**Bacteroides koreensis** sp. nov. and **Bacteroides kribbi** sp. nov., two new members of the genus **Bacteroides**

Yeseul Shin,† Soo-Je Park,‡ Jayoung Paek,¹ Joong-Su Kim,³ Moon-Soo Rhee,¹ Hongik Kim,⁴ Joong-Ki Kook⁵ and Young-Hyo Chang¹,*

**Abstract**

Three bacterial isolates from human faeces, YS-aM39ᵀ, R2F3-3-3ᵀ and R2F3-5-1, were characterized as Gram-negative, strictly anaerobic, non-spore-forming, non-motile, and rod-shaped. Isolate YS-aM39ᵀ formed a distinct line of descent, showing greatest 16S rRNA gene sequence relatedness with R2F3-3-3ᵀ (97.5 %), R2F3-5-1 (97.5 %), Bacteroides ovatus (98.8 %) and Bacteroides xylanisolvens (97.2 %). Isolates R2F3-3-3ᵀ and R2F3-5-1 also formed a distinct line of descent, sharing greatest 16S rRNA gene sequence relatedness with B. ovatus (98.2 %) and B. xylanisolvens (97.2 %). The DNA G+C content of YS-aM39ᵀ was 44.8 mol%, that of R2F3-3-3ᵀ was 42.4 mol% and that of R2F3-5-1 was 42.6 mol%. The respiratory quinone of all three isolates was menaquinone MK-10. Polar lipid analysis identified phosphatidylethanolamine as the major lipid. The predominant fatty acids in all three isolates were anteiso-C₁₅ : ₀, iso-C₁₅ : ₀, C₁₆ : ₀, 3-OH and iso-C₁₇ : ₀ 3-OH. The major end products of glucose fermentation were acetic acid, lactic acid and formic acid. DNA–DNA hybridization data indicated that two isolates, YS-aM39ᵀ and R2F3-3-3ᵀ, represent a species distinct from *B. ovatus* and *B. xylanisolvens*. Finally, in this study, the two isolates represented two new species in the genus *Bacteroides*, for which we propose the names *Bacteroides koreensis* sp. nov. (type strain, YS-aM39ᵀ=KCTC 15520ᵀ=JCM 31393ᵀ) and *Bacteroides kribbi* sp. nov. (type strain, R2F3-3-3ᵀ=KCTC 15460ᵀ=JCM 31391ᵀ).

The human colon contains a diverse microbial community (microbiota). Many 16S rRNA sequence-based studies have revealed that constituent microbes belong to hundreds of distinct species, including the genera *Bacteroides*, *Eubacterium*, *Clostridium*, *Faecalibacterium*, *Ruminococcus*, *Fusobacterium* and *Bifidobacterium* [1–3]. Among them, species belonging to the genus *Bacteroides*, the predominant genus within the human and animal gut microbiota, play an important role in degradation and fermentation of organic matter in the colon [4]. *Bacteroides* species are strictly anaerobic, Gram-negative, non-motile, rod-shaped and non-spore-forming bacteria, and comprise more than 92 species [5, 6].

Here, we obtained three isolates, YS-aM39ᵀ, R2F3-3-3ᵀ and R2F3-5-1, from the faeces of a healthy adult. Sequence analysis of 16S rRNA genes revealed that the isolates were most closely related to *Bacteroides ovatus* and *Bacteroides xylanisolvens* [7]. Here, we characterize the phenotypic, chemotaxonomic, and phylogenetic properties of these novel isolates belonging to the genus *Bacteroides*.

Faecal samples from a healthy adult were serially diluted in phosphate-buffered saline (pH 7.0), spread onto Reinforced Clostridial Medium (RCM; pH 6.8; Difco) plates and incubated at 37°C for 48 h under anaerobic conditions (Forma Anaerobic System; Thermo Fisher Scientific) in a gas phase of N₂/H₂/CO₂ (88:7:5 %, v/v/v). Isolates were stored at −80°C as skim milk suspensions (10 %, w/v). The type strains *B. ovatus* KCTC 5827ᵀ and *B. xylanisolvens* KCTC 15192ᵀ were used as reference strains based on comparison of their 16S rRNA gene sequences with those of the isolates. Morphological, physiological, molecular and chemotaxonomic studies were performed using cells grown in RCM agar (unless stated otherwise). For experimental validation, all data were

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**Keywords:** *Bacteroides koreensis* nov.; *Bacteroides kribbi* nov.; anaerobic; phylogenetic; new species.

†These authors contributed equally to this work.

The GeneBank accession numbers for the 16S rRNA gene sequences of strains YS-aM39ᵀ, R2F3-3-3ᵀ and R2F3-5-1 are KX025133, KX025134 and KX025135, respectively.

Three supplementary figures are available with the online Supplementary Material.
collected from three independent experiments conducted using the reference strains [8].

Cell morphology and Gram status were determined by bright field microscopy (Nikon Optiphot-2) [9]. Endospore formation was determined by staining with malachite green after heat shock. Catalase and oxidase activities were determined using 3 % (v/v) hydrogen peroxide solution and 1 % (v/v) p-tetramethyl phenylenediamine solution (bio-Mérieux), respectively. The growth temperature (10–50 °C) and NaCl concentration (0–7 %, w/v) ranges were evaluated on RCM over 3–5 days. Growth was assessed by monitoring optical density at 600 nm (Bio-Rad). The pH range for growth was determined in buffered RCM at 37 °C, using a 500 ml flask containing 250 ml of medium at pH 4.0–11.0 (0.5 pH unit increments). The medium was buffered with three different solutions: 50 mM succinic acid/NaOH (pH 4–6), 100 mM Na₂HPO₄/NaH₂PO₄ (pH 6–8) or 50 mM 2-amino-2-methyl-1,3-propanediol/HCl (pH 8–11). Cell motility was determined by incubating the cells on semi-solid RCM (0.4 % agar). Bile resistance was determined on RCM supplemented with 2 % (w/v) Bacto oxgall (Difco). A variety of biochemical and enzyme tests were performed using API 20A and 32A test strips (bioMérieux), according to the manufacturer’s instructions.

The 16S rRNA gene was PCR-amplified using universal primers 27F and 1492R [10] and the PCR products were used for sequence analysis, as described by Jung et al. [11]. The sequences of closely related species were retrieved from the EzTaxon and GenBank databases. Almost-complete 16S rRNA gene sequences of all three isolates were aligned manually against representatives from closely related genera (based on the bacterial 16S rRNA secondary structure model) [12]. The regions within all sequences (conserved and variable regions) showed unambiguous alignment and so were used to reconstruct phylogenetic trees. Alignment and neighbour-joining tree [13] analyses were performed using the PHYLIP [14] and jPHYDIT programs [15]. Phylogenetic trees were also reconstructed using the maximum-likelihood [16] and minimum-evolution [17] methods. An evolutionary distance matrix for the neighbour-joining tree was generated using the model of Jukes and Cantor [18]. The topology of all trees was determined by bootstrap analysis with 1000 replicates.

Fermentation end products in RCM broth (0.3 % glucose) cultures were identified using a gas chromatograph (Varian 3400 GC; Varian Associates) equipped with a flame ionization detector and a CP wax 52 CB column [11, 19].

Genomic DNA from all three isolates and from the reference strains was extracted using a Genomic DNA Extraction kit (Solgent). DNA–DNA hybridization was performed at 45 °C for 4 h, as described previously [20, 21], with P1 nuclease and photobiotin-labelled probes [22]. Fluorescence intensity was measured using a Fluoroskan Ascent Fluorescent plate reader (Thermo Life Science). The mean value for three replicate measurements per sample was calculated.

The G+C content (mol%) of genomic DNA was determined by reverse-phase high pressure liquid chromatography (HPLC), as described previously [23]. Relative values were calculated using E. coli KCTC 2441T DNA (50.8 mol%, Tm) as a standard. Polar lipids were extracted and separated from 100 mg of freeze-dried cell material using a two-stage method, as previously described [24]. Thin-layer chromatography plates were stained with 5 % molybdophosphoric acid to detect all lipids. The menaquinone was examined using reverse-phase HPLC, as described previously [25]. Cellular fatty acids produced by cells grown at 37 °C for 48 h on brain heart infusion agar supplemented with blood (BHIBLA) were determined using a standard protocol [26]. The extracts were analysed using an automated GC system (model 6890 N with autosampler 7683; Agilent Technologies) and the associated software package version 4.0 (BHIBLA library).

Isolates YS-aM39T (=KCTC 15520T=JCM 31393T), R2F3-3-3T (=KCTC 15460T=JCM 31391T) and R2F3-5-1T (=KCTC 15456T=JCM 31392T) were Gram-negative, strictly anaerobic, non-spore-forming, non-motile and rod-shaped bacteria. Colonies formed by the three isolates after growth on RCM agar for 3 days at 37 °C were circular, smooth and cream-coloured with a morphology similar to that of reference strains belonging to the genus Bacteroides. The isolates grew in the presence of bile. The results of tests for gelatin hydrolysis, nitrate reduction, sorbitol, arginine dihydrolase and urease reactions were different from those of the reference strains. The isolates were positive for melezitose, raffinose, α-glucosidase and raffinose, whereas B. ovatus KCTC 5827T was negative. R2F3-3-3T and R2F3-5-1T were positive for glutamic acid decarboxylase; however, YS-aM39T, B. ovatus KCTC 5827T and B. xylanisolvens KCTC 15192T were negative. These results suggested phenotypic differences between isolates and the reference strains of related Bacteroides species. The different characteristics of the isolates and reference strains are shown in Table 1.

Almost-complete 16S rRNA gene sequences of isolates YS-aM39T (1417 bp), R2F3-3-3T (1442 bp) and R2F3-5-1T (1430 bp) were obtained. Phylogenetic analysis clearly indicated that the isolates were related to members of the genus Bacteroides (Fig. 1). YS-aM39T was most closely related to R2F3-3-3T and R2F3-5-1T (97.5 % sequence similarity), followed by B. ovatus JCM 5824T (98.8 %) and B. xylanisolvens JCM 15633T (97.2 %). R2F3-3-3T and R2F3-5-1T were related to B. ovatus JCM 5824T (98.2 %) and B. xylanisolvens JCM 15633T (97.2 %). The degree of 16S rRNA gene sequence similarity between isolates R2F3-3-3T and R2F3-5-1T was 99.9 %. Therefore, they can be considered to represent a single species. The peculiar topology of the phylogenetic trees estimated by the neighbour-joining, maximum-likelihood (Fig. S1, available with the online Supplementary Material) and minimum-evolutionary (Fig. S2) algorithms indicated that isolate YS-aM39T clustered with B. ovatus JCM 5824T, whereas isolates R2F3-3-3T and R2F3-5-1T formed a distinct line of descent. Phylogenetic analysis indicated that the two
taxa represent novel species belonging to the genus *Bacteroides*.

The DNA–DNA hybridization experiments involving isolate YS-aM39\(^T\) revealed the following: 49% relatedness with isolate R2F3-5-1, 47% with isolate R2F3-3-3\(^T\), 35% with *B. xylanisolvens* and 33% with *B. ovatus*. The genomic relatedness between isolates R2F3-3-3\(^T\) and R2F3-5-1 was 89%. Isolates R2F3-3-3\(^T\) and R2F3-5-1 showed 48% relatedness with isolate YS-aM39\(^T\), 32% with *B. ovatus*, and 24% with *B. xylanisolvens*. These values were much lower than the suggested threshold value for species delineation [27], indicating that the isolates represent a novel species that is distinct from validly described species.

The DNA G+C content of isolates YS-aM39\(^T\), R2F3-3-3\(^T\) and R2F3-5-1 were 44.8, 42.4, and 42.6 mol\%, respectively, which supports their affiliation with the genus *Bacteroides*, members of which have a values of between 40 and 48 mol\% [28]. The respiratory quinone of isolates YS-aM39\(^T\), R2F3-3-3\(^T\) and R2F3-5-1 was MK-10. These properties are characteristic of members of the genus *Bacteroides* [28]. Polar lipid analysis of isolate YS-aM39\(^T\) identified phosphatidylethanolamine (PE) as the major lipid; minor lipids included aminophospholipid (PN), six phospholipids (PL1–PL6) and one unidentified aminolipid (AL2). Isolates R2F3-3-3\(^T\) and R2F3-5-1 had PE as the major lipid; minor lipids included PN, five phospholipids (PL1–PL5), and two unidentified aminolipids (AL1–AL2). The polar lipid content of the isolates was comparable to that of *B. ovatus* KCTC 5827\(^T\) and *B. xylanisolvens* KCTC 15192\(^T\) (Fig. S3). No AL2 was detected in *B. ovatus* KCTC 5827\(^T\) and *B. xylanisolvens* KCTC 15192\(^T\). By contrast, isolate YS-aM39\(^T\) showed a significantly different profile, with PL6 as the minor component and no AL1. The major cellular fatty acid produced by all isolates was anteiso-C\(_{15:0}\)(38.2–41.7%), iso-C\(_{15:0}\)(9.4–11.0%), C\(_{16:0}\) 3-OH (11.8–12.6%) and iso-C\(_{17:0}\) 3-OH (9.5–10.3%) (Table 2). These data indicate that the major cellular fatty acid composition of the isolates was basically consistent with that of other members of the genus *Bacteroides* [29]. The major end products of glucose fermentation by isolate YS-aM39\(^T\) were acetic acid (51.4 mM), lactic acid (8.6 mM) and formic acid (1.8 mM). Isolates R2F3-3-3\(^T\) and R2F3-5-1 produced acetic acid (51.1 and 53.3 mM, respectively), lactic acid (9.3 and 8.0 mM, respectively) and small amounts of formic acid (2.0 and 2.1 mM, respectively). Other *Bacteroides* species isolated from the human gut, such as *B. ovatus*, *Bacteroides thetaiotaomicron* and *Bacteroides fragilis*, also produce acetic acid from sugar fermentation [30, 31]; however, in contrast to the three isolates they did not produce lactic acid and formic acid. These results suggest that the isolates can be differentiated from other species belonging to the genus *Bacteroides*.

Based on these results, isolates YS-aM39\(^T\), R2F3-3-3\(^T\) and R2F3-5-1 were considered to be members of the genus *Bacteroides*. However, the isolates are different from their closest relatives *B. ovatus* KCTC 5827\(^T\) and *B. xylanisolvens* KCTC 15192\(^T\) in terms of phenotypic and genotypic features. Therefore, we propose that the isolates YS-aM39\(^T\), R2F3-3-3\(^T\) and R2F3-5-1 represent two novel species belonging to the genus *Bacteroides*. The names proposed are

### Table 1. Distinctive characteristics of isolates and the type strains of closely related *Bacteroides* species

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<td>Conditions for growth</td>
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<td>5.5–10</td>
<td>5.5–10</td>
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<td>–</td>
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<tr>
<td>Glutamic acid decarboxylase</td>
<td>–</td>
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<td>+</td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>44.8</td>
<td>42.4</td>
<td>42.6</td>
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**Bacteroides koreensis** sp. nov. YS-aM39^T (=KCTC 15520^T =JCM 31393^T) and **Bacteroides kribbi** sp. nov. R2F3-3-3^T (=KCTC 15460^T =JCM 31391^T).

**DESCRIPTION OF BACTEROIDES KOREENSIS SP. NOV.**

**Bacteroides koreensis** (ko.re.en’sis, N. L. masc. adj. koreensis, pertaining to Korea).

Cells are strictly anaerobic, Gram-negative, non-spore-forming, non-motile and rod-shaped (0.3–0.4×0.6–0.8 µm). Colonies on RCM agar plates are 1.0 mm in diameter, circular, smooth and creamy. The growth temperature ranges from 15 to 40 °C, with optimum growth at 37 °C; the growth pH ranges from 5.5 to 10, with optimum growth at pH 6.5; and the NaCl concentration range for growth is 1–2 % (w/v). The isolates grow in the presence of bile. No catalase or oxidase activity, indole production, nitrate reduction, or urease reaction is detected. Gelatin and aesculin are hydrolysed. Using API 20A, positive reactions are observed for glucose, mannitol, lactose, sucrose, maltose, xylose, arabinose, cellobiose, mannose, melezitose, raffinose, sorbitol, rhamnose and trehalose. Reactions for salicin and glycerol are negative. Using API 32A, positive reactions are observed for α-galactosidase, β-galactosidase, α-glucosidase and N-acetyl-β-glucosaminidase. Negative reactions are observed for arginine dihydrolase, β-galactosidase-6-phosphate, α-arabinosidase, β-glucuronidase, α-fucosidase, alkaline phosphatase, glutamic acid decarboxylase, arginine arylamidase, proline arylamidase, leucyl-glycine arylamidase, phenylalanine arylamidase, leucine arylamidase, pyroglutamic acid arylamidase, tyrosine arylamidase, alanine arylamidase, glycine...
Table 2. Cellular fatty acid contents (%) of isolates and related Bacteroides species

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<td>1.3</td>
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<td>C₁₄ : 0</td>
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<td>4.3</td>
<td>5.5</td>
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<td>iso-C₁₅ : 0</td>
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<td>10.3</td>
<td>11.9</td>
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<td>41.7</td>
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<td>-</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>C₁₅ : 0 3-OH</td>
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<td>1.1</td>
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<tr>
<td>C₁₆ : 0</td>
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<td>C₁₆ : 0 3-OH</td>
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<td>11.8</td>
<td>12.6</td>
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<td>-</td>
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<td>Summed features*</td>
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<td>10.3</td>
<td>10.7</td>
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</table>

*Summed features represent groups of two fatty acids that could not be separated by gas chromatography with the MIDI system. Summed feature 1 comprises C₁₅ : 0 cis 12 and/or C₁₆ : 0. Summed feature 3 comprises iso-C₁₅ : 0 and/or unknown 13.570. Summed feature 9 comprises iso-C₁₆ : 0 3-OH and/or unknown 17.157 DMA. Summed feature 11 comprises iso-C₁₇ : 0 3-OH and/or C₁₈ : 2 DMA.

arylamidase, histidine arylamidase, glutamate-glutaminic acid arylamidase and serine arylamidase. The DNA G+C content is 44.8 mol%. The sole quinone is MK-10. The major polar lipid is PE, and other minor lipids are PN, six PLs (PL1–PL6) and one unidentified AL (AL2). The predominant fatty acids are anteiso-C₁₅ : 0, iso-C₁₅ : 0, C₁₆ : 0 3-OH and iso-C₁₇ : 0 3-OH. The major end products of glucose fermentation are acetic acid, lactic acid and formic acid.

The type strain YS-aM39¹⁰ (KCTC 15520¹⁰ = JCM 31393¹⁰) was isolated from the faeces of a healthy adult.

DESCRIPTION OF BACTEROIDES KRBIBI SP. NOV.

Bacteroides kribbi (kribbi. N. L. neut. gen. n. kribbi, arbitrary name formed from KRIBB, Korea Research Institute of Bioscience and Biotechnology).

Cells are strictly anaerobic, Gram-negative, non-spore-forming, non-motile and rod-shaped (0.2–0.3×0.4–0.7 μm). Colonies on RCM agar plates are 1.0 mm in diameter, circular, smooth and creamy. The growth temperature ranges from 15 to 40 °C, with optimum growth at 37 °C; the growth pH ranges from 5.5 to 10, with optimum growth at pH 6.5; and the NaCl concentration range for growth is 1–2% (w/v). The isolates grow in the bile of no catalase or oxidase reaction, indole production, nitrate reduction or urease reaction is detected. Gelatin and aesculin are hydrolysed. Using API 20A, positive reactions are observed for glucose, mannitol, lactose, sucrose, maltose, xylose, arabinose, cellobiase, mannose, melezitose, raffinose, sorbitol, rhamnose and trehalose. Reactions for salicin and glycerol are negative. Using API 32A, positive reactions are observed for α-galactosidase, β-galactosidase, α-glucosidase, N-acetyl-β-glucosaminidase and glutamic acid decarboxylase.

Negative reactions are observed for arginine dihydrolase, β-galactosidase-6-phosphate, α-arabinosidase, β-glucuronidase, α-fucosidase, alkaline phosphatase, arginine arylamidase, proline arylamidase, leucyl-glycine arylamidase, phenylalanine arylamidase, leucine arylamidase, pyrogallate acid arylamidase, tyrosine arylamidase, alanine arylamidase, glycine arylamidase, histidine arylamidase, glutamate-glutaminic acid arylamidase and serine arylamidase.

The DNA G+C content is 42.4–42.6 mol%. The sole quinone is MK-10. The major polar lipid is PE, and other minor lipids are PN, five PLs (PL1–PL5) and two unidentified ALs (AL1–AL2). The predominant fatty acids are anteiso-C₁₅ : 0, iso-C₁₅ : 0, C₁₆ : 0 3-OH and iso-C₁₇ : 0 3-OH. The major end products of glucose fermentation are acetic acid, lactic acid and formic acid.

The type strain R2F3-3-3T (KCTC 15460T = JCM 31391T) was isolated from the faeces of a healthy adult.

Funding information

This work was supported by grants NRF-2013M3A9A5076603 and the KRIBB Research Initiative Program funded by the Ministry of Science, ICT, and Future Planning.

Acknowledgements

We thank Dr Stefano Ventura, National Research Council of Italy, for his help with nomenclature and Latin etymology.

Conflicts of interest

The authors declare that there are no conflicts of interest.

References

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