Bifidobacterium tsurumiense sp. nov., from hamster dental plaque

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Three novel micro-organisms, designated strains OMB115^T, OMB118 and OMB120, were isolated from dental plaque from golden hamsters fed with a high-carbohydrate diet. The three strains were Gram-positive, facultatively anaerobic rods that lacked catalase activity. Analysis of their partial 16S rRNA gene sequences indicated that these isolates belonged to the genus *Bifidobacterium*. They grew under aerobic conditions and each had a DNA G+C content of 53 mol%. On the basis of phylogenetic analyses involving phenotypic characterization and partial 16S rRNA gene sequencing, strain OMB115^T represents a novel species of the genus *Bifidobacterium*, for which the name *Bifidobacterium tsurumiense* sp. nov. is proposed. The type strain is OMB115^T (=JCM 13495^T =DSM 17777^T).

Bifidobacteria are Gram-positive, anaerobic, non-motile, non-spore-forming bacteria that are prevalent within the gastrointestinal tracts of humans and animals (Mikkelsen *et al.*, 2003). A role in human gut health has been recognized for members of the genus *Bifidobacterium*: they serve as probiotic micro-organisms, improving the microbial balance of intestinal flora (Caglar *et al.*, 2005). To date, bifidobacteria have not been found to cause human disease, although three species of bifidobacteria have been isolated from dental caries (Becker *et al.*, 2002; Beighton, 2005; Crociani *et al.*, 1996; Jian & Dong, 2002; Love & Jenkinson, 2002; Munson *et al.*, 2004). Phenotypic characteristics of members of the genus *Bifidobacterium* are as follows: a high DNA G+C content, an absolute requirement for anaerobic growth conditions, fructose-6-phosphate phosphoketolase (F6PPK) activity and the production of acetic and lactic acids (at a molar ratio of 3:2) as end products of glucose fermentation (Scardovi, 1986).

During our studies on experimental dental diseases from 1987 to 1997, we collected a number of oral isolates that have not been identified to genus level. Three of these isolates, which we have identified as *Bifidobacterium*-like organisms, grew under aerobic conditions, and their DNA G+C contents were slightly lower (53 mol%) than those

of members of the genus *Bifidobacterium*. Here, we report the taxonomic study of these novel isolates obtained from dental plaque from golden hamsters fed with a high-carbohydrate diet. The reference strains used in this study, *Bifidobacterium boum* JCM 1211^T, *Bifidobacterium thermacidophilum* JCM 11165^T, *Bifidobacterium indicum* JCM 1302^T and *Bifidobacterium dentium* JCM 1195^T, were obtained from the Japan Collection of Microorganisms (RIKEN BioResource Center). These organisms were maintained on Brucella HK agar plates (Kyokuto) in an Anaero Box (Forma Scientific) under an atmosphere comprising N₂/CO₂/H₂ (80:10:10) at 37 °C. Bacterial specimens were collected from dental plaque samples from hamsters that had been given a high-carbohydrate diet (D#2000; Jordan *et al.*, 1969) for 6 weeks. Diluted aliquots of the plaque samples were inoculated onto CFAT agar and incubated in an anaerobic box at 37 °C for 4 days. The CFAT agar used was a selective medium for human actinomycetes (Zylber & Jordan, 1982), its composition (l⁻¹) being as follows: trypticase soy broth (BBL Microbiology Systems), 30 g; glucose, 5 g; agar, 15 g; cadmium sulfate, 13 mg; sodium fluoride, 85 mg; neutral acriflavin, 1.2 mg; potassium tellurite, 2.5 mg; basic fuchsin, 1.3 mg; defibrinated sheep blood, 50 ml.

For chromosomal DNA preparations, culture cells were incubated with lysozyme (Seikagaku Kogyo) at 4 mg ml⁻¹, N-acetylmuramidase SG (Seikagaku Kogyo) at 50 µg ml⁻¹ and 1000 U achromopeptidase (Wako Pure Chemical Industry) for 3 h at 37 °C. Bacterial DNA was extracted according to the methods of Marmur & Doty (1961). The DNA concentration was determined spectrophotometrically from the A₂₆₀ and the purity of the samples was

Abbreviation: F6PPK, fructose-6-phosphate phosphoketolase.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA and *hsp60* gene sequences of strain OMB115^T are AB241106 and AB241108, respectively.

A neighbour-joining phylogenetic tree based on partial *hsp60* gene sequences is available as supplementary material with the online version of this paper.

(Miyake *et al.*, 1998; Sakata *et al.*, 2006; Weisburg *et al.*, 1991; Woese *et al.*, 1990). In recent years, several housekeeping genes, such as heat-shock protein genes (the *hsp60* gene; also known as *groEL* or *cpn60*, *groES* and *dnaK* genes), have been used for taxonomic studies of bifidobacterial species (Dalcenserie *et al.*, 2005; Jian *et al.*, 2001; Simpson *et al.*, 2004; Ventura *et al.*, 2004, 2005; Zhu *et al.*, 2003). *Bifidobacterium inopinatum* and *B. denticolens*, isolated from human dental caries, were transferred to *S. inopinata* and *Parascardovia denticolens*, respectively, on the basis of taxonomic studies involving *hsp60* sequences (Jian & Dong, 2002).

Likewise, we also constructed a phylogenetic tree based on a total of 36 sequences of *hsp60* genes including those of members of the genus *Bifidobacterium* and of related genera (see Supplementary Fig. S1 in IJSEM Online). Strain OMB115^T showed *hsp60* gene sequence similarities of 80.6, 79.7 and 79.3% with respect to the type strains of *P. denticolens*, *Gardnerella vaginalis* and *S. inopinata*, respectively. The levels of partial *hsp60* gene sequence similarity between strain OMB115^T and the type strains of *Bifidobacterium pullorum*, *Bifidobacterium merycicum* and *B. boum* were higher (83.7, 83.1 and 81.2%, respectively). However, the levels of *hsp60* gene sequence similarity among *Bifidobacterium* species were much lower than those for 16S rRNA gene sequences. Therefore, in this case, 16S rRNA gene sequences were more useful than *hsp60* gene sequences for determining the phylogeny of strain OMB115^T. It is likely that the similarity values observed for the *hsp60* gene among different *Bifidobacterium* species were the result of lateral transfer.

For the determination of phenotypic characteristics, API 50 CHL, API Rapid ID 32A and API ZYM tests were performed (in triplicate) using the methods recommended by the manufacturer (bioMérieux). Cells from agar plates were suspended in sterile water, inoculated onto the test strips and incubated in air for 4 h (for API Rapid ID 32A and API ZYM) or in an anaerobic box (for API 50 CHL) at 37 °C for 48 h. F6PPK activity was determined according to the method described by Scardovi (1986). Cell-wall peptidoglycan was prepared and hydrolysed using the methods of Kawamoto *et al.* (1981). The cell-wall amino acids were determined with an automatic amino acid analyser (model 835; Hitachi). Separation of amino acids was performed using previously published procedures (Einarsson *et al.*, 1987).

Table 1 shows the major characteristics of strain OMB115^T and related bacteria. Strain OMB115^T was found to be a Gram-positive, facultative anaerobe comprising short and irregularly shaped rods, was positive for F6PPK and was negative for catalase and oxidase. Of members of the family *Bifidobacteriaceae*, *Aeriscardovia aeriphila*, *Bifidobacterium psychraerophilum*, *Bifidobacterium scardovii* and *G. vaginalis* have been reported to be able to grow under aerobic conditions (Hoyle *et al.*, 2002; Simpson *et al.*, 2004; van Esbroeck *et al.*, 1996). *B. boum* and *B. thermophilum* have

been reported as having microaerophilic profiles (Kawasaki *et al.*, 2006). The optimal temperature for growth of strain OMB115^T was 37 °C; growth also occurred at 25 and 45 °C. The strain was able to grow on lactobacillus selective agar (LBS agar; Becton Dickinson). In contrast, growth of *S. inopinata*, *P. denticolens* and other members of the *Bifidobacteriaceae* was inhibited on this selective medium. The DNA G+C content of strain OMB115^T was 53 mol%, which is consistent with those of *P. denticolens* and *A. aeriphila* (Simpson *et al.*, 2004) but slightly lower than values reported previously for the genus *Bifidobacterium* (58–67 mol%) (Crociani *et al.*, 1996; Simpson *et al.*, 2004; Zhu *et al.*, 2003). However, the DNA G+C content of strain OMB115^T was higher than that of *S. inopinata* (45 mol%) (Crociani *et al.*, 1996). Isolates OMB118 and OMB120 showed the same characteristics as strain OMB115^T.

On the basis of this phylogenetic study using phenotypic characterization and 16S rRNA gene sequence similarity data, strains OMB115^T, OMB118 and OMB120 represent a novel species of the genus *Bifidobacterium*, for which the name *Bifidobacterium tsurumiense* sp. nov. is proposed.

Description of *Bifidobacterium tsurumiense* sp. nov.

Bifidobacterium tsurumiense (tsu.ru.mi.en'se. N.L. neut. adj. *tsurumiense* pertaining to Tsurumi University, Yokohama, Japan).

Colonies on Brucella HK blood agar plates are white, rough and slightly convex. Cells are short rods or coccoid when grown on Brucella HK blood agar under anaerobic conditions for 24 h. Cells are Gram-positive, F6PPK-positive, catalase- and oxidase-negative. Facultatively anaerobic. Growth occurs at 25 and 45 °C; optimal temperature for growth is 37 °C. Growth also occurs on LBS and CFAT agars. End products of glucose fermentation are acetic and lactic acids at a molar ratio of 3:2. The DNA G+C content is 53 mol%. L-Arabinose, D-ribose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, D-mannitol, methyl α -D-glucopyranoside, amygdalin, arbutin, salicin, cellobiose, maltose, D-lactose, melibiose, sucrose, trehalose, raffinose, starch, glycogen, gentiobiose, turanose, gluconate and 2-ketogluconate are fermented. Glycerol, erythritol, D-arabinose, L-xylose, D-adonitol, methyl β -D-xylopyranoside, L-sorbose, L-rhamnose, dulcitol, inositol, D-sorbitol, methyl α -D-mannopyranoside, N-acetylglucosamine, inulin, melezitose, xylitol, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol and 5-ketogluconate are not fermented. Positive for aesculin hydrolysis, α -galactosidase, β -galactosidase, α -glucosidase, β -glucosidase, α -arabinosidase, arginine arylamidase, proline arylamidase, leucyl glycine arylamidase, phenylalanine arylamidase, leucine arylamidase, tyrosine arylamidase, alanine arylamidase, glycine arylamidase, histidine arylamidase, serine arylamidase, esterase, esterase lipase, lipase, valine arylamidase, cysteine arylamidase and acid phosphatase. Negative for catalase, urease, arginine dihydrolase, β -galactosidase-6-phosphate,

Table 1. Phenotypic characteristics of strain OMB115^T and related type strains

Strains: 1, OMB115^T; 2, *B. boum* JCM 1211^T; 3, *B. thermacidophilum* JCM 11165^T; 4, *B. indicum* JCM 1302^T; 5, *B. dentium* JCM 1195^T. All strains produce acid from D-galactose, D-glucose, D-fructose, maltose, melibiose, sucrose and raffinose. All strains are positive for α -galactosidase, β -galactosidase, α -glucosidase, β -glucosidase, arginine arylamidase, proline arylamidase, phenylalanine arylamidase, leucine arylamidase, tyrosine arylamidase, alanine arylamidase, glycine arylamidase, histidine arylamidase, serine arylamidase and acid phosphatase activities. None of the strains produce acid from glycerol, erythritol, D-arabinose, L-xylose, D-adonitol, methyl β -D-xylopyranoside, L-sorbose, L-rhamnose, dulcitol, inositol, D-sorbitol, methyl α -D-mannopyranoside, N-acetylglucosamine, inulin, melezitose, xylitol, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol or 5-ketogluconate. All strains are negative for urease, arginine dihydrolase, β -galactosidase-6-phosphate, β -glucuronidase, N-acetyl- β -glucosaminidase, glutamic acid decarboxylase, α -fucosidase, nitrate reduction, indole production, alkaline phosphatase, pyroglutamate arylamidase, glutamyl glutamic acid arylamidase, alkaline phosphatase, trypsin, chymotrypsin and α -mannosidase. +, Positive; –, negative; w, weak.

Characteristic	1	2	3	4	5
Aerobic growth	+	–	–	–	–
Growth at/on:					
25 °C	+	+	+	+	w
45 °C	+	+	+	w	–
LBS agar	+	w	w	–	w
DNA G + C content (mol%)	53	60	58	60	61
API 50 CHL					
Aesculin hydrolysis	+	–	–	+	+
Acid from:					
L-Arabinose	+	–	+	–	+
D-Ribose	+	–	–	+	+
D-Xylose	+	–	–	–	+
D-Mannose	+	–	w	–	+
D-Mannitol	+	–	–	–	+
Methyl α -D-glucopyranoside	+	–	+	–	–
Amygdalin	+	–	–	+	+
Arbutin	+	–	–	+	+
Salicin	+	–	–	+	+
Cellobiose	+	–	–	+	+
D-Lactose	+	–	–	–	+
Trehalose	+	–	–	–	+
Starch	+	+	+	–	+
Glycogen	+	+	+	–	+
Gentiobiose	+	–	–	w	+
Turanose	+	+	+	–	+
Gluconate	+	–	–	–	+
2-Ketogluconate	w	–	–	–	+
API Rapid ID 32A					
α -Arabinosidase	+	–	–	+	+
Leucyl glycine arylamidase	+	+	+	+	–
API ZYM					
Esterase	+	+	+	–	+
Esterase lipase	+	+	+	–	+
Lipase	+	–	–	–	–
Valine arylamidase	+	+	+	+	–
Cysteine arylamidase	+	+	+	+	–
Phosphoamidase	–	+	+	+	+
β -Glucuronidase	–	–	–	–	+

β -glucuronidase, N-acetyl- β -glucosaminidase, glutamic acid decarboxylase, α -fucosidase, nitrate reduction, indole production, alkaline phosphatase, pyroglutamate arylamidase,

glutamyl glutamic acid arylamidase, trypsin, chymotrypsin, phosphoamidase, α -mannosidase and α -fucosidase. The cell-wall amino acids consist of Glu–Lys–Asp–(Ala)₂.

The type strain, OMB115^T (=JCM 13495^T =DSM 17777^T), was isolated from dental plaque from hamsters fed with a high-carbohydrate diet.

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