Slackia equolifaciens sp. nov., a human intestinal bacterium capable of producing equol

Jong-Sik Jin,1,2,3 Maki Kitahara,3 Mitsuo Sakamoto,3 Masao Hatton2 and Yoshimi Benno1

1Benno Laboratory, Center for Intellectual Property Strategies, RIKEN, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan
2Institute of Natural Medicine, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan
3Microbe Division/Japan Collection of Microorganisms, RIKEN BioResource Center, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan

An equol-producing bacterium, strain DZETT, which was isolated from human faeces, was characterized by morphological, biochemical and molecular methods. The isolate was Gram-positive, obligately anaerobic, non-spore-forming, asaccharolytic and rod-shaped. 16S rRNA gene sequence analysis showed 92.8, 91.0, 91.1 and 90.6 % similarities with Slackia faecicanis, Slackia exigua, Slackia heliotrinireducens and Slackia isoflavoniconvertens, respectively. Based on these data, we propose a novel species of the genus Slackia, Slackia equolifaciens sp. nov. The major cellular fatty acids are C14:0, C18:1ω9c and C18:1ω9c (dimethyl acetal). The DNA G+C content of the strain is 60.8 mol%. The type strain of S. equolifaciens sp. nov. is DZET (=JCM 16059T = CCUG 58231T).

Dietary isoflavones such as puerarin, daidzin and daidzein have recently aroused interest because of their potential health benefits (Anderson et al., 1999; Kurzer & Xu, 1997; Barnes, 1998; Setchell, 1998; Magee & Rowland, 2004). After ingestion from edible plants, these three compounds are converted to O-desmethylyangolensin and equol as end products (Supplementary Fig. S1, available in IJSEM Online) (Heinonen et al., 1999, 2003). Equol, in particular, has been extensively studied as an effective phytoestrogen. However, only 30–50 % of individuals in the general population are capable of producing equol from daidzein (Rowland et al., 2000; Kelly et al., 1995). Because equol is produced exclusively by the intestinal bacterial metabolism of isoflavones, isolation and characterization of equol-producing bacterial strains has been attempted. Several strains have been reported to date (Maruo et al., 2008; Minamida et al., 2008).

The genus Slackia belongs to the family Coriobacteriaceae and its members are Gram-positive, non-motile, obligate anaerobes (Wade et al., 1999). Four species, Slackia faecicanis (Lawson et al., 2005), Slackia exigua, Slackia heliotrinireducens (Wade et al., 1999) and Slackia isoflavoniconvertens (Matthies et al., 2009), have been described. S. isoflavoniconvertens, which was isolated from human faeces, is able to transform daidzein to equol.

We isolated a strain capable of transforming daidzein to equol from the faeces of a healthy female and reported its metabolic characteristics (Jin et al., 2008). A bacterial suspension from fresh faeces was repeatedly cultured in 2 ml of general anaerobic medium (GAM) broth (Nissui) containing 0.1 mM daidzein as a substrate at 37 °C in an anaerobic incubator. A portion of the culture, possessing metabolic activity, was seeded on GAM agar plates and incubated anaerobically for 72 h at 37 °C. Colonies were repeatedly screened for their activity of transforming daidzein, to obtain a single daidzein-metabolizing bacterium (strain DZET). The strains used in the present study were maintained on GAM agar incubated for 3 days at 37 °C in an anaerobic jar (Hirayama) filled with CO2. Physiological and biochemical reactions were determined with an API 20A anaerobe test kit and a Rapid ID 32A anaerobe identification kit, respectively, as recommended by the manufacturer (bioMérieux). All tests were performed in duplicate. The DNA G+C content was determined by using the HPLC method of Tamaoka & Komagata (1984). The elution solvent was a mixture of 0.02 M (NH4)2HPO4 and acetonitrile (20:1, v/v). Fatty acid methyl esters were obtained from approximately 40 mg of wet cells by saponification, methylation and extraction using minor modifications (Kuykendall et al., 1988) of the method of Miller (1982). Cells were cultured
anaerobically on GAM agar (0.5 % arginine-hydrochloride) for 96 h at 37 °C. The 16S rRNA gene sequence was analysed as described previously (Sakamoto et al., 2002). Almost 1500 bases of 16S rRNA gene sequence for strain DZE<sup>T</sup> were amplified by PCR with universal primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) using a Biometra Thermocycler Tgradient (Biometra). PCR products were purified by using an Ultraclean PCR Clean-up kit (MO BIO) and were sequenced by using a BigDye Terminator cycle sequencing kit (Applied Biosystems) and ABI PRISM 3130 Genetic Analyzer (Applied Biosystems). Phylogenetic relatives of the bacterium were determined by performing database searches, and sequences of related species were retrieved from the DDBJ, EMBL and GenBank nucleotide sequence databases. Sequences were aligned by using CLUSTAL X (version 2.0) (Thompson et al., 1997) and a phylogenetic tree was reconstructed according to the neighbour-joining method (Saitou & Nei, 1987). Minimum- and maximum-parsimony phylogenetic trees were inferred by using the software package MEGA version 4.0 (Tamura et al., 2007).

Analysis of 16S rRNA gene sequences revealed that the novel strain was a member of the family Coriobacteriaceae. Strain DZE<sup>T</sup> displayed highest sequence similarity with species of the genus Slackia [S. faecicanis (92.8 %), S. exigua (91.0 %), S. heliotri nariducens (91.1 %) and S. isoflavoniconvertens (90.6 %)] (Fig. 1). However, the strain exhibited substantially lower similarities with other members of the family Coriobacteriaceae [species of the genus Eggerthella (89.1–89.7 %), Denitrobacterium detoxificans (88.8 %) and Asaccharobacter celatus (91.1 %)]. Moreover, strain DZE<sup>T</sup> showed 91.4 % similarity with Adlercreutzia equolifaciens, which also converts daidzein to equol. Although it is not possible to distinguish species on the basis of 16S rRNA sequence similarities alone, it is clear that the greater than 7 % gene sequence divergence observed between strain DZE<sup>T</sup> and the four currently recognized species of the genus Slackia is consistent with separate species status. It is now generally accepted that organisms showing 3 % or greater 16S rRNA gene sequence divergence are not members of the same species (Lawson et al., 2005).

Physiological and biochemical reactions of strain DZE<sup>T</sup> were studied with S. faecicanis JCM 14555<sup>T</sup> and S. exigua JCM 11022<sup>T</sup> as references. The phenotypic and biochemical characteristics that are useful in differentiating strain DZE<sup>T</sup> from other members of the genus Slackia are shown in Table 1. The characteristics of strain DZE<sup>T</sup> were similar to those of S. exigua. However, the two reference strains, S. faecicanis JCM 14555<sup>T</sup> and S. exigua JCM 11022<sup>T</sup>, did not produce equol from daidzein. The long-chain cellular fatty acids of strain DZE<sup>T</sup> included C<sub>12:0</sub> (2.9 %), C<sub>14:0</sub> (15.1 %), C<sub>16:0</sub> (2.7 %) and C<sub>18:1ω9c</sub> (29.5 %).

Fig. 1. Phylogenetic tree showing the relationship between strain DZE<sup>T</sup> and the type strains of related species. The tree was reconstructed by the neighbour-joining method based on 16S rRNA gene sequences (approx. 1320 nucleotides). Numbers at nodes indicate percentage bootstrap values of 1000 replicates. Bootstrap values above 50 % are given at the branching points. Asterisks and hashes, respectively, indicate branches that were also recovered using the minimum-evolution and maximum-parsimony methods. Bar, 0.01 substitutions per nucleotide position. Accession numbers for 16S rRNA gene sequences are given for each strain.
Table 1. Characteristics of strain DZE<sup>T</sup> and other members of the genus Slackia

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Characteristic 1</th>
<th>Characteristic 2</th>
<th>Characteristic 3</th>
<th>Characteristic 4</th>
<th>Characteristic 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell shape</td>
<td>Rods</td>
<td>Rods</td>
<td>Rods</td>
<td>Rods</td>
<td>Rods</td>
</tr>
<tr>
<td>Size (µm)</td>
<td>0.5 × 0.8–1.0</td>
<td>0.5 × 1.0</td>
<td>0.5 × 1–2</td>
<td>0.8 × 0.8–1.2</td>
<td>0.4 × 2.4</td>
</tr>
<tr>
<td>Equol production from daidzein</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>NT</td>
</tr>
<tr>
<td>Fermentation products from glucose</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Acetate</td>
<td>ND</td>
</tr>
<tr>
<td>Enzyme activity</td>
<td>Arginine arylamidase</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Proline arylamidase</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Leucyl glycine arylamidase</td>
<td>V</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Phenylalanine arylamidase</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Leucine arylamidase</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Tyrosine arylamidase</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Alanine arylamidase</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Glycine arylamidase</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Histidine arylamidase</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Serine arylamidase</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>

*Data from this study were different from published data (Lawson et al., 2005; Wade et al., 1999). These characteristics are assumed to be variable.

( Supplementary Table S1). Based upon phylogenetic and phenotypic findings, we propose the name Slackia equolifaciens sp. nov. for this strain.

Description of Slackia equolifaciens sp. nov.

Slackia equolifaciens (e.quo.li.‘ci.ens. N.L. n. equol-olis equol; L. part. adj. faciens making; N.L. part. adj. equolifaciens equol-producing).

Cells are Gram-positive, rod-shaped (0.5 × 0.8–1.0 µm), anaerobic, non-motile and non-spore-forming. Colonies on GAM agar (0.5 % arginine-hydrochloride) plates are 1–2 mm in diameter and a translucent grey colour after 4 days at 37 °C. Growth is stimulated by arginine. Indole test is negative. Acid is not produced from L-arabinose, cellobiose, glucose, lactose, maltose, D-mannitol, D-mannose, melezitose, raffinose, L-rhamnose, salicin, D-sorbitol, sucrose, trehalose or D-xylene. Positive reactions are obtained using Rapid ID 32A for arginine dihydrolase, proline arylamidase, phenylalanine arylamidase, leucine arylamidase, tyrosine arylamidase, alanine arylamidase, glycine arylamidase, histidine arylamidase and serine arylamidase. Negative results are obtained for nitrate reduction, catalase, urease, α-galactosidase, β-galactosidase, β-galactosidase-6-phosphate, α-glucosidase, β-glucosidase, α-arabinosidase, β-gluco oxidase, N-acetyl-β-glucosaminidase, glutamic acid decarboxylase, α-fucosidase, arginine arylamidase, pyroglutamic acid arylamidase and glutamyl glutamic acid arylamidase. The leucyl glycine arylamidase reaction is variable. The major cellular fatty acids are C<sub>14:0</sub>, C<sub>18:1ω9c</sub> and C<sub>18:1ω9c</sub> DMA (dimethyl acetal). The DNA G+C content of the type strain is 60.8 mol%.

The type strain is DZE<sup>T</sup>(=JCM 16059<sup>T</sup>=CCUG 58231<sup>T</sup>), which was isolated from the faeces of a healthy human.

References


