Slackia piriformis sp. nov. and *Collinsella tanakaei* sp. nov., new members of the family *Coriobacteriaceae*, isolated from human faeces

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Three Gram-positive, strictly anaerobic, non-spore-forming, rod-shaped organisms (strains YIT 12062^T, YIT 12063^T and YIT 12064) were isolated from human faeces. Strain YIT 12062^T was asaccharolytic and possessed a DNA G+C content of 58.3 mol%. Cells of strain YIT 12062^T were negative for catalase, oxidase, urease, hydrolysis of aesculin and gelatin, nitrate reduction and indole production. Based on 16S rRNA gene sequence analysis, strain YIT 12062^T was assigned to the genus *Slackia* (91.7–96.0 % sequence similarities to type strains of *Slackia* species). Biochemical data showed that the isolate was phenotypically distinct from all recognized species of the genus *Slackia*. Strain YIT 12062^T therefore represents a novel species in the genus *Slackia*, for which the name *Slackia piriformis* sp. nov. is proposed. The type strain is YIT 12062^T (=DSM 22477^T=JCM 16070^T). Following 16S rRNA gene sequence analysis, strains YIT 12063^T and YIT 12064, which were isolated from different subjects, were shown to be most closely related to species of the genus *Collinsella* (93.8–95.1 % similarities to type strains). Although their phenotypic characteristics were very similar and they shared >99 % 16S rRNA gene sequence similarity and >97 ± 1.8 % DNA–DNA relatedness, the two isolates could be discriminated by RAPD fingerprints. The DNA G+C contents of strains YIT 12063^T and YIT 12064 were 60.8 and 61.0 mol%, respectively. They were saccharolytic in API test systems, positive for aesculin hydrolysis and negative for catalase, oxidase, urease, indole production, nitrate reduction and gelatin hydrolysis. The major end products of glucose fermentation of these strains were lactate, acetate and formate. Biochemical data supported the affiliation of strains YIT 12063^T and YIT 12064 to the genus *Collinsella* and showed that they were phenotypically distinct from all recognized species of the genus *Collinsella*. Strains YIT 12063^T and YIT 12064 therefore represent a novel species of the genus *Collinsella*, for which the name *Collinsella tanakaei* sp. nov. is proposed. The type strain is YIT 12063^T (=DSM 22478^T=JCM 16071^T).

Some species belonging to the genera *Slackia* and *Collinsella*, together with *Atopobium* and *Eggerthella*, have been isolated from faecal samples from normal healthy subjects (Kageyama & Benno, 2000; Matthies *et al.*, 2009) or detected by using culture-independent approaches based on 16S rRNA diversity (Kassinen *et al.*, 2007; Matsuki *et al.*, 2002; Wang *et al.*, 2004; Harmsen *et al.*, 2000). All of these

genera are within the family *Coriobacteriaceae* in the phylum *Actinobacteria*. Currently, five species of the genus *Slackia* have been described: *Slackia exigua*, *S. heliotrinireducens*, *S. faecicanis*, *S. isoflavoniconvertens* and *S. equolifaciens*. *S. exigua*, previously known as *Eubacterium exiguum* (Poco *et al.*, 1996; Wade *et al.*, 1999), was isolated from human oral lesions. *S. heliotrinireducens*, previously known as *Peptococcus heliotrinireducans*, and *S. faecicanis* were isolated from the rumen of a sheep and canine faeces, respectively (Lanigan, 1976; Lawson *et al.*, 2005). *S. isoflavoniconvertens* and *S. equolifaciens*, which produce equol, were recently isolated from human faeces by Matthies *et al.* (2009) and Jin *et al.* (2010), respectively. The genus *Collinsella* comprises only three recognized species, namely *Collinsella aerofaciens*, *C. stercoris* and *C. intestinalis*, all isolated from the faeces of healthy human subjects (Kageyama & Benno, 2000; Holdeman *et al.*, 1977).

Abbreviations: DMA, dimethyl acetal; ML, maximum-likelihood; MP, maximum-parsimony; RAPD, random amplified polymorphic DNA.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains YIT 12062^T, YIT 12063^T and YIT 12064 are AB490806, AB490807 and AB490808, respectively.

RAPD-PCR fingerprinting of the two novel *Collinsella* isolates (Fig. S1), fatty acid compositions of strain YIT 12062^T and the type strains of *Slackia* species (Table S1) and fatty acid compositions of strains YIT 12063^T, YIT 12064 and other members of the genus *Collinsella* (Table S2) are available with the online version of this paper.

To better understand the physiological characteristics and function of the majority of the human gastrointestinal microbiota, we have performed several intensive cultivation trials aimed at isolating as-yet uncultured bacteria from the human gastrointestinal tract (Sakon *et al.*, 2008; Morotomi *et al.*, 2008, 2009, 2010; Nagai *et al.*, 2009, 2010; Watanabe *et al.*, 2010). Here, the isolation of novel species of the genera *Slackia* and *Collinsella* from the faeces of healthy Japanese adults is reported. Interestingly, the isolates showed >99 % 16S rRNA gene sequence similarity to several cloned sequences originating from the faeces of human subjects and other mammals, suggesting that they are common members of the mammalian intestinal microbiota.

Faecal samples were collected from two healthy Japanese males (Subjects H and K; aged 57 and 27 years, respectively) and immediately transferred to anaerobic conditions. Each sample was weighed and diluted with pre-reduced 0.1 M PBS (pH 7) in an anaerobic glove box (Coy Laboratory Products), which contained 88 % nitrogen, 7 % hydrogen and 5 % carbon dioxide. Each dilution was then spread on modified Gifu anaerobic medium (GAM; Nissui Pharmaceutical) containing 1.5 % (w/v) agar and supplemented with bile, NaCl or antibiotics (described below) to isolate subdominant groups in the intestinal microbiota. The composition of the modified GAM agar was as described previously (Sakon *et al.*, 2008).

Plates were incubated at 37 °C for 3 days. Strain YIT 12062^T was isolated from a GAM agar plate supplemented with 6 % Bacto oxgall (Difco) and inoculated with a 10⁻⁵ serially diluted faecal sample from subject H. Strains YIT 12063^T and YIT 12064 were isolated from a GAM agar plate supplemented with 1 % (w/v) NaCl and fosfomycin (60 µg ml⁻¹) and inoculated with a 10⁻⁶ serially diluted faecal sample from subjects H and K, respectively. Single colonies were picked and streaked on the modified GAM agar until pure cultures were obtained. The end products of bacterial metabolism of glucose in pre-reduced peptone-yeast extract medium (Holdeman *et al.*, 1977) supplemented with 1 % glucose (PYG medium) were analysed by HPLC, as described previously (Chonan *et al.*, 1995). Cellular morphology was examined by phase-contrast light microscopy of 4-day-old modified GAM agar cultures. Biochemical characteristics were determined in duplicate by using the API Rapid ID32A, API ZYM and API 20A systems (bioMérieux) according to the manufacturer's instructions. Sensitivity to bile was determined by comparing growth of the strains on modified GAM agar with and without 2 % Bacto oxgall (Difco) after 5 days of incubation at 37 °C.

Cellular fatty acid methyl esters were obtained from lyophilized cells grown on modified GAM agar (*Slackia* species) or grown in modified GAM broth supplemented with 1 % glucose (*Collinsella* species) by saponification, methylation and extraction using minor modifications (Kuykendall *et al.*, 1988) of the method of Miller (1982).

Fatty acid methyl esters were determined by the MIDI system using MOORE5 of the MIS Standard Libraries.

Isoprenoid quinones were extracted as described by Komagata & Suzuki (1987) and were analysed by using an HPLC-atmospheric pressure chemical ionization-MS/MS system (API 3200; Applied Biosystems) with an L column ODS (2.1 × 150 mm; Chemicals Evaluation and Research Institute) according to a modification of the method of Katsuta *et al.* (2005).

The DNA G+C content was determined by hydrolysing the DNA enzymically and quantifying the nucleosides by HPLC according to the method of Ezaki *et al.* (1990). Fluorometric DNA-DNA hybridization in microdilution wells was carried out by the method of Ezaki *et al.* (1989). Details of isolation and purification of DNA were as described previously (Yuki *et al.*, 2000).

PCR-based random amplified polymorphic DNA (RAPD) fingerprinting was carried out by the method of Akopyanz *et al.* (1992) using primer 5'-AACGCGCAAC-3'. The PCR products were subjected to agarose gel electrophoresis.

The 16S rRNA genes of the isolates were amplified by PCR using universal primers 8F (5'-AGAGTTTGATCMTGG-CTCAG-3'; Miyake *et al.*, 1998) and 15R (5'-AAGGA-GGTGATCCARCCGCA-3'; Yuki *et al.*, 2000) and direct sequencing of the amplified DNA fragments was performed. Details of these procedures have been described previously (Miyake *et al.*, 1998). 16S rRNA gene sequences of reference species were retrieved from GenBank/EMBL/DBJ by using the FASTA program (Lipman & Pearson, 1985). Sequences were aligned and used to produce an unrooted phylogenetic tree according to the neighbour-joining method (Saitou & Nei, 1987) by using CLUSTAL_X (version 1.83) (Thompson *et al.*, 1997). The stability of the groupings was estimated by bootstrap analysis (1000 replications). All phylogenetic trees were visualized by using the program TreeView version 1.6.6 (Page, 1996). Maximum-parsimony (MP) and maximum-likelihood (ML) methods were used to confirm the phylogenetic placement of the aligned sequences. MP analysis was performed using the software package MEGA4 (Tamura *et al.*, 2007). The ML tree was constructed via the PHYML program (Guindon & Gascuel, 2003) by using Kimura's two-parameter nucleotide substitution model (Kimura, 1980). The input file was prepared via the program SEQBOOT within the PHYLIP package (Felsenstein, 2004).

Cells of YIT 12062^T were Gram-positive, obligately anaerobic and non-motile and formed chains of pear-shaped to irregularly shaped rods (0.4–1.1 × 0.9–2.6 µm). After 4 days anaerobic incubation at 37 °C on modified GAM agar, colonies were 0.1–1.0 mm in diameter, translucent to beige, and circular, with an uneven surface and irregular edges. Growth of strain YIT 12062^T in PYG broth was weak and no major acids were detected as end products of metabolism. Strain YIT 12062^T and *S. faecicanis* YIT 12015^T were able to grow on medium

containing 2 % oxgall, but the number of colonies decreased to about 50 % and 5 %, respectively, compared with the control medium without oxgall (Table 1). In contrast, the type strains of *S. exigua* and *S. heliotrinireducens* did not grow on the oxgall-containing medium (Table 1). Strain YIT 12062^T and the type strains of *S. exigua*, *S. heliotrinireducens* and *S. faecicanis* were asaccharolytic in the API test systems. Indole production, nitrate reduction, catalase, urease and hydrolysis of aesculin and gelatin were negative in all of these strains. Other biochemical characteristics obtained by using the API Rapid ID32A and API ZYM test systems are included in the species description of *Slackia piriformis* sp. nov.

The fatty acid profiles of strain YIT 12062^T and the type strains of species of the genus *Slackia* are detailed in

Supplementary Table S1 (available in IJSEM Online). All of these strains contained C_{18:1}ω₉c (32–52 %) and C_{18:1}ω₉c dimethyl acetal (DMA) (16–24 %) as the predominant fatty acids. Although some minor qualitative and quantitative differences in fatty acid content could be observed, the overall patterns of the composition were very similar among species of this genus. Respiratory quinones were not detected in strain YIT 12062^T or in the type strains of *S. exigua*, *S. heliotrinireducens* and *S. faecicanis*.

A 1463 bp region of the 16S rRNA gene of strain YIT 12062^T was sequenced. Database searches of recognized taxa revealed that strain YIT 12062^T was most closely related to species of the genus *Slackia*, with 16S rRNA gene sequence similarities of 91.7, 92.6, 92.6, 92.8 and 96.0 % to the type strains of *S. isoflavoniconvertens*, *S. exigua*, *S.*

Table 1. Major characteristics of strain YIT 12062^T and other members of the genus *Slackia*

Strains: 1, YIT 12062^T; 2, *S. exigua* YIT 10056^T (=JCM 11022^T); 3, *S. heliotrinireducens* YIT 12016^T (=JCM 14554^T); 4, *S. faecicanis* YIT 12015^T (=JCM 14555^T); 5, *S. isoflavoniconvertens* DSM 22006^T; 6, *S. equolifaciens* JCM 16059^T. Apart from *S. isoflavoniconvertens* (Matthies *et al.*, 2009) and *S. equolifaciens* (Jin *et al.*, 2010), data are from the present study unless otherwise indicated. +, Positive; –, negative; w, weak positive; v, variable; ND, no data available. In the API test systems, all strains were positive for arginine dihydrolase and naphthol-AS-BI-phosphohydrolase, but negative for alkaline phosphatase, α-arabinosidase, N-acetyl-β-glucosaminidase, chymotrypsin, α-fucosidase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, β-glucuronidase, glutamic acid decarboxylase, glutamyl glutamic acid arylamidase, lipase (C14), α-mannosidase, 6-phospho-β-galactosidase, pyroglutamic acid arylamidase, trypsin and urease.

Characteristic	1	2	3	4	5	6
Isolation source	Human faeces	Human oral lesions	Rumen of sheep	Canine faeces	Human faeces	Human faeces
Morphology	Pear-shaped to irregularly shaped rods	Short rods	Cocci and coccobacilli	Short rods	Short rods	Rod-shaped
Major products from PYG*	–	–	a	–	ND	ND
Bile resistant	w†	–	–	w†	ND	ND
DNA G + C content (mol%)	58.3	60‡	61‡	61.2‡	58.5‡	60.8‡
API tests (API ZYM, Rapid ID32A)						
Acid phosphatase	w	+	–	w	+	ND
Alanine arylamidase	+	+	+	–	–	+
Arginine arylamidase	–	+	–	–	–	–
Cystine arylamidase	+	+	v	–	–	ND
Esterase (C4)	–	–	–	–§	+	ND
Esterase lipase (C8)	–	–	–	–§	+	ND
Glycine arylamidase	+	+	+	–	–	+
Histidine arylamidase	+	+	+	–	–	+
Leucine arylamidase	+	+	+	–	–	+
Leucyl glycine arylamidase	–	–	–	–	–	v
Phenylalanine arylamidase	+	+	+	–	–	+
Proline arylamidase	+	+	+	–	–	+
Serine arylamidase	+	+	+	+§	–	+
Tyrosine arylamidase	+	+	+	–	–	+
Valine arylamidase	+	+	+	–	–	ND

*Lower-case letters indicate <1 mequiv. of acid per 100 ml; a, acetic acid.

†Cells grew on medium containing 2 % oxgall, but the number of colonies was decreased compared with the control medium without oxgall (see text for details).

‡Data for DNA G + C content for *S. exigua*, *S. heliotrinireducens*, *S. faecicanis*, *S. isoflavoniconvertens* and *S. equolifaciens* are from Poco *et al.* (1996), Wade *et al.* (1999), Lawson *et al.* (2005), Matthies *et al.* (2009) and Jin *et al.* (2010), respectively.

§Data from this study were different from published data (Lawson *et al.*, 2005). It is assumed that the culture conditions were different.

heliotrinireducens, *S. equolifaciens* and *S. faecicanis*, respectively (Fig. 1).

Interestingly, at the time of writing, the 16S rRNA gene sequences of 12 uncultured bacterial clones deposited in GenBank/EMBL/DDBJ had >99% sequence similarity to that of strain YIT 12062^T. The isolation sources of these clones were faecal samples of human and other mammals. Some of them are shown in Fig. 1. Strain YIT 12062^T displayed >99% 16S rRNA gene sequence similarity to some of the faecal uncultured clones from human subjects reported by Ley *et al.* (2006) (GenBank accession nos DQ800868 and DQ808704) and Turnbaugh *et al.* (2009) (accession no. FJ366859), and from armadillo (accession no. EU771928) reported by Ley *et al.* (2008) (Fig. 1).

Table 1 shows characteristics that can be used to differentiate strain YIT 12062^T from members of the genus *Slackia*. The DNA G+C content of strain YIT 12062^T was 58.3 mol%.

Cells of YIT 12063^T were Gram-positive, obligately anaerobic, non-motile rods (0.5–1.0 × 1.0–3.3 µm) most often occurring in long chains. Cells of YIT 12064 were similar to

those of YIT 12063^T, but occurred singly and only occasionally in pairs or short chains. Colonies of both strains after 4 days anaerobic incubation at 37 °C on modified GAM agar were 0.1–1.5 mm in diameter, entire, circular, beige or grey-beige and opaque. The major end products of glucose fermentation of strain YIT 12063^T in PYG broth were lactic (9.5 mM) and acetic (2.5 mM) acids; small amounts of formic and succinic acids were also detected. On the other hand, glucose fermentation by strain YIT 12064 in PYG broth was very weak and small amounts of lactic, acetic and formic acids were detected. However, when grown in modified GAM broth, this strain was stimulated by glucose: large amounts of lactic (28.9 mM), formic (10.8 mM) and acetic (9.8 mM) acids were detected after incubation in modified GAM broth supplemented with 1% glucose. Strains YIT 12063^T and YIT 12064 were both resistant to 20% bile. They were saccharolytic in the API test system, positive for aesculin hydrolysis and negative for catalase, urease, oxidase, indole production, nitrate reduction and gelatin hydrolysis. Other biochemical characteristics obtained by using the API systems (API Rapid ID32A, API ZYM and API 20A) are included in the species description of *Collinsella tanakaei* sp. nov.

Fig. 1. Phylogenetic tree showing the positions of strains YIT 12062^T, YIT 12063^T, YIT 12064 and the type strains of species of the family Coriobacteriaceae based on 16S rRNA gene sequence analysis. The tree was rooted with *Bifidobacterium bifidum* ATCC 29521^T and was constructed by using the neighbour-joining method and based on a comparison of approximately 1200 nt, with bootstrap values (>500) expressed as scores out of 1000 replications. Similar tree topologies were obtained by using the MP and ML methods (data not shown). Circles indicate that the corresponding nodes were also recovered in trees generated with the MP and ML methods. GenBank/EMBL/DDBJ accession numbers are shown in parentheses. Bar, 0.1 substitutions per nucleotide position.

The fatty acid profiles of strains YIT 12063^T and YIT 12064 and the type strains of species of the genus *Collinsella* are detailed in Supplementary Table S2 (available in IJSEM Online). All of these strains contained C_{18:1}ω9c (35–51 %) and C_{18:1}ω9c DMA (25–37 %) as the predominant fatty acids. The fatty acid profile of strain YIT 12063^T was very similar to that of *C. aerofaciens* and that of strain YIT 12064 was similar to those of *C. intestinalis* and *C. stercoris*. Respiratory quinones were not detected in strains YIT 12063^T, YIT 12064 or the type strains of *C. aerofaciens*, *C. intestinalis* and *C. stercoris*.

Regions of the 16S rRNA genes of YIT 12063^T (1491 bp) and YIT 12064 (1460 bp) were sequenced. They shared >99 % 16S rRNA gene sequence similarity and >97 ± 1.8 % DNA–DNA relatedness. Although their phenotypic characteristics were also very similar, they could be discriminated by RAPD fingerprinting (Supplementary Fig. S1, available in IJSEM Online). Sequence database searches of recognized taxa revealed that strains YIT 12063^T and YIT 12064 were related most closely to species of the genus *Collinsella*, with 16S rRNA gene sequence similarities of 93.8, 95.0 and 95.1 % to the type strains of *C. aerofaciens*, *C. intestinalis* and *C. stercoris*, respectively (Fig. 1).

However, the most similar 16S rRNA gene sequences were derived from uncultured bacterial clones. At the time of writing, the 16S rRNA gene sequences of nine uncultured bacterial clones deposited in GenBank/EMBL/DDBJ had >99 % similarity to that of strain YIT 12063^T. Some of these were faecal uncultured clones from a human (GenBank accession no. EF399657), reported by Li *et al.* (2008), an armadillo (accession no. EU467584), a squirrel (accession no. EU779003) and a lion (accession no. EU776612) reported by Ley *et al.* (2008) (Fig. 1).

Table 2 shows the characteristics that can be used to differentiate between isolates YIT 12063^T and YIT 12064 and members of the genus *Collinsella*. The DNA G+C contents of strains YIT 12063^T and YIT 12064 were 60.8 and 61.0 mol%, respectively.

Although there is no direct evidence that members of the species represented by strains YIT 12062^T and YIT 12063^T have been isolated from human faeces as common members of the human indigenous microbiota, as described above, evidence in favour of this comes from the fact that many clones from uncultured human intestinal bacteria with highly similar 16S rRNA gene sequences to those of strains YIT 12062^T and YIT 12063^T have been deposited in GenBank/EMBL/DDBJ (Fig. 1).

It is evident from the results presented that strains YIT 12062^T and YIT 12063^T recovered from human faeces represent novel species within the genera *Slackia* and *Collinsella*, respectively, for which the names of *Slackia piriformis* sp. nov. and *Collinsella tanakaei* sp. nov. are proposed.

Emended description of the genus *Slackia* Wade *et al.* (1999)

The genus description is as given by Wade *et al.* (1999) with the addition that sensitivity to bile is variable and DNA G+C content is 58–64 mol%.

Description of *Slackia piriformis* sp. nov.

Slackia piriformis (pi.ri.for'mis. L. n. *pirum* pear; L. adj. suffix *-formis* -like, in the shape of; N.L. fem. adj. *piriformis* pear-shaped, referring to the cell shape).

Cells are Gram-positive, obligately anaerobic, non-motile, approximately 0.4–1.1 × 0.9–2.6 µm in size and form chains of pear-shaped to irregularly shaped rods. Colonies after 4 days of growth at 37 °C on modified GAM agar under anaerobic conditions are 0.1–1.0 mm in diameter, translucent to beige and circular, with an uneven surface and irregular edges. Cells are asaccharolytic and weakly resistant to 20 % bile. Negative for nitrate reduction, catalase, urease, oxidase, and hydrolysis of aesculin and gelatin. Indole is not produced. In the API test systems (API Rapid ID32A and API ZYM), positive reactions are obtained for alanine arylamidase, arginine dihydrolase, cystine arylamidase, glycine arylamidase, histidine arylamidase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase, phenylalanine arylamidase, proline arylamidase, serine arylamidase, tyrosine arylamidase and valine arylamidase, and a weakly positive reaction is observed for acid phosphatase; *N*-acetyl-β-glucosaminidase, alkaline phosphatase, α-arabinosidase, chymotrypsin, esterase (C4), esterase lipase (C8), α-fucosidase, α-galactosidase, β-galactosidase, 6-phospho-β-galactosidase, α-glucosidase, β-glucosidase, β-glucuronidase, glutamic acid decarboxylase, glutamyl glutamic acid arylamidase, leucyl glycine arylamidase, lipase (C14), α-mannosidase, pyroglutamic acid arylamidase and trypsin activities are not detected. The major cellular fatty acids are C_{18:1}ω9c and C_{18:1}ω9c DMA. Respiratory quinones are not detected.

The type strain is YIT 12062^T (=DSM 22477^T=JCM 16070^T), isolated from human faeces. The DNA G+C content of the type strain is 58.3 mol%.

Description of *Collinsella tanakaei* sp. nov.

Collinsella tanakaei (ta.na.ka'e.i. N.L. masc. gen. n. *tanakaei* of Tanaka, to honour Ryuichiro Tanaka, a Japanese microbiologist, for his contribution to increased knowledge about human intestinal microbiota and probiotics).

Cells are Gram-positive, obligately anaerobic, non-motile, approximately 0.5–1.0 × 1.0–3.3 µm in size and occur singly or in long chains of short rods. Colonies after 4 days of growth at 37 °C on modified GAM agar under anaerobic conditions are 0.1–1.5 mm in diameter, entire, circular, beige or grey-beige, and opaque. The major end

Table 2. Major characteristics of strains YIT 12063^T and YIT 12064 and other members of the genus *Collinsella*

Taxa: 1, strain YIT 12063^T; 2, strain YIT 12064; 3, *C. aerofaciens* YIT 10235^T (=DSM 3979^T); 4, *C. intestinalis* YIT 10051^T (=JCM 10643^T); 5, *C. stercoris* YIT 10052^T (=JCM 10641^T). Data are from the present study unless otherwise indicated. +, Positive; –, negative; v, variable. In the API ZYM and Rapid ID32A test systems, all strains were positive for arginine arylamidase, arginine dihydrolase, glycine arylamidase, histidine arylamidase, leucine arylamidase, leucyl glycine arylamidase and naphthol-AS-BI-phosphohydrolase, but negative for α -arabinosidase, chymotrypsin, cystine arylamidase, esterase (C4), esterase lipase (C8), α -fucosidase, α -galactosidase, glutamic acid decarboxylase, glutamyl glutamic acid arylamidase, lipase (C14), α -mannosidase, phenylalanine arylamidase, pyroglutamic acid arylamidase, trypsin, urease and valine arylamidase. In the API 20A test system, all strains produced acid from glucose and D-mannose, but not from L-arabinose, glycerol, D-mannitol, melezitose, raffinose, L-rhamnose, D-sorbitol or D-xylose.

Characteristic	1	2	3	4	5
Morphology	Rods	Rods	Cocci to rods	Rods	Rods
Major products from PYG*	lafs	laf (LFA)†	lf	lf	lf
DNA G + C content (mol%)	60.8	61.0	61.0‡	64.4‡	61.2‡
Acid produced from (API 20A):					
Cellobiose	+	+	–	+	+
Lactose	+	+	+	–	+
Maltose	+	+	+	–	+
Salicin	+	+	+§	–§	+
Sucrose	+	+	+	–	+§
Trehalose	+	+	–	–	+§
API tests (API ZYM, Rapid ID32A)					
Acid phosphatase	+	+	–	+	+
Alanine arylamidase	–	–	–	–	+
Alkaline phosphatase	+	+	–	+	+
β -Galactosidase	–	–	+	–	+
α -Glucosidase	–	–	+	–	–
β -Glucosidase	+	+	–	v	+
β -Glucuronidase	+	–	–	–	–
N-Acetyl β -glucosaminidase	–	–	–	+	+
6-Phospho- β -galactosidase	–	–	–	+	–
Proline arylamidase	+	+	+	–	–
Serine arylamidase	–	–	–	–	+
Tyrosine arylamidase	–	–	–	–	+

*Lower-case letters indicate <1 mequiv. acid per 100 ml; a, acetic acid; l, lactic acid; s, succinic acid; f, formic acid. Data for strains YIT 12063^T and YIT 12064 are from this study and those for *C. aerofaciens*, *C. intestinalis* and *C. stercoris* are from Kageyama *et al.* (1999) and Kageyama & Benno (2000).

†Letters in parentheses indicate products formed when grown in GAM broth supplemented with 1 % glucose (see text for details).

‡Data for DNA G + C content for *C. aerofaciens* are from Kageyama *et al.* (1999) and those for *C. intestinalis* and *C. stercoris* are from Kageyama & Benno (2000).

§Data from this study (tested by using API 20A) were different from the published data (Kageyama *et al.*, 1999 and Kageyama & Benno, 2000, tested by using the conventional culture method as described by Holdeman *et al.*, 1977).

products of glucose metabolism are lactic, acetic and formic acids. Cells are resistant to 20 % bile and positive for aesculin hydrolysis. Nitrate reduction, indole, oxidase, urease, catalase and gelatin hydrolysis are negative. In API 20A tests, positive for acid production from cellobiose, glucose, lactose, maltose, D-mannose, salicin, sucrose and trehalose; acid is not produced from L-arabinose, glycerol, D-mannitol, melezitose, raffinose, L-rhamnose, D-sorbitol or D-xylose. By using the API Rapid ID32A and API ZYM test systems, acid phosphatase, alkaline phosphatase, arginine arylamidase, arginine dihydrolase, β -glucosidase, β -glucuronidase, glycine arylamidase, histidine arylamidase, leucine arylamidase, leucyl glycine arylamidase,

naphthol-AS-BI-phosphohydrolase and proline arylamidase activities are detected; N-acetyl- β -glucosaminidase, alanine arylamidase, α -arabinosidase, chymotrypsin, cystine arylamidase, esterase lipase (C8), esterase (C4), α -fucosidase, α -galactosidase, β -galactosidase, 6-phospho- β -galactosidase, α -glucosidase, glutamic acid decarboxylase, glutamyl glutamic acid arylamidase, lipase (C14), α -mannosidase, phenylalanine arylamidase, pyroglutamic acid arylamidase, serine arylamidase, trypsin, tyrosine arylamidase and valine arylamidase activities are not detected. The major cellular fatty acids are C_{18:1} ω 9c and C_{18:1} ω 9c DMA. Respiratory quinones are not detected. The DNA G + C content range is 60.8–61.0 mol%.

The type strain is YIT 12063^T (=DSM 22478^T=JCM 16071^T), isolated from human faeces. Strain YIT 12064 (=DSM 22514=JCM 16103) is also assigned to this species.

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