Asaccharobacter celatus gen. nov., sp. nov., isolated from rat caecum

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An obligately anaerobic and equol-producing bacterium, designated strain do03^T, was isolated from the caecal content of a rat. Cells were Gram-positive, non-spore-forming rods. The results from a phylogenetic analysis based on 16S rRNA gene sequences showed that strain do03^T formed a separate line of descent in the phylogenetic cluster of the family *Coriobacteriaceae*. The strain was unable to metabolize glucose or other carbohydrates as sole carbon sources; growth was enhanced in the presence of arginine. The cell wall contained meso-diaminopimelic acid. The major fatty acid was C_{18:1}cis9 (54.0%). The strain had one unidentified predominant (91.9%) quinone that was not menaquinone, methylmenaquinone, demethylmenaquinone, ubiquinone or rhodoquinone. The DNA G+C content was 63 mol%. The data presented in this work show that strain do03^T differs from members of the related recognized genera *Eggerthella* and *Denitrobacterium* at both the phylogenetic and phenotypic level. Therefore, the strain constitutes a novel genus and species, for which the name *Asaccharobacter celatus* gen. nov., sp. nov. is proposed. The type strain of the type species is do03^T (=JCM 14811^T=DSM 18785^T=AHU 1763^T).

The family *Coriobacteriaceae* was proposed by Stackebrandt *et al.* (1997) and, at the time of writing, comprises the following eight genera: *Olsenella* (Dewhirst *et al.*, 2001), *Atopobium* (Collins & Wallbanks, 1992), *Slackia* (Wade *et al.*, 1999), *Cryptobacterium* (Nakazawa *et al.*, 1999), *Eggerthella* (Kageyama *et al.*, 1999; Wade *et al.*, 1999), *Denitrobacterium* (Anderson *et al.*, 2000), *Coriobacterium* (Haas & König, 1988) and *Collinsella* (Kageyama & Benno, 2000). A Gram-positive bacterium, designated strain do03^T, capable of converting daidzein to equol via dihydrodaidzein was isolated from the caecal content of a rat (Minamida *et al.*, 2006a). The 16S rRNA gene sequence (1428 bp) of the novel strain showed highest similarity (99%) with that of the human intestinal bacterium SNU-Julong 732 (GenBank accession no. AY310748), which was capable of metabolizing dihydrodaidzein to S-equol (Wang *et al.*, 2005). The levels of similarity with respect to members of the three recognized species of the genus *Eggerthella*, *Eggerthella sinensis* HKU14^T (AY321958), *Eggerthella hongkongensis* HKU10^T (AY288517) and *Eggerthella lenta* ATCC 25559^T (AF292375), were in the range of 93 to 94%. On the basis of the phylogenetic analysis, strain do03^T differed from members of the genus *Eggerthella* and belonged to a novel genus of the family *Coriobacteriaceae* (Minamida *et al.*, 2006a). In this paper,

we describe additional taxonomic characteristics of the novel strain in comparison with phylogenetically close genera, and classify it as a novel genus and species within the family *Coriobacteriaceae*.

Cell morphology and size after anaerobic cultivation at 37 °C for 2 days in Gifu anaerobic medium (GAM) broth (Nissui) were examined using phase-contrast microscopy (Nikon). Gram-staining was performed with 3-day-old culture by using a neo-B&M kit (Wako). Colonial morphologies were viewed after cultivation on GAM agar at 37 °C for 2 days in an anaerobic chamber (N₂/H₂/CO₂, 85:5:10; Coy Laboratory). Biochemical traits were determined using both conventional methods (Holdeman *et al.*, 1977) and the API 50 CH system (bioMérieux). Enzyme activities were analysed with the API ZYM system (bioMérieux) using bacteria harvested from GAM broth supplemented with 0.5% arginine. Cells were incubated anaerobically at 37 °C for 48 h (API 50 CH) and 4 h (API ZYM). For the analysis of short-chain fatty acids by HPLC (Minamida *et al.*, 2006b), the novel strain was grown in PYG broth (Holdeman *et al.*, 1977). To investigate the effects of arginine on growth, cells precultured in GAM broth were inoculated (1%, v/v) into GAM broth supplemented with 0, 0.5, 1.0 and 2.0% arginine (pH 7.0) and then incubated anaerobically at 37 °C. Absorbance values (OD₆₀₀) were measured using Spectronic 20D+ spectrophotometers (Thermo Electron). Fatty acid methyl esters were obtained from wet biomass (approx. 40 mg) by saponification, methylation and extraction

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain do03^T is AB266102.

according to the method described by Miller (1982), modified as described by Kuykendall *et al.* (1988). The fatty acids were separated according to the method described by Nishimukai *et al.* (2003) and were identified using authentic fatty acid methyl ester standards (Supelco). DNA G+C contents were determined using HPLC (Tamaoka & Komagata, 1984) after enzyme digestion of DNA to the deoxyribonucleosides. DNA was extracted and purified as described by Saito & Miura (1963). An equimolar mixture of four deoxyribonucleotides from a GC kit (Yamasa Shoyu) was used as the quantitative standard. Isoprenoid quinones and diamino acids were investigated by the TechnoSuruga Laboratory Co., Ltd (Shizuoka, Japan). Isoprenoid quinones were extracted as described by Nishijima *et al.* (1997) and analysed using HPLC apparatus (Waters) equipped with an Inertsil ODS-3 column (4.6 × 150 mm; GL Science) and a photodiode array detector (210–400 nm; Waters). The elution solvent was a mixture of methanol and 2-propanol (3:1, v/v). Cell-wall peptidoglycan was prepared and hydrolysed as described by Komagata & Suzuki (1987). The amino acid composition was determined by HPLC using the method of the TechnoSuruga Laboratory. Samples were derivatized with phenylisothiocyanate and UV detection (at 254 nm) was used.

The novel strain was found to be an obligately anaerobic, Gram-positive, rod-shaped, non-spore-forming bacterium. After 2 days incubation on GAM plates, colonies were 1 mm in diameter, smooth, clear and colourless. The strain did not grow in the presence of oxygen. Catalase was not produced and nitrate was not reduced. The cell-wall peptidoglycan contained *meso*-diaminopimelic acid, alanine and glutamic acid (molar ratio approx. 0.9:1.7:1.0), indicating the A1 γ -type polymer (Schleifer & Kandler, 1972). Two unidentified quinones (91.9 and 4.1 %) and one unidentified ubiquinone (4.0 %) were detected. The unidentified quinones were similar to menaquinones in the UV-spectrum pattern but differed from the saturated and unsaturated menaquinones found in actinobacteria to date. They were not methylmenaquinones, demethylmenaquinones, ubiquinones or rhodoquinones. After 22 h cultivation, the OD₆₀₀ values (means $\pm$ SD) in GAM broth supplemented with 0, 0.5, 1.0 and 2.0 % arginine were 0.24 (pH 7.1), 0.85 $\pm$ 0.02 (pH 7.8),

1.16 $\pm$ 0.01 (pH 8.0) and 1.09 $\pm$ 0.05 (pH 8.1), respectively. The optimum arginine concentration was 1.0 %. The novel strain tested negative for all carbohydrates in the API 50 CH system and showed strong naphthol-AS-BI-phosphohydrolase activity, medium acid phosphatase activity and weak alkaline phosphatase and esterase (C4) activities.

The strain differed from closely related genera in the family *Coriobacteriaceae* with validly published names in terms of the features listed in Table 1. On the basis of the results of the 16S rRNA gene analysis (Minamida *et al.*, 2006a) and the differences in phenotypic characteristics (Table 1), strain do03^T represents a novel genus and species in the family *Coriobacteriaceae*, for which the name *Asaccharobacter celatus* gen. nov., sp. nov. is proposed.

Description of *Asaccharobacter* gen. nov.

Asaccharobacter (A.sac.cha.ro.bac' ter. Gr. pref. *a-* not; Gr. n. *saccharon* sugar; N.L. masc. n. *bacter* a rod; N.L. masc. n. *Asaccharobacter* rod that does not digest sugar).

Cells are Gram-positive, rod-shaped and non-sporing. Obligately anaerobic and catalase-negative. Asaccharolytic; produce trace amounts of organic acids (lactic, acetic and succinic) in medium containing peptone, yeast extract and glucose. Growth is enhanced in the presence of arginine, but not with Tween 80. Can grow in 20 % bile. Cell-wall peptidoglycan contains *meso*-diaminopimelic acid, alanine and glutamic acid. The predominant cellular fatty acid is C_{18:1}*cis*9. The predominant lipoquinone is an unidentified quinone that is not menaquinone, methylmenaquinone, demethylmenaquinone, ubiquinone or rhodoquinone. The DNA G+C content is approximately 63 mol%. The type species is *Asaccharobacter celatus*. The genus *Asaccharobacter* is a member of the family *Coriobacteriaceae*.

Description of *Asaccharobacter celatus* sp. nov.

Asaccharobacter celatus (ce.la'tus. L. masc. adj. *celatus* conceal, hide, keep secret).

In addition to possessing the characteristics given in the genus description, cells are 0.45 μ m in width and

Table 1. Differential phenotypic characteristics for strain do03^T and closely related genera

Taxa: 1, strain do03^T (data from this study); 2, *Eggerthella* (Kageyama *et al.*, 1999; Lau *et al.*, 2004; Moore & Holdeman-Moore, 1986; Wade *et al.*, 1999); 3, *Denitrobacterium* (Anderson *et al.*, 2000). +, Positive; –, negative; ND, not determined.

Characteristic	1	2	3
Organic acids	Acetic acid, lactic acid, succinic acid	(Acetic acid, lactic acid, succinic acid)*	ND
Cell-wall diamino acid	<i>meso</i> -Diaminopimelic acid	LL-Diaminopimelic acid	ND
Major cellular fatty acids	C _{18:1} <i>cis</i> 9	Branched C _{15:0}	C _{16:0}
Major quinone	Unknown†	MK-6, MMK-6	ND
DNA G+C content (mol%)	63	62	56–60

*Products in parentheses indicate strain variation.

†Major quinone was not menaquinone, methylmenaquinone, demethylmenaquinone, ubiquinone or rhodoquinone.

2.3–2.7 µm in length. Colonies are smooth, clear and colourless on GAM agar, reaching 1 mm in diameter after cultivation at 37 °C for 2 days. Does not reduce nitrate. Cells do not produce acid from or show negative test results in the API 50 CH system with the following substrates: glycerol, glucose, erythritol, D-arabinose, L-arabinose, ribose, D-xylose, L-xylose, adonitol, methyl β-D-xyloside, galactose, fructose, mannose, sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, methyl α-D-mannoside, methyl α-D-glucoside, N-acetylglucosamine, amygdalin, arbutin, aesculin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, inulin, melezitose, raffinose, starch, glycogen, xylitol, gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate, 2-ketogluconate and 5-ketogluconate. Cells show strong naphthol-AS-BI-phosphohydrolase activity, medium acid phosphatase activity and weak alkaline phosphatase and esterase (C4) activities, but do not show any of the following activities: esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase or α-fucosidase. Capable of converting daidzein to equol. The major cellular fatty acids are C_{18:1} cis9 (54.0 %), C_{18:0} (11.7 %) and C_{16:0} (8.4 %). The DNA G + C content is 63 mol%.

The type strain, do03^T (=JCM 14811^T=DSM 18785^T=AHU 1763^T), was isolated from the caecal content of a rat.

Acknowledgements

The authors gratefully acknowledge Mr Ongol Martin Patrick for his help during the preparation of this article.

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