Clostridium bolteae</i>	<i>Clostridium asparagiforme</i>
Cell shape	Elongated, pointed ends	Elongated, pointed ends	Elongated, pointed ends	Ovoid	Curved	Straight	Curved	Curved	Straight	Straight	Straight, tapered ends
Motility	–	–	–	+	+	+	+	+	+	ND	ND
Spore formation	–	–	–	–	–	Spore-like structures	–	–	+	+	+
Indole	+	+	+	+	–	ND	ND	–	v	–	ND
Acid from:											
Lactose	–	–	–	–	–	+	d	+	v	–	ND
Maltose	–	–	–	–	–	–	–	+	+	+	ND
Metabolic end products*	A, B, l	a, B, L	A, B, L	A, L	F, A, L, e	F, A, e	F, A, B, L	F, B, (a, l)	A	A, L	A, L, e
DNA G+C content (mol%)	50.2	ND	ND	42.4	ND	51.4	36–37	41	47–49	50.5	53
Isolation source	Human buttock abscess	Human intra-abdominal abscess	Human thigh abscess	Human oral cavity	Rumen of ruminants	Pig intestine	Human faeces	Rumen of ruminants; human, rabbit, horse faeces	Humans and animals	Human faeces, blood, intra-abdominal abscess	Human faeces
Reference	This paper	This paper	This paper	Carlier <i>et al.</i> (2004)	Bryant (1986a)	Cornick <i>et al.</i> (1994)	Bryant (1986b)	Bryant (1986b)	Cato <i>et al.</i> (1986)	Song <i>et al.</i> (2003)	Mohan <i>et al.</i> (2006)

*A, Acetate; B, butyrate; E, ethanol; F, formate; L, lactate. Parentheses indicate variable production. Upper-case letters indicate major products.

Fig. 2. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequence comparisons over 1375 aligned bases indicating the relationships between the three new clinical strains and related species. The sequence of *Propionibacterium acnes* DSM 1897^T was used as an outgroup. Numbers on the tree are bootstrap percentages. GenBank accession numbers are given in parentheses. Bar, 10% sequence divergence.

microaerophilic or aerobic conditions. Catalase and urease reactions are negative. Nitrate is not reduced. Indole-positive and weakly saccharolytic. The major metabolic end products are acetic, butyric and lactic acids. Phylogenetically, the genus belongs to the *C. coccooides* rRNA cluster (rRNA cluster XIVa; Collins *et al.*, 1994). The type species is *Moryella indoligenes*.

Description of *Moryella indoligenes* sp. nov.

Moryella indoligenes (in.do.li'ge.nes. N.L. n. *indolum* indole; N.L. suff. -*genes* producing from Gr. v. *gennaô* to produce; N.L. part. adj. *indoligenes* indole-producing).

Displays the following properties in addition to those given in the genus description. Cells are sometimes warped, about 0.8–1.7 µm long and 0.5–0.6 µm wide, occurring singly, in pairs or occasionally in short chains. Colonies are circular, convex and about 0.5–1 mm in diameter on Wilkins–Chalgren blood agar after 24–48 h incubation, non-pigmented and non-haemolytic. Indole production is the main biochemical characteristic of the species. Glucose, galactose, maltose and ribose fermentation are variable. Acid is not produced from any of the following carbohydrates: raffinose, sucrose, aesculin, arabinose, cellobiose, fructose, glycerol, inositol, lactose, mannitol, mannose, melezitose, melibiose, rhamnose, salicin, sorbitol, starch, trehalose or xylose. Aesculin is not hydrolysed. Gelatin is not liquefied and milk is not modified. The type strain is susceptible to

ampicillin, amoxicillin, penicillin G, imipenem, cefalotin, cefotaxim, cefoxitin, latamoxef and metronidazole, moderately resistant to tetracycline and resistant to trimethoprim-sulfamethoxazole, erythromycin and rifampicin. Strains AIP 241.03 and MDA2477 are resistant only to trimethoprim-sulfamethoxazole and erythromycin. The DNA G+C content is 50.2 mol%. Habitat is not known, but likely to be the human gut.

The type strain, AIP 220.04^T (=CIP 109174^T=CCUG 52648^T), was isolated from a clinical specimen from an intra-abdominal abscess. Strains MDA2477 (=ATCC BAA-695) and AIP 241.03 are additional strains of this species.

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