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

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An obligately anaerobic and equol-producing bacterium, designated strain do03T, 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 do03T 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 C18:1ω9c (54.0%). The strain had one unidentified predominant (91.9%) quinone that was not menaquinone, methylmenaquinone, demethylmenaquinone, ubiquinone or rho-diquinone. The DNA G+C content was 63 mol%. The data presented in this work show that strain do03T 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 do03T (=JCM 14811T=DSM 18785T=AHU 1763T).

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 do03T, 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 HKU14T (AY321958), Eggerthella hongkongensis HKU10T (AY288517) and Eggerthella lenta ATCC 25559T (AF292375), were in the range of 93 to 94%. On the basis of the phylogenetic analysis, strain do03T 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 analyzed 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 novel strain was found to be an obligately anaerobic, Gram-positive, rod-shaped, non-sporing 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. The predominant cellular fatty acid is C18:1. The peptidoglycan contains meso-diaminopimelic acid, alanine and glutamic acid (molar ratio approx. 0.9:1.7:1.0), indicating the A1 contained meso-diaminopimelic acid, alanine and glutamic acid. The predominant cellular fatty acid is C18:1. The peptidoglycan contains meso-diaminopimelic acid, alanine and glutamic acid (molar ratio approx. 0.9:1.7:1.0), indicating the A1 contained meso-diaminopimelic acid, alanine and glutamic acid. The predominant cellular fatty acid is C18:1.

The predominant lipoquinone is an unidentified quinone that is not menaquinone, methylmenaquinone, demethylmenaquinone, ubiquinone or rhodoquinone. They were not methylmenaquinones, demethylmenaquinones, ubiquinones or rhodoquinones. 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 OD 600 values (means ± SD) in GAM broth supplemented with 0, 0.5, 1.0 and 2.0 % arginine were 0.24 (pH 7.1), 0.85 ± 0.02 (pH 7.8), 1.16 ± 0.01 (pH 8.0) and 1.09 ± 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 do03T 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.char.o.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 C18:1. 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 μm in width and 4.6 μm 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 contained meso-diaminopimelic acid, alanine and glutamic acid. The predominant cellular fatty acid is C18:1.

### Table 1. Differential phenotypic characteristics for strain do03T and closely related genera

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic acids</td>
<td>Acetic acid, lactic acid, succinic acid</td>
<td>(Acetic acid, lactic acid, succinic acid)*</td>
<td>ND</td>
</tr>
<tr>
<td>Cell-wall diamino acid</td>
<td>meso-Diaminopimelic acid</td>
<td>LL-Diaminopimelic acid</td>
<td>ND</td>
</tr>
<tr>
<td>Major cellular fatty acids</td>
<td>C18:1, C69</td>
<td>Branched C12:0</td>
<td>C16:0</td>
</tr>
<tr>
<td>Major quinone</td>
<td>Unknown†</td>
<td>MK-6, MKM-6</td>
<td>ND</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>63</td>
<td>62</td>
<td>56–60</td>
</tr>
</tbody>
</table>

*Products in parentheses indicate strain variation.

†Major quinone was not menaquinone, methylmenaquinone, demethylmenaquinone, ubiquinone or rhodoquinone.
Olsenella profusa as Lactobacillus uli cultivation at 37°C, colourless on GAM agar, reaching 1 mm in diameter after 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-xylene, L-xylene, adonitol, methyl β-D-xyloside, galactose, fructose, mannose, sorbose, rhamnose, dulcitol, inositol, mannotol, 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 and 5-ketogluconate, 2-ketogluconate and 5-ketogluconate. Cells show strong naphthol-AS-Bl-phosphohydrolase activity, medium acid phosphatase activity and weak alkaline phosphatase activity 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 C₁₈:₁ cis9 (54.0%), C₁₈:₀ (11.7%) and C₁₆:₀ (8.4%). The DNA G + C content is 63 mol%.

The type strain, dof03 (=JCM 14811T =DSM 18785T =AHU 17631T), was isolated from the caecal content of a rat.

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