Bacteroides barnesiae sp. nov., Bacteroides salanitronis sp. nov. and Bacteroides gallinarum sp. nov., isolated from chicken caecum

Pham Thi Ngoc Lan,1,2 Mitsuo Sakamoto,2 Shinji Sakata2 and Yoshimi Benno2

1Institute of Biotechnology, Vietnamese Academy of Science and Technology, Hanoi, Vietnam
2Microbe Division/Japan Collection of Microorganisms, RIKEN BioResource Center, Wako, Saitama 351-0198, Japan

Eight bacterial strains isolated from the caecum of chicken, BL2T, BL66, EG3, EG6, M27, BL78T, C35T and C43, were characterized by determining their phenotypic characteristics, cellular fatty acid profiles, menaquinone profiles and phylogenetic positions based on 16S rRNA gene sequence analysis. 16S rRNA gene sequence analysis showed that these isolates belonged to the genus Bacteroides. One group of five strains (BL2T, BL66, EG3, EG6 and M27) was related most closely to Bacteroides coprocola JCM 12979T, with approximately 93 % 16S rRNA gene sequence similarity, and to Bacteroides plebeius JCM 12973T, with about 92 % similarity, and shared ≥99-6 % similarity with each other. Strain BL78T exhibited 90·5 % similarity to B. plebeius JCM 12973T and 89·8 % similarity to B. coprocola JCM 12979T and differed from the above group of five strains at ≥10 % sequence divergence. Strains C35T and C43 were related most closely to Bacteroides eggertii JCM 12986T, with 95·1 % sequence similarity, to Bacteroides stercoris JCM 9496T, with 94·6 % similarity, and to Bacteroides uniformis JCM 5828T, with 94·4 % similarity, and shared 100 % similarity with each other. From results of phenotypic examination, cellular fatty acid composition analysis, menaquinone composition analysis and DNA G + C contents, the group of five strains as well as strain BL78T were shown to differ from the type strains of B. coprocola and B. plebeius. Strain BL78T differed from the others based on its menaquinone composition, which included MK-11 and MK-12. Strains C35T and C43 could also be differentiated from the type strains of B. eggertii, B. stercoris and B. uniformis. The group of five strains, strain BL78T, B. coprocola JCM 12979T and B. plebeius JCM 12973T showed low levels of DNA–DNA relatedness (<35 %) with each other. High levels of DNA–DNA relatedness were obtained within the group of five strains (>75 %). Strains C35T and C43 exhibited a high level of DNA–DNA relatedness (>88 %) with each other, but low levels with B. eggertii JCM 12986T (<40 %), B. stercoris JCM 9496T (<37 %) and B. uniformis JCM 5828T (<16 %). On the basis of these data, three novel Bacteroides species are proposed: Bacteroides barnesiae sp. nov. (type strain BL2T = JCM 13652T = DSM 18169T), Bacteroides salanitronis sp. nov. (type strain BL78T = JCM 13657T = DSM 18170T) and Bacteroides gallinarum sp. nov. (type strain C35T = JCM 13658T = DSM 18171T).

Gastrointestinal microbes are important factors that influence animal production. The genus Bacteroides represents one of the predominant anaerobic genera found in the chicken caecum (Barnes et al., 1978; Salanitro et al., 1974a, b). However, relatively few Bacteroides species have been isolated from chicken intestine. Bacteroides species are thought to play a fundamental role in the breakdown of complex molecules (such as polysaccharides) into simpler compounds that are used by the animal host as well as the micro-organisms themselves (Reeves et al., 1997; Degnan et al., 1997), in the utilization of nitrogenous substances and in the biotransformation of bile acids and other steroids.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of B. barnesiae strains JCM 13652T, JCM 13653, JCM 13654, JCM 13655 and JCM 13656, B. salanitronis JCM 13657T and B. gallinarum strains JCM 13658T and JCM 13659 are AB253726–AB253733.

Tables giving the phenotypic characteristics, cellular fatty acid compositions and menaquinone compositions of the novel strains and related Bacteroides species are available as supplementary material in IJSEM Online.
Eight strains (BL2T, BL66, EG3, EG6, M27, BL78T, C35T and C43) were isolated from the caecum of a healthy chicken. Isolation of strictly anaerobic bacteria was performed according to a standard anaerobic technique in our laboratory (Mitsuoka et al., 1969; Lan et al., 2002). The isolated strains were maintained on Eggerth Gagnon (EG) agar (Merck) supplemented with 5% (v/v) horse blood for 2 days at 37°C in an anaerobic jar (Hirayama Manufacturing Corp.) filled with 100% CO2. Strains BL2T, BL66 and BL78T were isolated on BL agar (Nissui Pharmaceutical Co., Ltd). Strains EG3 and EG6 were isolated on EG agar. Strains M27, C35T and C43 were isolated on medium 10 (Caldwell & Bryant, 1966) by using the ‘plate-in-bottle’ procedure (Mitsuoka et al., 1969).

Physiological and biochemical characteristics were determined by using an API 20A anaerobic test kit and API Rapid ID 32A enzymic tests in duplicate as recommended by the manufacturer (bioMérieux). Bile resistance was tested by growing the bacteria on Bacteroides bile aesculin agar plates (Shah, 1992). Fermentation metabolic end products were prepared as described by Holdeman et al. (1977) and analysed as described previously (Sakamoto et al., 2004, 2005). 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 using the MIDI microbial identification system (Microbial ID). Isoprenoid quinones were extracted as described by Komagata & Suzuki (1987) and analysed as described previously (Sakamoto et al., 2004, 2005). Chromosomal DNA was isolated by using the methods of Marmur (1961) and Saito & Miura (1963), with some modifications. The DNA G+C content was determined by using the HPLC method of Tamaoka & Komagata (1984), again with some modifications. DNA–DNA hybridization experiments were carried out in microplate wells, as described by Ezaki et al. (1989). Hybridization was performed at 44°C for 16 h. 16S rRNA gene sequences were obtained and analysed as described previously (Lan et al., 2002; Sakamoto et al., 2002). Reference 16S rRNA gene sequences used for comparisons in this study were retrieved from the DDBJ, EMBL and GenBank nucleotide sequence databases. Sequence data were aligned using the CLUSTAL W program (Thompson et al., 1994) and corrected by manual inspection. Nucleotide substitution rates (Ksub values) were calculated (Kimura, 1980) after gaps and unknown bases had been eliminated. A phylogenetic tree was constructed based on the neighbour-joining method (Saitou & Nei, 1987). Bootstrap resampling analysis (Felsenstein, 1985) was performed to estimate the confidence of tree topologies.

Cells of the eight isolates investigated were obligately anaerobic, non-spore-forming, non-motile, Gram-negative pleomorphic rods. On EG agar, cells of strains BL2T, BL66, EG3, EG6 and M27 were 0·5–1·4 × 0·8–10·6 μm, cells of strain BL78T were 0·4–0·7 × 0·8–5·6 μm and cells of strains C35T and C43 were 0·4–0·6 × 0·8–6·5 μm. Cells of all eight strains occurred either singly or in pairs. Colonies of all eight strains were white–greyish. Colonies of strains BL2T, BL66, EG3, EG6 and M27 on EG agar were 1·5–3·0 mm in diameter, circular, raised, convex and smooth. Colonies of strain BL78T on EG agar were 2·0–3·0 mm in diameter, rounded and smooth, and colonies of strains C35T and C43 on EG agar were 1·0–1·5 mm in diameter, polished and circular. All eight strains were able to hydrolyse aesculin on Bacteroides bile aesculin agar. The results of phenotypic tests are given in the species descriptions below. Phenotypic characteristics of the test strains and the type strains of related Bacteroides species are given in Supplementary Table S1 available in IJSEM Online. From the API 20A tests, five strains (BL2T, BL66, EG3, EG6 and M27) exhibited identical sugar fermentation patterns; these strains could be differentiated from Bacteroides coprocola JCM 12979T by their inability to ferment L-rhamnose and could be distinguished from Bacteroides plebeius JCM 12973T by their ability to ferment salicin and inability to ferment L-arabinose or L-rhamnose. Strain BL78T could be differentiated from B. coprocola JCM 12979T by its ability to ferment L-arabinose and from B. plebeius JCM 12973T by its ability to ferment salicin. Strains C35T and C43 could be differentiated from Bacteroides eggerthii JCM 12986T by their ability to ferment sucrose and D-raffinose, from Bacteroides stercoris JCM 9496T by their ability to ferment L-arabinose and D-cellulbiose and from Bacteroides uniformis JCM 5828T by their ability to ferment L-rhamnose and inability to ferment salicin. From the API rapid ID 32A tests, the group of five strains differed from B. coprocola JCM 12979T in that they were negative for β-galactosidase 6-phosphate and glutamic acid decarboxylase. These five strains differed from B. plebeius JCM 12973T based on the absence of activities for β-galactosidase 6-phosphate, β-glucuronidase, glutamic acid decarboxylase and arginine, phenylalanine, leucine, glycine and histidine arylamidases. The enzymic pattern of strain BL78T differed from that of B. coprocola JCM 12979T in that it was negative for β-galactosidase 6-phosphate, N-acetyl-β-glucosaminidase, glutamic acid decarboxylase and α-fucosidase. The enzymic pattern of strain BL78T differed distinctively from that of B. plebeius JCM 12973T in that it was negative for β-galactosidase 6-phosphate, β-glucuronidase, N-acetyl-β-glucosaminidase,
glutamic acid decarboxylase, \( \alpha \)-fucosidase and arginine, phenylalanine, leucine, glycine and histidine arylamidases. Isolates C35\(^T\) and C43 could be differentiated from \emph{B. eggerthii} JCM 12986\(^T\) by their activity for glutamic acid decarboxylase and raffinose fermentation, from \emph{B. stercoris} JCM 9496\(^T\) by activity for \( \alpha \)-arabinosidase and glutamic acid decarboxylase and of lack of activity for \( \alpha \)-fucosidase and from \emph{B. uniformis} JCM 5828\(^T\) by lack of activity for \( \alpha \)-galactosidase, \( \beta \)-galactosidase 6-phosphate and \( \alpha \)-fucosidase. Differential characteristics between the novel strains and the type strains of related \emph{Bacteroides} species are summarized in Table 1.

The cellular fatty acid composition of \emph{Bacteroides} species has been determined previously (Mayberry \textit{et al.}, 1982; Miyagawa \textit{et al.}, 1979; Shah & Collins, 1980) and was reviewed for the classification of the genus \emph{Bacteroides} by Shah & Collins (1983). In the present study, the major cellular fatty acids of five strains (BL2\(^T\), BL66, EG3, EG6 and M27) included anteiso-C\(_{15:0}\), iso-C\(_{15:0}\), C\(_{16:0}\) 3-OH and C\(_{16:0}\). The major cellular fatty acids of strain BL78\(^T\) were anteiso-C\(_{15:0}\), iso-C\(_{15:0}\), C\(_{16:0}\) 3-OH and iso-C\(_{17:0}\) 3-OH. The major cellular fatty acids of strains C35\(^T\) and C43 were anteiso-C\(_{15:0}\), iso-C\(_{15:0}\) and iso-C\(_{17:0}\) 3-OH. Strains C35\(^T\) and C43 showed slightly higher levels of anteiso-C\(_{15:0}\) (37\% and 38.4\%, respectively) than \emph{B. eggerthii} JCM 12986\(^T\) (33\%), \emph{B. stercoris} JCM 9496\(^T\) (30\%) and \emph{B. uniformis} JCM 5828\(^T\) (33\%). The cellular fatty acid compositions of the novel strains and of the type strains of related \emph{Bacteroides} species are given in Supplementary Table S2 in IJSEM Online.

Isoprenoid quinones (principally menaquinones and ubiquinones) are important in the functioning of respiratory electron transport systems. The usefulness of quinone analysis for bacterial classification and identification has been reviewed by Collins & Jones (1981). The major menaquinones of members of the genus \emph{Bacteroides} are MK-10 and MK-11 (Shah, 1992). Indeed, five of the studied strains (BL2\(^T\), BL66, EG3, EG6 and M27) and two related

### Table 1. Differential characteristics of strains BL2\(^T\), BL78\(^T\) and C35\(^T\) and the type strains of related \emph{Bacteroides} species

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fermentation of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Salicin</td>
<td>+</td>
<td>w</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>L-Arabinose</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>D-Cellobiose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>W</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>D-Raffinose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>L-Rhamnose</td>
<td>–</td>
<td>w</td>
<td>+</td>
<td>w</td>
<td>w</td>
<td>w</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>API rapid ID 32A results</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \alpha )-Galactosidase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>( \beta )-Galactosidase 6-phosphate</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>( \beta )-Glucosidase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>W</td>
<td>+</td>
</tr>
<tr>
<td>( \alpha )-Arabinosidase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>( \beta )-Glucuronidase</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>W</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>N-Acetyl-( \beta )-glucosaminidase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glutamic acid decarboxylase</td>
<td>–</td>
<td>–</td>
<td>W</td>
<td>W</td>
<td>W</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>( \alpha )-Fucosidase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Arginine arylamidase</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Phenylalanine arylamidase</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Leucine arylamidase</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Glycine arylamidase</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Histidine arylamidase</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Distinctive major cellular fatty acid(s)*</td>
<td>C(<em>{16:0}), C(</em>{16:0}) 3-OH</td>
<td>C(<em>{16:0}), C(</em>{16:0}) 3-OH</td>
<td>C(<em>{16:0}), C(</em>{16:0}) 3-OH</td>
<td>C(<em>{16:0}), C(</em>{16:0}) 3-OH</td>
<td>C(<em>{16:0}), C(</em>{16:0}) 3-OH</td>
<td>C(<em>{17:0}), C(</em>{17:0}) 3-OH</td>
<td>C(<em>{17:0}), C(</em>{17:0}) 3-OH</td>
<td>C(<em>{17:0}), C(</em>{17:0}) 3-OH</td>
</tr>
<tr>
<td>Distinctive predominant menaquinone*</td>
<td>MK-10</td>
<td>MK-12</td>
<td>MK-10</td>
<td>MK-10</td>
<td>MK-10</td>
<td>MK-10</td>
<td>MK-10</td>
<td>MK-10</td>
</tr>
<tr>
<td>Metabolic end products (acetic acid: propionic acid)</td>
<td>1:0</td>
<td>1:0</td>
<td>1:0</td>
<td>1:0</td>
<td>1:0</td>
<td>1:0</td>
<td>1:0</td>
<td>1:0</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>46–47:1</td>
<td>46:9</td>
<td>42:8</td>
<td>44:6</td>
<td>47:0–47:3</td>
<td>44:2</td>
<td>46:3</td>
<td>46:1</td>
</tr>
</tbody>
</table>

*Anteiso-C\(_{15:0}\), iso-C\(_{15:0}\) and MK-11 are major components in all strains.
Bacteroides species (*B. coprocola* JCM 12979^T^ and *B. plebeius* JCM 12973^T^) possess a high level of MK-10 (58–67 %) and MK-11 (25–34 %). However, strain BL78^T^ exhibited MK-11 (43 %) and MK-12 (43 %) as the main components (see Supplementary Table S3 in IJSEM Online). Strains C35^T^ and C43 and three reference type strains (*B. eggerthii* JCM 12986^T^, *B. stercoris* JCM 9496^T^ and *B. uniformis* JCM 5828^T^) also possess MK-10 and MK-11 as the major menaquinones.

Approximately 1500 bases of the 16S rRNA gene sequence were determined for each of the eight new isolates. For the phylogenetic analysis, 1338 bp sequences (positions 61–1375 of the *Escherichia coli* numbering system) of each organism were used. Five strains (BL2^T^, BL66, EG3, EG6 and M27) were found to be genetically closely related to each other, with ≥99.6 % 16S rRNA gene sequence similarity, and created a separate branch; these five strains differed from strain BL78^T^ with ≤10 % sequence divergence. Strain BL78^T^ and the group of five strains comprised a new subcluster, which displayed the closest phylogenetic relationship with the subcluster of *B. plebeius* JCM 12973^T^ and *B. coprocola* JCM 12979^T^.

The DNA G+C contents of the group of five strains (BL2^T^, BL66, EG3, EG6 and M27) ranged from 46.4 to 47.1 mol%. The DNA G+C content of strain BL78^T^ was 46.9 mol%. In comparison, the DNA G+C contents of *B. coprocola* JCM 12979^T^ and *B. plebeius* JCM 12973^T^ were 42.8 and 44.6 mol%, respectively. The group of five strains showed a level of DNA–DNA relatedness of >75 % with each other, <30 % with strain BL78^T^, <22 % with *B. coprocola* JCM 12979^T^ and <35 % with *B. plebeius* JCM 12973^T^.

On the basis of the data presented here we propose the creation of three novel *Bacteroides* species: *Bacteroides barnesiae* sp. nov., *Bacteroides salanitronis* sp. nov. and *Bacteroides gallinarum* sp. nov.
these three species and of related Bacteroides species are shown in Table 1.

Description of Bacteroides barnesiae sp. nov.

Bacteroides barnesiae (barne’siae. N.L. gen. fem. n. barnesiae of Barnes, named after Ella M. Barnes, a British microbiologist, who has contributed much to our knowledge of intestinal bacteriology and anaerobic bacteriology in general).

Cells are strictly anaerobic, non-spor-forming, non-motile, Gram-negative, pleomorphic rods, 0.5–1.4 μm wide and 0.8–10.6 μm long, which occur singly or in pairs. Surface colonies on EG blood agar plates after 2 days are 1.5–3.0 mm in diameter, white-greyish, circular, raised and convex. Optimum growth temperature is 37°C. Grows in the presence of bile. Indole is not produced. Catalase- and urease-negative. Nitrate is not reduced to nitrite. Gelatin is not liquefied. Aesculin is hydrolysed. Using the API 20A system, produces acid from glucose, lactose, sucrose, maltose, D-xylene, L-arabinose, D-cellobiose, D-mannose and D-raffinose, but not from D-mannitol, glycerol, D-melezitose, D-sorbitol or D-trehalose. Salicin and L-rhamnose are fermented weakly. Using the rapid ID 32A system, positive reactions are obtained for x-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, α-arabinosidase, N-acetyl-β-glucosaminidase, α-fucosidase, alkaline phosphatase, leucyl glycine arylamidase, alanine arylamidase and glutamyl glutamic acid arylamidase. Negative reactions are obtained for L-arginine dihydrolase, β-galactosidase β-phosphate, β-glucuronidase, N-acetyl-β-glucosaminidase, glutamic acid decarboxylase, x-fucosidase and arginase, proline, phenylalanine, leucine, pyrogallol glutamic acid, tyrosine, glycine, histidine and serine arylamidas. The major end products [from 1 % (w/v) peptone/1 % (w/v) yeast extract/1 % (w/v) glucose broth culture] are succinic and acetic acids; a small amount of isovaleric acid is also produced. The major cellular fatty acid is anteiso-C15 : 0 (32 % of the total). Significant amounts of iso-C15 : 0 (14 %), C16 : 0 3-OH (12 %) and iso-C17 : 0 3-OH (10 %) are also present. The principal respiratory quinones are menaquinones MK-11 (43 %) and MK-12 (43 %). Menaquinones MK-10 (5 %) and MK-13 (7 %) are present as minor components. The DNA G+C content of the type strain is 46-9 mol%.

The type and only strain, BL78T (= JCM 13657T = DSM 18170T), was isolated from caecum of a healthy chicken.

Description of Bacteroides gallinarum sp. nov.

Bacteroides gallinarum (gall.i.na’rum. L. gen. fem. pl. n. gallinarum from/of chickens or hens).

Cells are strictly anaerobic, non-spor-forming, non-motile, Gram-negative rods, 0.4–0.6 μm wide and 0.8–6.5 μm long, which occur singly or in pairs. Surface colonies on EG blood agar plates after 2 days are 1.0–1.5 mm in diameter, white-greyish, polished and circular. Optimum growth temperature is 37°C. Grows well in the presence of bile and can produce indole. Catalase- and urease-negative. Nitrate is not reduced to nitrite. Gelatin is not liquefied. Aesculin is hydrolysed. Using the API 20A system, produces acid from glucose, lactose, sucrose, maltose, D-xylene, L-arabinose, D-cellobiose, D-mannose and D-raffinose, but not from D-mannitol, salicin, glycerol, D-melezitose, D-sorbitol or D-trehalose. L-Rhamnose is fermented weakly. Using the rapid ID 32A system, positive reactions are obtained for β-galactosidase, α-glucosidase, β-glucosidase, α-arabinosidase, alkaline phosphatase, leucyl glycine arylamidase, alanine arylamidase and glutamyl glutamic acid arylamidase. Negative reactions are obtained for L-arginine dihydrolase, β-galactosidase β-phosphate, β-glucuronidase, N-acetyl-β-glucosaminidase, glutamic acid decarboxylase, x-fucosidase and arginase, proline, phenylalanine, leucine, pyrogallol glutamic acid, tyrosine, glycine, histidine and serine arylamidas. The major end products [from 1 % (w/v) peptone/1 % (w/v) yeast extract/1 % (w/v) glucose broth culture] are succinic and acetic acids; a small amount of isovaleric acid is also produced. The major cellular fatty acid is anteiso-C15 : 0 (32 % of the total). Significant amounts of iso-C15 : 0 (10–15 %), C16 : 0 (10–15 %) and C16 : 0 3-OH (10–14 %) are also present. The principal respiratory quinones are menaquinones MK-11 (43 %) and MK-12 (43 %). Menaquinones MK-10 (5 %) and MK-13 (7 %) are present as minor components. The DNA G+C content of the type strain is 46-8 mol%.

The type strain, BL2T (= JCM 13652T = DSM 18169T), was isolated from caecum of a healthy chicken.


**References**


**Acknowledgements**

We are grateful to Professor Masafumi Fukuyama (Department of Microbiology, Azabu University, Japan) for his kind help in collecting samples. We are also grateful to Professor H. G. Truper, University of Bonn, Germany, for his suggestions regarding bacterial nomenclature. We thank colleagues at the JCM (Japan Collection of Microorganisms) for their help and for useful discussion. This study was supported by a research project of the JCM. It was also supported in part by a Grant-in-Aid for Scientific Research (No. 16255001) from the Japan Society for the Promotion of Science.
