Bacteroides sartorii is an earlier heterotypic synonym of *Bacteroides chinchillae* and has priority

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Strains of the recently proposed species *Bacteroides chinchillae* share more than 99.4 % 16S rRNA gene sequence similarity with the type strain of *Bacteroides sartorii* although these two species do not appear to be similar from their published descriptions. The aim of this study was to perform phenotypic and genetic analyses of both species to clarify their taxonomic position. *B. chinchillae* JCM 16497^T exhibited high *hsp60* gene sequence similarity with *B. sartorii* JCM 17136^T (100 %) as well as *B. chinchillae* JCM 16498 (100 %). The *hsp60* gene sequence analysis and levels of DNA–DNA relatedness observed demonstrated *B. sartorii* JCM 17136^T, *B. chinchillae* JCM 16497^T, and *B. chinchillae* JCM 16498 are members of a single species. Based on these data, we propose *Bacteroides chinchillae* as a later heterotypic synonym of *Bacteroides sartorii*. An emended description of *B. sartorii* is provided.

Clavel *et al.* (2010) described *Bacteroides sartorii*, which was isolated from the caecum of a TNF^{deltaARE} mouse, as a novel species within the genus *Bacteroides*. *B. sartorii* was closely related to *Bacteroides massiliensis*, *Bacteroides dorei* and *Bacteroides vulgatus* (≤ 94 % 16S rRNA gene sequence similarity between strains). Kitahara *et al.* (2011) isolated a novel *Bacteroides* species, *Bacteroides chinchillae*, from chinchilla (*Chinchilla lanigera*) faeces. This species was related to the above-mentioned three species as well as to *B. sartorii*. From their published descriptions, *B. sartorii* and *B. chinchillae* appear to be different species. However, the two type strains exhibit 99.6 % 16S rRNA gene sequence similarity; they differ by only 5 bases in their almost full-length sequences (1490 nt). The present study was designed to determine the taxonomic status of *B. sartorii* and *B. chinchillae* by using a polyphasic taxonomic approach.

Bacteroides sartorii JCM 17136^T, and *Bacteroides chinchillae* JCM 16497^T and JCM 16498 were used in this study. The strains were maintained on Eggerth Gagnon (EG) agar (Merck) supplemented with 5 % (v/v) horse blood for 2 days at 37 °C in an atmosphere containing 100 % CO₂. *Bacteroides* bile aesculin agar (Shah, 1992) was used to check whether the growth of the strains was inhibited on this medium.

Physiological reactions were determined with an API 20A anaerobe test kit in duplicate as recommended by the

manufacturer (bioMérieux). Fatty acid methyl esters were obtained from about 40 mg wet cells by saponification, methylation and extraction using minor modifications (Kuykendall *et al.*, 1988) of the method of Miller (1982). Cellular fatty acid profiles were determined by the Sherlock Microbial Identification System (MIDI) version 2.99B with database BHIBLA (version 3.80). Isoprenoid quinones were extracted (Komagata & Suzuki, 1987) and analysed (Sakamoto *et al.*, 2002) as described previously. Biochemical reactions were determined with the Rapid ID 32A anaerobe identification kit in duplicate as recommended by the manufacturer (bioMérieux). Chromosomal DNA was isolated by previously described methods (Marmur, 1961; Saito & Miura, 1963), with some modifications. The DNA G+C content was determined by the HPLC method of Tamaoka & Komagata (1984). The elution solvent was a mixture of 0.02 M NH₄H₂PO₄ and acetonitrile (20:1, v/v). The DNA–DNA hybridization experiments were carried out in microplate wells, as described by Ezaki *et al.* (1989). Hybridization was performed at 42.5 °C for 16 h. The 16S rRNA gene was analysed as described previously (Sakamoto *et al.*, 2002). The partial *hsp60* gene was analysed as described previously (Sakamoto & Ohkuma, 2010; Sakamoto *et al.*, 2010). Related sequences were aligned with the program CLUSTAL_X 2.0 (Larkin *et al.*, 2007) and corrected by manual inspection. Nucleotide substitution rates (K_{nuc} values) were calculated (Kimura, 1980) after gaps and unknown bases were eliminated. The phylogenetic tree was reconstructed by the neighbour-joining method (Saitou & Nei, 1987). Bootstrap resampling analysis (Felsenstein, 1985) was performed to estimate the confidence of tree topologies.

B. sartorii JCM 17136^T, and *B. chinchillae* JCM 16497^T and JCM 16498 were obligately anaerobic, non-spore-forming,

The GenBank/EMBL/DDJB accession numbers for the 16S rRNA gene sequences of *B. sartorii* JCM 17136^T, and *B. chinchillae* JCM 16497^T and JCM 16498 are AB572597, AB547636 and AB547637, respectively, and those for their partial *hsp60* gene sequences are AB641323, AB547549 and AB547550, respectively.

Table 1. Characteristics of *Bacteroides sartorii* and *Bacteroides chinchillae*

Strains: 1, *B. sartorii* JCM 17136^T; 2, *B. chinchillae* JCM 16497^T; 3, *B. chinchillae* JCM 16498. All strains produced acid from L-arabinose, cellobiose, glucose, lactose, maltose, D-mannose, raffinose, L-rhamnose and D-xylose. All strains failed to produce acid from glycerol, D-mannitol, melezitose, salicin, D-sorbitol and trehalose. All strains were positive for aesculin hydrolysis. All strains were negative for indole production, gelatin digestion, and urease and catalase activities. All strains were positive in Rapid ID 32A tests for β -N-acetylglucosaminidase, alkaline phosphatase, α -arabinosidase, α -fucosidase, α -galactosidase, β -galactosidase, α -glucosidase, β -glucosidase, mannose and raffinose fermentation, alanine arylamidase, glutamyl glutamic acid arylamidase, glycine arylamidase and leucyl glycine arylamidase. All strains were negative in Rapid ID 32A tests for indole production, nitrate reduction, urease, arginine dihydrolase, β -galactosidase-6-phosphate, β -glucuronidase, glutamic acid decarboxylase, histidine arylamidase, phenylalanine arylamidase, proline arylamidase, pyroglutamic acid arylamidase, serine arylamidase and tyrosine arylamidase.

Characteristic	1	2	3
Acid production from sucrose	–	+	+
Rapid ID 32A tests			
Arginine arylamidase	+	–	–
Leucine arylamidase	+	–	–

non-motile, Gram-negative short rods or rods. Cells on EG agar were 1.0–1.5 μ m wide and variable in length, mostly 2.0–15.0 μ m. Colonies on EG agar plates after 48 h of incubation at 37 °C under anaerobic conditions were 1–2 mm in diameter, circular, entire, slightly convex, smooth, white-greyish and translucent. *B. sartorii* and *B. chinchillae* were able to grow on Bacteroides bile aesculin agar and aesculin hydrolysis was positive. The phenotypic and biochemical characteristics of the two strains of *B. chinchillae* were almost the same as that of the type strain of *B. sartorii*; the only differential characteristics were sucrose fermentation and arginine arylamidase and leucine arylamidase activities (Table 1).

The cellular fatty acid compositions of the two strains of *B. chinchillae* were similar to that of the type strain of *B. sartorii* (Table 2). The major fatty acids were anteiso-C_{15:0}, C_{18:1} ω 9c and iso-C_{17:0} 3-OH in both species. Amounts of anteiso-C_{15:0} were slightly lower in *B. chinchillae* than in *B. sartorii*. The major menaquinone of *B. sartorii* JCM 17136^T, and *B. chinchillae* JCM 16497^T and JCM 16498 was MK-10 (95.5, 94.8 and 95.5 %, respectively). The minor menaquinone of the three strains was MK-9 (4.5, 5.2 and 4.5 %, respectively).

Approximately 1500 bases of the 16S rRNA gene sequence were determined for each strain. For the phylogenetic analysis, 1465 bp sequences of each species were used. *B. sartorii* JCM 17136^T, and *B. chinchillae* JCM 16497^T and JCM 16498 formed a single cluster and a distinct line of descent (Fig. 1). These strains exhibited more than 99.4 % 16S rRNA gene sequence similarity with each other and

Table 2. Cellular fatty acid compositions of *Bacteroides sartorii* and *Bacteroides chinchillae*

Strains: 1, *B. sartorii* JCM 17136^T; 2, *B. chinchillae* JCM 16497^T; 3, *B. chinchillae* JCM 16498. tr, Trace amount (<0.5 %); –, not detected. Values are percentages of total fatty acids.

Fatty acid	1	2	3
Saturated straight-chain			
C _{13:0}	–	–	tr
C _{14:0}	1.7	1.2	3.3
C _{15:0}	1.3	0.6	2.3
C _{16:0}	4.1	3.9	5.3
C _{18:0}	tr	0.9	0.6
Unsaturated straight-chain			
C _{16:1} ω 7c	–	tr	–
C _{18:1} ω 9c	10.1	13.2	15.5
C _{18:2} ω 6,9c	0.9	1.3	1.4
Hydroxy acids			
C _{14:0} 3-OH	–	tr	tr
C _{15:0} 3-OH	tr	tr	0.9
C _{16:0} 3-OH	4.7	9.0	11.1
C _{17:0} 3-OH	–	–	tr
iso-C _{15:0} 3-OH	tr	tr	–
iso-C _{16:0} 3-OH	0.9	1.3	1.3
iso-C _{17:0} 3-OH	14.1	15.9	10.5
anteiso-C _{17:0} 3-OH	4.1	8.5	5.1
Saturated branched-chain			
iso-C _{13:0}	0.5	tr	tr
anteiso-C _{13:0}	0.7	tr	tr
iso-C _{14:0}	0.8	tr	0.9
iso-C _{15:0}	6.7	2.9	3.5
anteiso-C _{15:0}	43.7	34.7	31.1
iso-C _{16:0}	tr	–	–
anteiso-C _{17:0}	tr	tr	tr
Summed features*			
1	0.7	1.0	2.1
3	2.8	2.6	2.4
10	0.6	1.4	1.2

*Summed features represent groups of two or three fatty acids that could not be separated using the MIDI system. Summed feature 1 contains C_{13:1} ω 1c and/or C_{14:0} ALDE. Summed feature 3 contains one or more of an unknown fatty acid of ECL 13.570 and/or iso-C_{15:0} ALDE. Summed feature 10 contains one or more of an unknown fatty acid of ECL 17.834 and/or C_{18:1} ω 11c/9t/6t fatty acid methyl ester.

were most closely related to *B. massiliensis* JCM 13223^T as described previously (Clavel *et al.*, 2010; Kitahara *et al.*, 2011). In addition, *hsp60* gene sequence analysis suggested that the three strains were members of the same species (100 % *hsp60* gene sequence similarity) (Fig. 1). The *hsp60* gene has been found to be an alternative phylogenetic marker for the classification of species of the genus *Bacteroides* (Sakamoto *et al.*, 2010).

The DNA G + C contents of *B. chinchillae* JCM 16497^T and JCM 16498 were 44.1 and 44.4 mol%, respectively. These

Fig. 1. Phylogenetic tree based on 16S rRNA (a) and *hsp60* (b) gene sequences showing the relationships between *Bacteroides sartorii* and *Bacteroides chinchillae*. The tree was reconstructed by the neighbour-joining method. Numbers at nodes indicate the bootstrap values (%) of 1000 replicates. Bars, 0.01 (a) or 0.02 (b) substitutions per nucleotide position.

Table 3. DNA G+C contents and levels of DNA relatedness

Strain	DNA G+C content (mol%)	DNA relatedness (%) with:		
		1	2	3
1. <i>B. sartorii</i> JCM 17136 ^T	45.5	100	79	74
2. <i>B. chinchillae</i> JCM 16497 ^T	44.1	79	100	94
3. <i>B. chinchillae</i> JCM 16498	44.4	87	105	100

values are slightly lower than that of *B. sartorii* JCM 17136^T (45.5 mol%). The levels of DNA–DNA relatedness among *B. sartorii* JCM 17136^T, *B. chinchillae* JCM 16497^T and JCM 16498 are shown in Table 3 and demonstrate that these three strains represent a single species. Consequently, *B. chinchillae* (Kitahara *et al.*, 2011) is a later heterotypic synonym of *B. sartorii* (Clavel *et al.*, 2010).

Emended description of *Bacteroides sartorii* Clavel *et al.* 2010

The description is as given by Clavel *et al.* (2010) and Kitahara *et al.* (2011) with the following modifications: the predominant respiratory quinone is MK-10 (94.8 to 95.5 %); a minor amount of MK-9 (4.5 to 5.2 %) is present; and the DNA G+C content is in the range 44.1–45.5 mol%.

Acknowledgements

We thank Ms Natsuko Suzuki and Ms Misako Matsuda for their technical assistance. This work was supported by a research grant from IFO (Institute for Fermentation, Osaka, Japan) (2009–2011), and also by a Grant-in-Aid from the Scientific Research from the Japan Society for the Promotion of Science to M. S. (no. 23580126).

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