Emendation of genus *Collinsella* and proposal of *Collinsella stercoris* sp. nov. and *Collinsella intestinalis* sp. nov.

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***Collinsella aerofaciens*-like strains isolated from human faeces were characterized by biochemical tests, cell wall murein analysis and 16S rDNA analysis. The results indicated that these strains are phylogenetically a member of the family Coriobacteriaceae and close to the genus *Collinsella*. Their phenotypic characters resembled those of *Collinsella aerofaciens*. Determination of DNA–DNA relatedness showed that these strains could be divided into two groups (groups 1 and 2). *Collinsella aerofaciens* and both new groups have A4-type cell wall murein. Based on their phenotypic and phylogenetic characters, two new species of the genus *Collinsella* are proposed for the isolated strains: *Collinsella stercoris* for group 1 and *Collinsella intestinalis* for group 2. Species-specific PCR primer sets for these two species were also constructed. Using these primer sets, *Collinsella stercoris* and *Collinsella intestinalis* can be identified easily and rapidly.**

Keywords: *Collinsella stercoris* sp. nov., *Collinsella intestinalis* sp. nov., 16S rDNA, cell wall, PCR

INTRODUCTION

Collinsella aerofaciens belongs to the family Coriobacteriaceae and is close to the genus *Coriobacterium* (Hass & König, 1988) or *Eggerthella* (Wade *et al.*, 1999; Kageyama *et al.*, 1999c). In 1971, Moore *et al.* reported that these organisms belonged to the genus *Eubacterium* because they are Gram-positive, obligatory anaerobic, non-spore-forming rods which do not produce propionic acid as a major product (*Propionibacterium*); lactic acid as a sole major acid product (*Lactobacillus*); large amounts of succinic (in the presence of CO₂) and lactic acids, sometimes with acetic and formic acids (*Actinomyces*); or acetic and lactic acids (acetic > lactic), with or without formic acid, as sole major acid products (*Bifidobacterium*) (Moore *et al.*, 1971). The genus *Eubacterium* has a broad definition and has over the years acted as a repository for a large number of phenotypically diverse species (Andreesen, 1992). Currently, some species that belonged to the genus *Eubacterium* have been

transferred to other genera or new genera with specific phylogenetic characters (Kageyama *et al.*, 1999a, b; Ludwig *et al.*, 1992; Wade *et al.*, 1999). In particular, *Eubacterium aerofaciens* was reclassified on the basis of phylogenetic characters and cell wall peptidoglycan type, and transferred to the new genus *Collinsella* with one species (Kageyama *et al.*, 1999a). *Collinsella aerofaciens* is the most abundant bacterium in the human intestine (Benno *et al.*, 1986; Finegold & Sutter, 1978; Moore & Holdeman, 1974). However, some strains identified as *Collinsella aerofaciens* by biochemical tests or morphology were actually new species.

In this paper we report on the phenotypic and phylogenetic characterization of 10 *Collinsella aerofaciens*-like strains from human faeces. On the basis of the results presented, we propose that these organisms be classified as *Collinsella stercoris* sp. nov. and *Collinsella intestinalis* sp. nov. Furthermore, species-specific PCR primer sets for these two new species were constructed. Using these primer sets, we can identify the two new species rapidly and easily.

METHODS

Bacterial strains studied and cultivation. Strains RCA55-4 (JCM 10639), RCA55-5 (JCM 10708), RCA55-27 (JCM

The DDBJ accession numbers for the 16S rDNA sequences of *Collinsella stercoris* JCM 10639–10642 and JCM 10708–10711, and for *Collinsella intestinalis* JCM 10646 and 10647 are AB030159–AB030162, AB037381, AB037382, AB037384 and AB037385, and AB031063 and AB037383, respectively.

10709), RCA55-34 (JCM 10640), RCA55-49 (JCM 10710), RCA55-54 (JCM 10641^T), RCA55-56 (JCM 10642), RCA55-58 (JCM 10711), RCA56-68 (JCM 10646^T) and RCA56-80 (JCM 10647) used in this study were isolated from human faeces. All bacterial strains were cultivated for 2 d at 37 °C on EG agar [premixed EG agar (Eiken Chemical) supplemented with 5% horse blood containing 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 Na₂HPO₄, 0.5 g soluble starch, 0.5 g Tween 80, 0.5 g silicone and 15 g agar in 1000 ml distilled water; pH 7.7] in an anaerobic jar with 100% CO₂.

Phenotypic characteristics. Acid production from carbohydrates was tested as described previously (Holdeman *et al.*, 1977; Kaneuchi *et al.*, 1976). Activities of 18 enzymes were tested by API ZYM (bioMérieux).

Chemotaxonomy. Cells for chemotaxonomic analyses were obtained from cultures grown in EGF broth [containing 2.4 g Lab-Lemco powder (Oxoid), 10 g proteose peptone no. 3 (Difco), 5 g yeast extract (Difco), 4 g Na₂HPO₄, 40 ml sterilized horse blood, 5 g glucose, 0.5 g soluble starch (Sigma) and 0.5 g L-cysteine.HCl in 960 ml distilled water; pH 7.6] in an anaerobic chamber at 37 °C.

Cell wall peptidoglycan was prepared and hydrolysed by the methods of Kawamoto *et al.* (1981), and the amino acid composition was analysed with an automatic amino acid analyser (Hitachi; model 835). The neutral amino acid fraction was reacted with a chiral reagent [(+)-1-(9-fluorenyl)ethyl chloroformate] and used for HPLC as described by Einarsson & Josefsson (1987).

DNA studies. DNA was isolated as described by Saito & Miura (1983). DNA base composition was estimated by HPLC (Tamaoka & Komagata, 1984). Levels of DNA–DNA relatedness were determined by the method of Ezaki *et al.* (1989) using photobiotin and microplates.

16S rDNA sequencing. Nearly complete 16S rDNA gene sequences of all isolated strains were determined. The 16S rDNA gene was amplified using the PCR method and prokaryotic 16S rDNA universal primers 27F (5'-AGAG-TTGATCTGGCTCAG-3') and 1492R (5'-GGTAC-CTTGTTACGACTT-3'). 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).

Phylogenetic analysis. Species related to the new isolates were determined by performing a sequence database search using FASTA and RDP. 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) (Fig. 1). The DNAML program in the PHYLIP 3.5c package (Felsenstein, 1985) was used for the maximum-likelihood analysis, with the default transition/transversion ratio of 2.000000 (Fig. 2). The sequence similarity values given in Table 4 were determined visually. The DDBJ accession numbers for the 16S rDNA sequences of *Collinsella stercoris* JCM 10639–10642 and JCM 10708–10711, and for *Col-*

linsella intestinalis JCM 10646 and 10647 are AB030159–AB030162, AB037381, AB037382, AB037384 and AB037385, and AB031063 and AB037383, respectively.

Primer design. To design the species-specific primer, we compared 16S rDNA sequences of all isolated strains and *Collinsella aerofaciens* JCM 10188^T, JCM 7790 and JCM 7791, and then searched for specific positions. We designed two sets of primers: STER-F and STER-R, and INTE-F and INTE-R. The specificity was evaluated by performing a sequence database search using FASTA and RDP.

PCR reaction. PCR was performed using a DNA thermal cycler with 25 cycles consisting of denaturation at 94 °C for 60 s, primer annealing at 58 °C for 150 s and primer extension at 72 °C for 150 s with each of the two sets of primers as mentioned above. The sample DNA was prepared by suspending the cultured colony in distilled water, heating at 100 °C for 5 min and then rapidly cooling it down. After PCR, an 8 µl aliquot of amplified sample from each PCR tube was electrophoresed through 2% agarose gel (Sigma) in TAE buffer for 30 min at 100 V. Amplification products were visualized and photographed under a UV light transilluminator after 30 min of ethidium bromide staining. The molecular masses of the amplicons were determined by comparison with commercial DNA molecular mass markers.

RESULTS

Morphological characteristics

All of the strains which we studied were Gram-positive, non-sporing, anaerobic rods. Colonies were white or grey in the centre with clear margins on EG agar. Cells were 0.3–0.5 × 1.2–2.4 µm long and occurred in chains of 2–20 cells.

Biochemical and physiological tests

The 10 isolated strains of *Collinsella aerofaciens*-like micro-organisms were characterized. They were divided into two groups: group 1 consisted of eight strains (RCA55-4, RCA55-5, RCA55-27, RCA55-34, RCA55-49, RCA55-54, RCA55-56 and RCA55-58) and group 2 consisted of two strains (RCA56-68 and RCA56-80). Group 1 strains produced acid from glucose, mannose, galactose, fructose, maltose, cellobiose and lactose. None of them produced acid from L-arabinose, D-xylose, rhamnose, ribose, sucrose, raffinose, melezitose, starch, glycogen, mannitol, sorbitol, inositol, erythritol or aesculin (Table 1). They showed strong activity of leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase and N-acetyl-β-glucosaminidase, and weak activity of alkaline phosphatase, α-glucosidase and β-glucosidase. They did not show activity of lipase (C4), valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, α-galactosidase, β-glucuronidase, α-mannosidase or α-fucosidase (Table 2). Group 2 strains produced acid from glucose, mannose, galactose, fructose and cellobiose. None of them produced acid from L-arabinose, D-xylose, rhamnose, sucrose, maltose, lactose, trehalose, raffinose, melezitose, starch, glycogen, mannitol, sorbitol, inositol,

Table 1. Acid production by *Collinsella aerofaciens*-like strains and *Collinsella aerofaciens*

+, Positive; –, negative; V, variable.

Acid from:	<i>Collinsella aerofaciens</i> -like strains*		<i>Collinsella aerofaciens</i> †
	Group 1	Group 2	
Rhamnose	–	–	V
Ribose	–	V	V
Sucrose	–	–	V
Maltose	+	–	+
Cellobiose	+	+	V
Lactose	+	–	+
Trehalose	V	–	V
Raffinose	–	–	V
Aesculin	–	V	V
Salicin	V	V	V
Amygdalin	V	–	V

*Data based on reactions of 10 strains isolated in this study.

†Data based on reactions of 181 strains (Table 1, Kageyama *et al.*, 1999a).

erythritol or amygdalin (Table 1). They showed strong activity of leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and *N*-acetyl- β -glucosaminidase, and weak activity of alkaline phosphatase. They did not show activity of lipase (C4), valine arylamidase, cystine arylamidase, trypsin,

chymotrypsin, α -galactosidase, β -glucuronidase, α -mannosidase or α -fucosidase (Table 2).

Chemotaxonomic characteristics

The structure of the cell wall peptidoglycan of RCA55-54, RCA56-68 and RCA56-80 was determined. In RCA55-54, the peptidoglycan contained, in addition to muramic acid and glucosamine, the amino acids glutamic acid, aspartic acid, ornithine and alanine at a molar ratio of 1:1:1:2. Ornithine occurred in the L configuration and aspartic acid in the D configuration. This indicated that the structural type was the A4 β -type, (L-Ala)-D-Glu-L-Orn-D-Asp. In RCA56-68 and RCA56-80, the peptidoglycan contained, in addition to muramic acid and glucosamine, the amino acids glutamic acid, lysine and alanine at a molar ratio of 2:1:2. Glutamic acid occurred in the D configuration and lysine in the L configuration. This indicated that the structural type was the A4 α -type, (L-Ala)-D-Glu-L-Lys-D-Glu.

DNA base composition and DNA–DNA relatedness

The DNA base composition of RCA55-4, RCA55-54, RCA55-56, RCA56-68 and RCA56-80 was 61.2–64.4 mol% G+C according to the HPLC nucleoside method (Table 3). The levels of DNA–DNA relatedness among these five isolated strains and *Collinsella aerofaciens* JCM 10188^T were determined, and the values among RCA55-4, RCA55-54 and RCA55-56 were 83.1–88.3% and between RCA56-68 and RCA56-80 were 100–101%. Therefore, these two groups were independent species. There was a low level

Table 2. Enzymic activity of *Collinsella aerofaciens*-like strains and *Collinsella aerofaciens*

+, Positive; W, weak; –, negative; V, variable.

Enzyme	<i>Collinsella aerofaciens</i> -like strains*		<i>Collinsella aerofaciens</i> †
	Group 1	Group 2	
Alkaline phosphatase	W	W	–
Esterase lipase (C8)	–	–	V
Leucine arylamidase	+	+	W
Acid phosphatase	+	+	W
Naphthol-AS-BI-phosphohydrolase	+	+	V
β -Galactosidase	+	–	V
α -Glucosidase	W	–	V
β -Glucosidase	W	–	V
<i>N</i> -Acetyl- β -glucosaminidase	+	+	–

*Data based on reactions of 10 strains isolated in this study.

†Data based on reactions of strains JCM 10188^T, JCM 7790, JCM 7791, JCM 10777, JCM 10788 and JCM 10800.

Table 3. DNA base composition and levels of DNA–DNA relatedness among isolated *Collinsella aerofaciens*-like strains and *Collinsella aerofaciens* JCM 10188^T

Strain	G + C content (mol%)	Percentage DNA–DNA reassociation with:		
		RCA55-54	RCA56-68	RCA56-80
RCA55-4	63.8	88.3	28.5	31.4
RCA55-54	61.2	100	24.7	22.2
RCA55-56	63.4	83.1	26.7	25.7
RCA56-68	64.4	22.6	100	101
RCA56-80	63.6	18.2	100	100
<i>Collinsella aerofaciens</i> JCM 10188 ^T	60.8	8.3	8.3	9.0

Fig. 1. Phylogenetic tree derived from 16S rDNA sequences. The tree was created using the neighbour-joining method and K_{nuc} values. The numbers on the tree indicate bootstrap values for the branch points. Only values above 50% significance are indicated.

of DNA–DNA relatedness between the two groups, but it was not enough to assign them to the same species.

16S rDNA sequence analysis

More than 1400 bases of the 16S rDNA sequences (positions 28–1491; *Escherichia coli* numbering system) of all isolated strains were determined, and these sequences have been deposited in the DDBJ database. Fig. 1 shows a phylogenetic tree based on calculated K_{nuc} values created by using our sequences and those obtained from public databases. Fig. 2 shows a phylogenetic tree based on maximum-likelihood

analysis. A database search demonstrated that these isolated strains belong to the family *Coriobacteriaceae*, which contains the genera *Coriobacterium*, *Atopobium* (Collins & Wallbanks, 1992), *Eggerthella* and *Collinsella*. A phylogenetic analysis showed that these isolated strains were closest to *Collinsella aerofaciens* JCM 10188^T. The sequence similarity values among these strains are listed in Table 4.

Primer design and specificity

We compared 16S rDNA sequences of the 10 isolated strains and *Collinsella aerofaciens* JCM 10188^T, JCM 7790 and JCM 7791, and designed two sets of

Fig. 2. Phylogenetic tree derived from 16S rDNA sequences. The tree was created using maximum-likelihood analysis.

Table 4. Percentage similarity of 16S rDNA sequences of isolated *Collinsella aerofaciens*-like strains and *Collinsella aerofaciens* JCM 10188^T

	1	2	3	4	5	6	7	8	9	10
1. RCA55-4										
2. RCA55-5	98.5									
3. RCA55-27	98.4	99.1								
4. RCA55-34	98.4	98.7	99.1							
5. RCA55-49	98.8	99.0	99.2	99.0						
6. RCA55-54	99.2	99.1	99.5	99.1	99.5					
7. RCA55-56	98.8	99.0	98.8	98.9	98.7	99.2				
8. RCA55-58	98.2	98.6	98.7	98.7	98.9	98.8	98.4			
9. RCA56-68	96.8	97.4	97.4	97.3	97.3	97.5	97.3	96.7		
10. RCA56-80	96.5	97.2	97.3	97.2	97.1	97.3	97.1	96.7	99.6	
11. <i>Collinsella aerofaciens</i> JCM 10188 ^T	91.6	92.6	92.5	92.6	92.1	92.5	92.4	92.1	92.4	92.1

primers: STER-F (5'-TGCAAGTCGAACGGCAC-CCG-3') (positions 56–75; *Escherichia coli* numbering system) and STER-R (5'-CCGTCTGGGCTTTG-CCGCC-3') (positions 223–204), and INTE-F (5'-CTTACCAGGGCTTGACATGA-3') (positions 980–999) and INTE-R (5'-TTAGGCGCCTCCC-CCCAGAT-3') (positions 1469–1450). Using primers STER-F and STER-R, a 158 bp amplicon was obtained, and using primers INTE-F and INTE-R,

a 486 bp amplicon was obtained. The specificity of the primers was checked by performing PCR with the 10 isolated strains, *Collinsella aerofaciens* JCM 10188^T, *Coriobacterium glomerans* JCM 10262^T and *Eggerthella lenta* JCM 9970^T (Table 5). STER-F and STER-R were positive only for RCA55-4, RCA55-5, RCA55-27, RCA55-34, RCA55-49, RCA55-54, RCA55-56 and RCA55-58. INTE-F and INTE-R were positive only for RCA56-68 and RCA56-80.

Table 5. Results of PCR reactions

+, Positive; –, negative.

Strains	PCR reaction	
	STER-F + STER-R	INTE-F + INTE-R
RCA55-4, RCA55-5, RCA55-27, RCA55-34, RCA55-49, RCA55-54, RCA55-56, RCA55-58	+	–
RCA56-68, RCA56-80	–	+
<i>Collinsella aerofaciens</i> JCM 10188 ^T	–	–
<i>Coriobacterium glomerans</i> JCM 10262 ^T	–	–
<i>Eggerthella lenta</i> JCM 9970 ^T	–	–

DISCUSSION

Collinsella aerofaciens is the most abundant bacterium in the human intestine [about 10^{10} cells (g faeces)^{–1}], and is found in more than 90 % of human intestines (Benno *et al.*, 1986; Finegold & Sutter, 1978; Moore & Holdeman, 1974). This organism has been reclassified on the basis of phylogenetic characters and cell wall peptidoglycan type, and transferred from *Eubacterium aerofaciens* to the new genus *Collinsella* with one species (Kageyama *et al.*, 1999a). Moore & Holdeman (1974) reported that *Eubacterium aerofaciens* isolated from human intestine could be divided into three main groups (I–III) according to sucrose and cellobiose fermentation patterns. In our previous study, 181 strains of this organism isolated from human faeces over 10 years were divided into four groups (I–IV) on the basis of sucrose and cellobiose fermentation patterns. Three groups, I, II and III, were the same as those of Moore & Holdeman (1974), and an additional group, IV, was described (Kageyama *et al.*, 1999a).

On the other hand, identification of *Collinsella aerofaciens* was difficult and required considerable time. Previously, a rapid and easy identification method using species-specific PCR primers for *Collinsella aerofaciens* was described (Kageyama *et al.*, 2000), and 181 *Collinsella aerofaciens*-like strains were identified using this method. However, some *Collinsella aerofaciens*-like strains were not positive for this method. The morphological and biochemical characters of the strains, however, were almost the same as those of *Collinsella aerofaciens*. We consider that these strains are different species and interesting candidates for study.

The phylogenetic positions of these strains were determined, and some strains were found to be close to *Collinsella aerofaciens*. The 16S rDNA sequence of all isolated strains was determined. A database search revealed that these strains belong to class *Coriobacteriales*, family *Coriobacteriaceae* (Stackebrandt *et al.*, 1997), and are most closely related to *Collinsella aerofaciens*. Phylogenetic analysis including all species

of the family *Coriobacteriaceae* was performed by two different methods, a neighbour-joining method and maximum-likelihood analysis (Figs 1 and 2, respectively). Both trees showed that the isolated strains are closest to *Collinsella aerofaciens*. All isolated strains were closely related to each other, but RCA56-68 and RCA56-80 were slightly separated from the other eight strains. The sequence similarity values among the eight strains were 98.2–99.5 %, but the sequence similarity values between these eight strains and RCA56-68 and RCA56-80 were 96.5–97.5 %, which is not very high (Table 4). Therefore, we checked the similarity of these strains based on DNA–DNA hybridization. The results showed that the two bacterial groups were both new species (Table 3). Strains RCA56-68 and RCA56-80 (group 2) belonged to one species, and the other strains (group 1) belonged to another species.

It is clear that the isolated strains were divided into two species and that they are related to *Collinsella aerofaciens*. The phenotypic differential characters of these three species were fermentation of maltose and lactose and activity of *N*-acetyl- β -glucosaminidase.

Collinsella aerofaciens contains A4 β -type peptidoglycan and the G + C content of the DNA is 60–61 mol %. RCA55-54 (group 1) contains A4 β -type peptidoglycan, and RCA56-68 (group 2) and RCA56-80 (group 2) contain A4 α -type peptidoglycan. The G + C contents of the DNA are 61–65 mol % (group 1) and 64–65 mol % (group 2).

We noted that *Collinsella aerofaciens* and the two new species have different cell wall peptidoglycan types and phylogenetic characters. However, identification using these differential characters is very difficult and time-consuming. Therefore, a different, rapid and easy method was required for identification of these microorganisms. Species-specific primers were designed by comparing 16S rDNA sequences of the isolated strains and *Collinsella aerofaciens*. The specificity of these primers was checked by performing PCR with the 10 isolated strains, *Collinsella aerofaciens* JCM 10188^T,

Coriobacterium glomerans JCM 10262^T and *Eggerthella lenta* JCM 9970^T. The results showed that the primer sets were accurate in identification of the two new species.

From phenotypic characters and phylogenetic analysis, it is evident that these two new species belong to the genus *Collinsella* and we propose the names *Collinsella stercoris* sp. nov. and *Collinsella intestinalis* sp. nov.

Emended description of the genus *Collinsella* (Kageyama, Benno and Nakase)

Cells occur in chains of rod-shaped cells. Gram-positive and obligately anaerobic. Spores and flagella are absent. Fermentation products of glucose are H₂, ethanol, formate and lactate. Cell wall contains A4-type peptidoglycan. DNA G+C content is 60–65 mol%. Type species is *Collinsella aerofaciens*. The genus *Collinsella* is a member of the *Coriobacteriaceae* and exhibits a close phylogenetic association with the genus *Coriobacterium*.

Description of *Collinsella stercoris* sp. nov.

Collinsella stercoris (ster'co.ris. L. n. *stercus* faeces; L. gen. n. *stercoris* of faeces, referring to the source of the isolate).

Cells are 0.3–0.4 × 1.3–2.4 µm in size and occur in chains of 2–20 cells. Colonies are white or grey in the centre with clear margins on EG agar. Gram-positive and obligately anaerobic. Spores and flagella are absent. Can be cultivated for 2 d at 37 °C on EG agar in an anaerobic jar with 100% CO₂. Cells produce acid from glucose, mannose, galactose, fructose, maltose, cellobiose, lactose and salicin, but not from L-arabinose, D-xylose, rhamnose, ribose, sucrose, trehalose, raffinose, melezitose, starch, glycogen, mannitol, sorbitol, inositol, erythritol, aesculin or amygdalin. Cells show strong activity of leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase and *N*-acetyl-β-glucosaminidase, but do not show any activity of lipase (C4), valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, α-galactosidase, β-glucuronidase, α-mannosidase or α-fucosidase. Cell wall contains A4β-type peptidoglycan with an (L-Ala)-D-Glu-L-Orn-D-Asp peptide subunit, and interpeptide bridge consists only of D-Asp. DNA G+C content is 61.2 mol%. Isolated from human faeces. Type strain is JCM 10641^T (= DSM 13279^T).

Description of *Collinsella intestinalis* sp. nov.

Collinsella intestinalis (in'test.in.alis. N.L. adj. *intestinalis* pertaining to the intestine).

Cells are 0.3–0.5 × 1.2–2.2 µm in size and occur in chains of 2–20 cells. Colonies are white or grey in the centre with clear margins on EG agar. Gram-positive

and obligately anaerobic. Spores and flagella are absent. Can be cultivated for 2 d at 37 °C on EG agar in an anaerobic jar with 100% CO₂. Cells produce acid from ribose, glucose, mannose, galactose, fructose, cellobiose and salicin, but not from L-arabinose, D-xylose, rhamnose, sucrose, maltose, lactose, trehalose, raffinose, melezitose, starch, glycogen, mannitol, sorbitol, inositol, erythritol, aesculin or amygdalin. Cells show strong activity of leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and *N*-acetyl-β-glucosaminidase, but do not show any activity of lipase (C4), valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, α-galactosidase, β-glucuronidase, α-mannosidase or α-fucosidase. Cell wall contains A4α-type peptidoglycan with an (L-Ala)-D-Glu-L-Lys-D-Glu peptide subunit, and interpeptide bridge consists only of D-Glu. DNA G+C content is 64.4 mol%. Isolated from human faeces. Type strain is JCM 10643^T (= DSM 13280^T).

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