Bacteroides intestinalis sp. nov., isolated from human faeces

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During studies of the microbiota of human faeces, five strains of Gram-negative anaerobic rods were isolated following growth in a polyamine-deficient medium. These strains belonged to the genus *Bacteroides* on the basis of 16S rRNA gene sequence data. 16S rRNA gene sequence similarity between one of the strains, 341^T, and recognized species within the genus *Bacteroides* was <95%. The DNA G+C content (44 mol%) and major fatty acid composition (anteiso-C_{15:0}, 32.0%) supported the affiliation of strain 341^T to the genus *Bacteroides*. Biochemical tests and DNA–DNA hybridization analysis demonstrated that strain 341^T was distinct from *Bacteroides uniformis* and *Bacteroides helcogenes*, to which it was related most closely. On the basis of these data, a novel *Bacteroides* species, *Bacteroides intestinalis* sp. nov., is proposed with strain 341^T (=JCM 13265^T=DSM 17393^T) as the type strain.

Polyamines such as putrescine, spermidine and spermine are organic cations required for animal cell growth and differentiation and in various steps of DNA, RNA and protein synthesis. Both prokaryotic and eukaryotic cells synthesize polyamines (Capano *et al.*, 1998; McCormack *et al.*, 1998). The polyamines found in the intestinal lumen originate from endogenous and exogenous sources. Polyamines derived from food are absorbed in the small intestine and thus are unable to fulfil the high metabolic demand for polyamines of the mucosal tissue in the large bowel (Bardocz *et al.*, 1993). Bacteria present in the human gut serve as a possible source of polyamines (Satink *et al.*, 1989). Members of the genera *Bacteroides* and *Fusobacterium* and anaerobic cocci are able to synthesize large amounts of putrescine and spermidine in rats (Noack *et al.*, 1998). The use of polyamine-deficient media facilitates the isolation of polyamine-producing bacteria (Noack *et al.*, 2000). During an investigation of micro-organisms grown on polyamine-deficient medium, we isolated the strains reported here.

Five bacterial strains (strains 276, 281, 341^T, 342 and 344) were isolated from the faeces of healthy Japanese adults (strains 276 and 281 were from a 40-year-old male and the others from a 32-year-old female). The polyamine-deficient medium of Noack *et al.* (1998), with minor modification, and a standard dilution plate method were used for isolation. The composition of the medium was as follows (mmol l⁻¹ unless indicated): MOPS (40), Tricine (4), K₂HPO₄ (1.3), NaHCO₃ (119), NaCl (85.5), NH₄Cl (9.5), glucose (27.3), L-amino acid mixture [alanine (3.6), arginine (1.6), asparagine (1.9), aspartic acid (1.5), cystine (0.12), cysteine (3.3), glutamic acid (2.7), glutamine (3.4), glycine (8.5), histidine (0.6), isoleucine (1.7), leucine (2.8), lysine (12.3), methionine (0.7), ornithine (2.0), phenylalanine (1.3), proline (4.9), serine (2.3), threonine (1.7), tryptophan (0.2), tyrosine (0.7) and valine (2.4)], purines and pyrimidines [adenine, guanine, cytosine and uracil (0.2 each)], sodium succinate (9.0), haemin (0.015), vitamins [(μmol l⁻¹): *p*-aminobenzoic acid (0.4), folic acid (0.05), biotin (0.08), nicotinic acid (0.3), calcium pantothenate (0.1), thiamine hydrochloride (0.15), pyridoxine hydrochloride (0.49) and menadione (1.1)], mineral mixture [(μmol l⁻¹): MgSO₄·7H₂O (121.7), MnSO₄·4H₂O (22.4), CaCl₂·2H₂O (6.8), ZnSO₄·7H₂O (3.5), CoCl₂·6H₂O (4.2), Fe(II)SO₄·7H₂O (3.6), H₃BO₃ (1.6), Na₂MoO₄·2H₂O (0.41) and NiCl₂·6H₂O (0.13)], Tween 80 (1.0 ml l⁻¹) and agar (20.0 g l⁻¹). An AnaeroPack (Mitsubishi Gas) was used for creating anaerobic conditions and the incubation period was 72–120 h at 37 °C. The strains were subcultured

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 341^T is AB214328.

Biochemical characteristics determined using the API systems, cellular fatty compositions, DNA G+C contents and levels of DNA–DNA hybridization among the novel strains and related *Bacteroides* species are available as supplementary tables in IJSEM Online.

on Eggerth Gagnon (EG) agar (Merck) supplemented with 5 % horse blood for 2 days at 37 °C in an anaerobic jar (Hirayama) filled with 100 % CO₂.

Amplification of the 16S rRNA gene of the studied strains was conducted using a PCR machine (Biometra). Universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTACCTGTGACGACTT-3') were used for the amplification of a fragment of approximately 1500 bases of the 16S rRNA gene of the isolated strains. The amplified product was purified by using a Montage PCR₉₆ filter plate (Millipore). A BigDye Terminator cycle sequencing kit (Applied Biosystems) and ABI PRISM 3100 Genetic Analyzer (Applied Biosystems) were used for sequencing, following the manufacturers' instructions. 16S rRNA gene sequences related closely to those of the isolated strains were retrieved from DDBJ, EMBL and GenBank. The sequences were aligned using CLUSTAL X (version 1.8) (Thompson *et al.*, 1997). A phylogenetic tree was constructed based on the neighbour-joining method (Saitou & Nei, 1987). Stability of the groupings was estimated by bootstrap analysis (1000 replications). Almost complete (1484–1488 bp) 16S rRNA gene sequences of the five strains were determined. 16S rRNA gene sequence similarity between strains 341^T and 342 was 100 %. Phylogenetic analysis indicated that strains 341^T and 342 were related most closely to *Bacteroides uniformis* (94.8 %) and *Bacteroides helcogenes* (92.0 %) (Fig. 1). These low sequence similarities (<97 %) indicated that strains 341^T and 342 represented a novel genomic species (Stackebrandt & Goebel, 1994). The 16S rRNA gene sequence similarity between strain 344 and *B. uniformis* was 99.6 %. Sequence similarity between strains 276 and 281 was 100 % and the two strains showed 97.4 % 16S rRNA gene sequence similarity to *B. uniformis*.

The API 20 A and API rapid ID 32 A systems (bioMérieux) were used for biochemical tests according to the manufacturer's instructions. Strains 341^T and 342 produced the same biochemical reactions, except for the production of 6-phospho-β-galactosidase (negative for strain 341^T but positive for strain 342). Tests that are useful in distinguishing the novel taxon from some other *Bacteroides* species are summarized in Table 1 and results from the two API systems are listed in Supplementary Table S1 (available in IJSEM Online). Results of phenotypic analyses are listed in the species description below. Strain 341^T demonstrated several differences from *B. uniformis*, including differences in acid production from salicin and L-rhamnose and production of 6-phospho-β-galactosidase. Biochemical characteristics of strain 341^T that differed from those of *B. helcogenes* included indole production, acid production from salicin, L-arabinose and L-rhamnose and production of 6-phospho-β-galactosidase, α-arabinosidase, proline arylamidase, phenylalanine arylamidase and leucine arylamidase. The strains were grown on GAM (Nissui) agar plates supplemented with 2 % Bacto oxgall (Difco) to test

Fig. 1. Phylogenetic positions of the isolated strains in the genus *Bacteroides*. The tree was constructed by using the neighbour-joining method based on 16S rRNA gene sequences. Bootstrap values >50 % based on 1000 replications are listed as percentages at branching points. Bar, 1 % sequence divergence.

for bile resistance. All the studied strains grew well in the presence of bile.

Cellular fatty acid compositions of the strains were determined following the method described by Sakamoto *et al.* (2002). The results are given in Supplementary Table S2. No significant differences were found in the fatty acid profiles of strains 341^T and 342. The major cellular fatty acid was anteiso-C_{15:0} (32.0 %), in agreement with data for the genus *Bacteroides* as described by Miyagawa *et al.* (1979). The closely related type strains of *B. uniformis* JCM 5828^T and *B. helcogenes* JCM 6297^T also contained anteiso-C_{15:0} as the major cellular fatty acid (32.5 and 30.0 %, respectively).

Based on the results of physiological, biochemical, cellular fatty acid and phylogenetic analysis, two strains (276 and 341^T) were selected for determination of the G + C content and for DNA–DNA hybridization experiments with reference strains *B. uniformis* JCM 5828^T and *B. helcogenes* JCM 6297^T. For this purpose, bacterial cells were cultured in EGF

Table 1. Biochemical characteristics that are useful in differentiating *Bacteroides intestinalis* sp. nov. from some members of the genus *Bacteroides*

Species: 1, *B. intestinalis* sp. nov.; 2, *B. uniformis*; 3, *B. helcogenes* (data in columns 1–3 from this study); 4, *B. caccae* (data from Jousimies-Somer *et al.*, 2003); 5, *B. coprocola* (Kitahara *et al.*, 2005); 6, *B. distasonis*; 7, *B. eggerthii*; 8, *B. fragilis* (data in columns 6–8 from Jousimies-Somer *et al.*, 2003); 9, *B. massiliensis* (Fenner *et al.*, 2005); 10, *B. merdae* (Jousimies-Somer *et al.*, 2003); 11, *B. nordii* (Song *et al.*, 2004); 12, *B. ovatus* (Jousimies-Somer *et al.*, 2003); 13, *B. plebeius* (Kitahara *et al.*, 2005); 14, *B. pyogenes* (Benno *et al.*, 1983); 15, *B. salyersiae* (Song *et al.*, 2004); 16, *B. stercoris*; 17, *B. thetaiotaomicron*; 18, *B. vulgatus* (data in columns 16–18 from Jousimies-Somer *et al.*, 2003). Characteristics are scored as: +, positive; –, negative; v, variable; +[–], most strains positive; –⁺, most strains negative; ND, not determined.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Indole production (L-tryptophan)	+	+	–	–	–	–	+	–	–	–	+	+	–	–	+	+	+	–
Aesculin hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	– ⁺
Acid production from:																		
L-Arabinose	+	+	–	+	–	– ⁺	+	–	–	– ⁺	–	+	+	–	+	– ⁺	+	+
D-Cellobiose	+	+	+	+	+	+	– ⁺	+	–	v	+	+	+	+	+	– ⁺	+	–
L-Rhamnose	+	–	–	+	+	v	+	–	–	+	+	+	+	–	+	+	+	+
Salicin	–	+	+	– ⁺	+	+	–	–	–	+	–	+	–	– ⁺	–	– ⁺	– ⁺	–
Sucrose	+	+	+	+	+	+	–	+	+	+	+	+	+	– ⁺	+	+	+	+
D-Trehalose	–	–	–	+	–	+	–	–	–	+	–	+	–	–	–	–	+	–
D-Xylose	+	+	+	+	+	+	+	+	–	+	+	+	+	–	+	+	+	+
α-Fucosidase activity	+	+	+	+	+	–	–	+	+	–	–	+	+	ND	–	v	+	+

broth at 37 °C for 12 h and then harvested. DNA was purified following the method of Saito & Miura (1963). HPLC was used for determination of the DNA G+C content of the strains as described by Kitahara *et al.* (2001). The DNA G+C contents of strain 341^T, *B. uniformis* JCM 5828^T and *B. helcogenes* JCM 6297^T were 44, 48 and 46 mol%, respectively (Supplementary Table S3). The G+C content of strain 341^T also supports its affiliation to the genus *Bacteroides*, members of which have G+C contents of between 40 and 48 mol% (Shah, 1992). The level of DNA–DNA hybridization was determined by the method of Ezaki *et al.* (1989). Strain 341^T showed a level of DNA–DNA hybridization of <9% with *B. uniformis* JCM 5828^T and *B. helcogenes* JCM 6297^T (Supplementary Table S3). The low DNA–DNA relatedness value (<70%) with the most closely related *Bacteroides* species again indicated the novel species status of strain 341^T (Stackebrandt & Goebel, 1994). Strain 276 showed over 84% DNA–DNA relatedness with *B. uniformis* JCM 5828^T. 16S rRNA gene sequence similarity between strains 276 and 281 was 100%. Therefore, they were identified as representing *B. uniformis*.

On the basis of the results presented here, strain 341^T should be classified as the type strain of a novel species of the genus *Bacteroides*, for which the name *Bacteroides intestinalis* sp. nov. is proposed.

Description of *Bacteroides intestinalis* sp. nov.

Bacteroides intestinalis (in.tes.ti.na'lis. N.L. masc. adj. *intestinalis* pertaining to the intestine).

Cells are strictly anaerobic, non-spore-forming, non-motile, Gram-negative rods, about 0.8 µm wide and 1–5 µm long, and occur singly. Surface colonies on EG blood agar

plates after 2 days are 1–3 mm in diameter, circular, translucent-whitish, raised and convex. The optimum temperature for growth is about 37 °C. Grows in the presence of bile. Indole-positive and able to hydrolyse aesculin. Nitrate is not reduced. No activity is detected for urease and gelatin is not hydrolysed. Acid is produced from glucose, lactose, sucrose, maltose, xylose, arabinose, cellobiose, mannose, raffinose and rhamnose. Acid is not produced from mannitol, salicin, glycerol, melezitose, sorbitol or trehalose. Positive reactions are obtained using API rapid ID 32 A for α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, α-arabinosidase, N-acetyl-β-glucosaminidase, glutamic acid decarboxylase, α-fucosidase, alkaline phosphatase, leucyl glycine arylamidase, alanine arylamidase and glutamyl glutamic acid arylamidase. Mannose and raffinose are fermented. Negative reactions are obtained for arginine dihydrolase, β-glucuronidase, arginine arylamidase, proline arylamidase, phenylalanine arylamidase, leucine arylamidase, pyroglutamic acid arylamidase, tyrosine arylamidase, glycine arylamidase, histidine arylamidase and serine arylamidase. The result for 6-phospho-β-galactosidase is variable. The major fatty acids are anteiso-C_{15:0} (32.0–33.6%) and iso-C_{17:0} 3-OH (18.5–19.2%). The DNA G+C content is 44 mol%.

The type strain, 341^T (=JCM 13265^T=DSM 17393^T), was isolated from human faeces. Strain 342 (=JCM 13266) is included in this species.

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