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

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An equol-producing bacterium, strain DZE^T, 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 C_{14:0}, C_{18:1ω9c} and C_{18:1ω9c} DMA (dimethyl acetal). The DNA G + C content of the strain is 60.8 mol%. The type strain of *S. equolifaciens* sp. nov. is DZE^T (=JCM 16059^T =CCUG 58231^T).

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-desmethylangolensin 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 DZE^T). 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)H₂PO₄ 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

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DZE^T is EU377663.

The proposed metabolic pathway of puerarin and daidzin by human intestinal bacteria, and cellular fatty acid compositions of strain DZE^T and the type strains of closely related species are available with the online version of this paper.

Table 1. Characteristics of strain DZE^T and other members of the genus *Slackia*

Strains: 1, *S. equolifaciens* sp. nov. DZE^T; 2, *S. exigua* JCM 11022^T; 3, *S. faecicanis* JCM 14555^T; 4, *S. heliotrinireducens* JCM 14554^T; 5, *S. isoflavoniconvertens* DSM 22006^T. +, Positive; –, negative; ND, not detected; NT, not tested; v, variable. Data for *S. heliotrinireducens* and *S. isoflavoniconvertens* from Lawson *et al.* (2005), Wade *et al.* (1999) and Matthies *et al.* (2009).

Characteristic	1	2	3	4	5
Cell shape	Rods	Rods	Rods	Cocci and coccibacilli	Rods
Size (µm)	0.5 × 0.8–1.0	0.5 × 1.0	0.5 × 1–2	0.8 × 0.8–1.2	0.4 × 2.4
Equol production from daidzein	+	–	–	NT	+
Nitrate reduction	–	–	–*	+	–
Fermentation products from glucose	ND	ND	ND	Acetate	ND
Enzyme activity					
Arginine arylamidase	–	–*	–	–	–
Proline arylamidase	+	+	–	+	–
Leucyl glycine arylamidase	v	–	–	–	–
Phenylalanine arylamidase	+	+	–	+	–
Leucine arylamidase	+	+	–	+	–
Tyrosine arylamidase	+	+	–	+	–
Alanine arylamidase	+	+	–	+	–
Glycine arylamidase	+	+	–	+	–
Histidine arylamidase	+	+	–	+	–
Serine arylamidase	+	+	–	+	–

*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.fa'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-xylose. 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-glucuronidase, 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_{14:0}, C_{18:1ω9c} and C_{18:1ω9c} DMA (dimethyl acetal). The DNA G + C content of the type strain is 60.8 mol%.

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

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