Bacteroides caecigallinarum sp. nov., isolated from caecum of an Indonesian chicken

Sugiyono Saputra,† Tomohiro Irisawa,‡ Mitsuo Sakamoto,‡ Maki Kitahara, Sulistiani, Titin Yulinery, Moriya Ohkuma and Achmad Dinoto

†Microbiology Division, Research Center for Biology, Indonesian Institute of Sciences (LIPI), Jl. Raya Bogor Km. 46, Cibinong, 16911, Indonesia
‡Microbe Division/Japan Collection of Microorganisms, RIKEN BioResource Center, Tsukuba, Ibaraki 305-0074, Japan

Three strains of anaerobic Gram-stain-negative, short to longer rod-shaped bacteria isolated from the caecum of chicken in Indonesia were studied using a polyphasic taxonomic approach. These strains belonged to the genus Bacteroides, based on sequence analysis of 16S rRNA and hsp60 (groEL) genes, with similarities of 93.2–94.1 and 89.8–90.8 %, respectively, to the closest recognized species, Bacteroides coprocola JCM 17929T. Sugar fermentation and enzyme characteristics, cellular fatty acid profiles, menaquinone profiles and metabolic end products were also investigated. Furthermore, DNA–DNA hybridization studies confirmed that the three novel strains are different from the closest related species. The strains were also found to be distinct from each other on the basis of ribotype profiles. The DNA G+C contents of the three strains were 41.1–41.8 mol%. Based on phenotypic and phylogenetic characteristics, a novel species, Bacteroides caecigallinarum sp. nov., is proposed (type strain C13EG111T=LIP12-4-Ck773T=JCM 17929T).

The microbiota of the broiler chicken’s gastrointestinal tract plays an important role in health and protection against intestinal colonization by pathogenic bacteria. Generally, the intestine of chickens can be divided into three sections: the duodenum and ileum, the caecum, and the large intestine. Each part has its own role in microbial interactions and dominant bacterial colonization (Stanley et al., 2014). Based on culture-dependent methods, the caecum has received most attention because of its microbial diversity. Bacterial amounts in 1 g (wet wt) of caecal content are approximately 10¹¹ c.f.u., depending on the age and diet of the animal. Bacterial density in the caecum, where considerable microbial fermentation occurs, is markedly higher (up to 1000-fold), compared with the duodenum and ileum (Barnes, 1972; Salanitro et al., 1978). The distribution of the culturable bacterial groups is varied, but the majority of caecal bacteria are obligate anaerobes (Salanitro et al., 1974).

Culture-independent approaches based on 16S rRNA gene sequencing have made it possible to clarify the microbiota of the chicken caecum (Lan et al., 2002; Zhu et al., 2002; Lu et al., 2003). These studies demonstrated that one of the major groups in the caecum comprises members of the genus Bacteroides. However, only approximately 28 % of the sequences obtained in these studies showed >96 % similarity to their closest relatives included in public databases. This indicates that our knowledge of culturable Bacteroides is very limited.

During our studies of the bacterial composition in caecum of chickens sampled in Bogor, Indonesia, three strains were isolated. Partial 16S rRNA gene sequences of these strains showed >94 % similarity to Bacteroides coprocola JCM 17929T. Here we describe the taxonomic characteristics of the isolates belonging to the genus Bacteroides and propose a novel species.

Three strains (C13EG111T, C13EG153 and C13EG172) were isolated from the caecum of a healthy chicken (90 days old). The isolation of strictly anaerobic bacteria was performed in an anaerobic jar filled with Anaerocult (Merck) using Eggerth Gagnon (EG) agar (Merck) supplemented with 5 % (v/v) horse blood. The isolates were also maintained on the same medium for 2 days at 37 °C in an anaerobic jar (Hirayama Manufacturing) filled with 100 % CO₂.

Physiological and biochemical characteristics were determined by using the API 20A anaerobic test kit and API...
Table 1. Differential characteristics between strains C13EG111<sup>T</sup>, C13EG153 and C13EG172 and the type strains of related *Bacteroides* species

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2&lt;sup&gt;a&lt;/sup&gt;</th>
<th>3&lt;sup&gt;b&lt;/sup&gt;</th>
<th>4&lt;sup&gt;c&lt;/sup&gt;</th>
<th>5&lt;sup&gt;b&lt;/sup&gt;</th>
<th>6&lt;sup&gt;a&lt;/sup&gt;</th>
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<td>Fermentation of:</td>
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<tr>
<td>D-Mannitol</td>
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<td>–</td>
<td>+</td>
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</tr>
<tr>
<td>Salicin</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<td>–</td>
<td>w</td>
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<td>Glycerol</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Cellobiose</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Melizitose</td>
<td>–</td>
<td>–</td>
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<tr>
<td>D-Sorbitol</td>
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<tr>
<td>l-Rhamnose</td>
<td>–</td>
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<td>+</td>
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<td>Trehalose</td>
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<tr>
<td>Arginine dihydrolase</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<tr>
<td>β-Glucosidase</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<td>+</td>
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<tr>
<td>α-Arabinosidase</td>
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<td>α-Fucosidase</td>
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<td>–</td>
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<tr>
<td>Arginine arylamidase</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<td>–</td>
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<tr>
<td>Phenylalanine arylamidase</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Leucine arylamidase</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<td>–</td>
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<tr>
<td>Tyrosine arylamidase</td>
<td>+</td>
<td>–</td>
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<td>w</td>
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<td>Glycine arylamidase</td>
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<tr>
<td>Histidine arylamidase</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<td>–</td>
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<tr>
<td>Serine arylamidase</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>w</td>
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<td>–</td>
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<tr>
<td>Distinctive major cellular fatty acid(s)</td>
<td>anteiso-C&lt;sub&gt;15&lt;/sub&gt;:0</td>
<td>anteiso-C&lt;sub&gt;15&lt;/sub&gt;:0</td>
<td>anteiso-C&lt;sub&gt;15&lt;/sub&gt;:0</td>
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<td></td>
<td>iso-C&lt;sub&gt;15&lt;/sub&gt;:0</td>
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<td>C&lt;sub&gt;16&lt;/sub&gt;:0</td>
<td>C&lt;sub&gt;16&lt;/sub&gt;:0</td>
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<td>C&lt;sub&gt;16&lt;/sub&gt;:0 3-OH</td>
<td>C&lt;sub&gt;16&lt;/sub&gt;:0 3-OH</td>
<td>C&lt;sub&gt;16&lt;/sub&gt;:0 3-OH</td>
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<td></td>
<td>iso-C&lt;sub&gt;17&lt;/sub&gt;:0 3-OH</td>
<td>C&lt;sub&gt;18&lt;/sub&gt;:1&lt;sup&gt;ω9c&lt;/sup&gt;</td>
<td>iso-C&lt;sub&gt;17&lt;/sub&gt;:0 3-OH</td>
<td>iso-C&lt;sub&gt;17&lt;/sub&gt;:0 3-OH</td>
<td>iso-C&lt;sub&gt;17&lt;/sub&gt;:0 3-OH</td>
<td>iso-C&lt;sub&gt;17&lt;/sub&gt;:0 3-OH</td>
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*Data for the reference type strains were taken from the original description: a, Lan et al. (2006); b, Kitahara et al. (2005); c, Hayashi et al. (2007).*

Rapid ID 32A enzyme tests as recommended by the manufacturer (bioMérieux). A 48 h anaerobic culture on EG agar supplemented with 5 % (v/v) horse blood was suspended in 3 ml of suspension medium (bioMérieux). Panels were incubated at 37 °C for 48 h for the API 20A and 4 h for the Rapid ID 32A. After incubation, the strip was read with reference to the reading table provided with the kit. Bile resistance was tested by growing the bacteria on Bacteroides bile aesculin agar plates (Shah, 1992). Aesculin reaction was detected based on a dark brown to black area surrounding colonies. Metabolic end products were analysed as described previously (Sakamoto et al., 1982) and these were reviewed for the classification of the genus *Bacteroides* given by Shah & Collins (1983). Isoprenoid quinones were extracted and analysed using HPLC as described by Sakamoto et al. (2004).

DNA was extracted using a Qiagen Genomic tip 100/G following the manufacturer’s instructions. For phylogenetic analysis of the isolates, the 16S rRNA gene was amplified by using two universal primers: 27F and 1492R. The partial *groEL* gene was amplified by PCR using primers H729 (5′-CGCCAGGGTTTTTCCAGTCAGCAAIIIGC IGGGAYGGIACIACIAC-3′) and H730 (5′-AGCGGAT AACATTTTCACACAGGAYKIYKITCICCRAAAICIGGIGGC YTT-3′). Amplification conditions and sequencing reactions were as previously described (Sakamoto et al., 2010). Phylogenetic analysis was performed with the CLUSTAL X (version 2.0) program. A phylogenetic tree was reconstructed by the neighbour-joining method and evaluated by bootstrap analysis of 1000 replicates as described by Kitahara et al. (2005). Maximum-likelihood analysis was also performed using version 3.0 of the PhyML program (Guindon & Gascuel, 2003) and optimal models of nucleotide substitution were
estimated, within jModelTest version 2.1.4 (Darriba et al., 2012), using the Akaike information criterion; the models selected were TrN+I+G for the 16S rRNA gene and TIM3+I+G for the hsp60 (groEL) gene. Levels of DNA–DNA relatedness between the isolates and phylogenetic relatives were determined by the method of Ezaki et al. (1989) using photobiotin and microplates. The automated ribotyping device RiboPrinter microbial characterization system (Qualicon) was used with EcoRI as a restriction enzyme. Ribopatterns were analysed as described by Kitahara et al. (2010), using BioNumerics version 2.5 software (Applied Maths). The G+C content of the DNA was determined by using the HPLC method (Tamaoka & Komagata 1984; Kitahara et al., 2001).

The three isolates investigated were obligately anaerobic, non-spore-forming, non-motile, Gram-stain-negative short to longer rods (0.8–24 μm). Colonies were circular, white to greyish, had a butyrous texture and were 0.5–1.0 mm in diameter. All three strains were able to hydrolyse aesculin on Bacteroides bile aesculin agar plates. The major end products of metabolism were succinate and acetate. The results of phenotypic analysis based on API 20A and API rapid ID 32A systems are listed in the species description below. Strain C13EG153 differed from the two others (C13EG111T and C13EG172) in acid production from cellobiose and production of β-glucosidase. All three strains showed the same results for the other characteristics. In addition, there were several distinct phenotypic differences between the novel Parabacteroides distasonis JCM 5825T (AB238922) Bacteroides caecigallinarum C13EG111T (AB861981) Bacteroides caecigallinarum C13EG172 (AB861983) Bacteroides caecigallinarum C13EG153 (AB861982)

Fig. 1. Phylogenetic relationship of the caecum strains and closely related species based on 16S rRNA gene sequences. The tree was reconstructed by the neighbour-joining method. Parabacteroides distasonis JCM 5825T was used as an outgroup. Bootstrap percentages above 70 % are given at branch points. Bar, 1 % sequence divergence.
C13EG111T, C13EG153 and C13EG172 was slightly lower
ings that the major menaquinones of members of the
MK-11 (15.9–18.8 %). This result supports previous find-
in the online Supplementary Material). The major menaqui-

strains and the type strains of related Bacteroides species
(Table 1).

The major cellular fatty acids of the three strains investigated
here included anteiso-C\textsubscript{15}:0 (20.8–26.7 %), iso-C\textsubscript{15}:0
(14.1–18.6 %), C\textsubscript{16}:0 3-OH (9.6–12.6 %) and iso-C\textsubscript{17}:0
3-OH (8.7–11.6 %). Different proportions were found for
(14.1–18.6 %), C\textsubscript{16}:0 3-OH (9.6–12.6 %) and iso-C\textsubscript{17}:0
3-OH (8.7–11.6 %). Different proportions were found for

Fig. 2. Phylogenetic relationship of the caecum strains and closely related species based on hsp60 (groEL) gene sequences.
The tree was reconstructed by the neighbour-joining method. Parabacteroides distasonis JCM 5826\textsuperscript{T} was used as an
outgroup. Bootstrap percentages above 70 % are given at branch points. Bar, 2 % sequence divergence.

compared with those in the nucleotide sequence databases
DDBJ, EMBL and GenBank. Based on 16S rRNA gene
sequences, the three novel strains showed 93.2–94.1 %
similarity with B. coprocola JCM 12979\textsuperscript{T}. Lower similarity
was found for the hsp60 (groEL) gene, with the three strains
sharing 89.8–90.8 % with B. coprocola JCM 12979\textsuperscript{T}.
As reported by Sakamoto et al. (2010), the hsp60 (groEL)
A phylogenetic
tree was evaluated by using bootstrap analysis based on
1000 replicates. In both the neighbour-joining tree and
the maximum-likelihood tree, the three strains formed a
cluster separate from closely related species (Figs. 1
and 2; see also Figs S1 and S2). The novel strains shared
>98.7 % with >97.8 % similarity in 16S rRNA and hsp60
(groEL) gene sequences, respectively. DNA–DNA relatedness
between the three strains and B. coprocola JCM 12979\textsuperscript{T}
was low (8–11 %), which clearly indicated that they belonged
to a different species. The three chicken caecum strains
showed high levels of DNA–DNA relatedness (77.4–100 %)
with one another, indicating that they represented a single
species (Wayne et al., 1987). The intraspecies relationship
of the strains was investigated using RiboPrinter. The dendrogram created from the ribotype patterns of the strains demonstrated that the strains were different from each other (Fig. 3). Strains C13EG111T and C13EG172 were most similar to each other while strain C13EG153 formed a different cluster. The DNA G+C contents of strains C13EG111T, C13EG172 and C13EG153 were 41.7, 41.8 and 41.1 mol%, respectively.

Based on these results, strains C13EG111T, C13EG153 and C13EG172 are considered to represent a novel species of the genus Bacteroides, for which the name Bacteroides caecigallinarum sp. nov. is proposed.

**Description of Bacteroides caecigallinarum sp. nov.**

*Bacteroides caecigallinarum* {caei.gal.li.na’rum. N.L. n. *caecum* [from Latin *caecum* (or *caecum*) intestinum, *caecum*] caecum; L. gen. pl. n. *gallinarum* of hens; N.L. gen. pl. n. *caecigallinarum* of/from caecum (caecum) of hens.

Cells cultivated on EG blood agar plates are strictly anaerobic, Gram-stain-negative, non-spore-forming and non-motile. Cells are short to longer rod-shaped, 0.8 μm in diameter and variable in length, generally in the range 0.8–24 μm. Colonies on EG blood agar plates are 0.5–1.0 mm in diameter, disc-shaped and greyish white. No growth in 24 h. Produces acid from D-glucose, lactose, sucrose, maltose, D-xyllose, L-arabinose, aesculin, D-mannose and raffinose. Aesculin is hydrolysed. Gelatin is not hydrolysed. Indole and urease are not produced. Catalase is negative. Using the Rapid ID 32A kit, positive reactions are observed for arginine dihydrolase, α-galactosidase, β-galactosidase, α-glucosidase, N-acetyl-β-glucosaminidase, alkaline phosphatase, arginine arylamidase, leucyl glycine arylamidase, phenylalanine arylamidase, leucine arylamidase, tyrosine arylamidase, alanine arylamidase, glycine arylamidase, histidine arylamidase, glutaminyl glutamic acid arylamidase and serine arylamidase. Negative reactions for urease, β-galactosidase phosphate, β-glucosidase, β-glucuronidase, glutamic acid decarboxylase, α-fucosidase and pyroglycemic acid arylamidase. Succinate and acetate are the metabolic end products. The major menaquinones are MK-10 and MK-11. The major fatty acids are anteiso-C<sub>15</sub>:0, iso-C<sub>15</sub>:0, C<sub>16</sub>:0 3-OH, C<sub>16</sub>:0 and iso-C<sub>17</sub>:0 3-OH.

The type strain, C13EG111T (=LIP112-4-Ck773<sup>T</sup> = JSAT12-4-Ck773<sup>T</sup> = InaCC B455<sup>T</sup> = NBRC 110959<sup>T</sup>), was isolated from the caecum of an Indonesian local chicken. Two additional strains, C13EG153 (=LIP112-4-Ck814<sup>T</sup> = JSAT12-4-Ck814 = InaCC B456<sup>T</sup> = NBRC 110961) and C13EG172 (=LIP112-4-Ck832<sup>T</sup> = JSAT12-4-Ck832 = InaCC B457<sup>T</sup> = NBRC 110962), are included in the description.

**Acknowledgements**

We thank Ms Sayuri Nakamura, Japan Collection of Microorganisms, RIKEN BioResource Center, for her technical assistance. This work was supported by Science and Technology Research Partnership for Sustainable Development (SATREPS) which is a research programme in collaboration with the Japan Science and Technology Agency (JST) and the Japan International Cooperation Agency (JICA).

**References**


