Anaerostipes butyricus sp. nov., an anaerobic, butyrate-producing bacterium from Clostridium cluster XIVa isolated from broiler chicken caecal content, and emended description of the genus Anaerostipes

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Four butyrate-producing isolates were obtained from the caecal content of a 4-week-old broiler chicken. The 16S rRNA gene sequences were determined and confirmed the close relatedness of the four isolates, which suggested that they were derived from a single bacterial clone. Phylogenetic analysis based on 16S rRNA gene sequences showed that its closest relatives were members of cluster XIVa of the Clostridium subphylum of Gram-positive bacteria and that the closest related type strain was Anaerostipes caccae L1-92T (94.5 % similarity). Similarity levels of 96–98 % with sequences from uncultured bacteria from human stool samples were observed. On the basis of morphological, biochemical and phylogenetic characteristics, this strain is assigned to a novel species in the genus Anaerostipes, for which the name Anaerostipes butyricus sp. nov. is proposed. The type strain is 35-7T (\(5\)LMG 24724\(^T\) = DSM 22094\(^T\)). An emended description of the genus Anaerostipes is also provided.

It has been well documented that butyric acid, which is produced in the intestinal lumen by microbial fermentation of dietary carbohydrates (Topping & Clifton, 2001), exerts a wide variety of effects on intestinal function. Indeed, butyrate enhances proliferation of normal intestinal epithelial cells, has anti-inflammatory effects and reinforces the colonic defence barrier by increasing the production of mucus, antimicrobial peptides and tight junction proteins (Mariadason et al., 1997; Barcelo et al., 2000; Schauber et al., 2003; Bordin et al., 2004; Peng et al., 2007). Due to these characteristics, butyrate, and thus butyrate-producing bacteria, is considered to have a protective role against several colonic diseases (Raz et al., 2007; Sokol et al., 2008). Many studies on the caecal bacterial community of chickens have shown the predominance of sequences representing bacteria of the phylum Firmicutes (Selim, 2006). Although butyrate-producing bacteria are known to belong to several genera within this phylum, there is limited information about butyrate-producing strains in the chicken gut. During a study on the phylogenetic relationship of butyrate-producing bacteria from the chicken caecum (data not shown), we observed that all isolates belonged to the low-G+C-content Gram-positive bacteria of clostridial cluster XVI, cluster XIV subclusters a and b and cluster IV (Collins et al., 1994). We previously characterized an organism belonging to cluster IV, Butyricicoccus pullicaecorum (Eeckhaut et al., 2008), and showed that it could synthesize considerable amounts of butyrate under anaerobic conditions at pH 6, which is the ambient pH in the chicken caecum (van der Wielen et al., 2000). In the present study, a novel butyrate-producing organism belonging to cluster XIVa is described.

Isolates 4-7, 16-7, 35-7\(^T\) and 50-7 were obtained from the caecum of a 4-week-old healthy chicken as follows. Caecal content (1 g) was diluted in 9 ml M2GSC medium (Miyazaki et al., 1997) at pH 6 under anaerobic conditions (Barcenilla et al., 2000). Tenfold dilutions were spread onto agar plates containing M2GSC medium with 1.5 % agar. Plates were incubated for 48 h in an anaerobic chamber.
(Ruskinn Technology) with 84 % N₂, 8 % CO₂ and 8 % H₂ at 41 °C, which is the temperature of the chicken caecum (van der Wielen et al., 2000). Sixty-one pure colonies were grown overnight in M2GSC broth culture and tested for the production of short-chain fatty acids using GLC (GC14; Shimadzu) as described by Van Nevel & Demeyer (1977). Isolates 4-7, 16-7, 35-7T and 50-7 were studied in the production of short-chain fatty acids using GLC grown overnight in M2GSC broth culture and tested for with reference sequences using the CLUSTAL W program et al. Gamma*, 3 and O* on an ABI PRISM 310 Genetic the BigDye Terminator sequencing kit with primers pD, universal eubacterial primers fD1 and rD1 (Weisburg isolates were sequenced after amplification using the Approximately 1400 bases of the 16S rRNA gene of all four tract of the sampled chicken. 

**Table 1.** Fermentation products of the four caecal isolates

Data were taken from this study after overnight growth in M2GSC broth at 41 °C. Values are mean ± SD concentrations (mM) from three replications.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Acetate</th>
<th>Propionate</th>
<th>Butyrate</th>
<th>H₂</th>
<th>CO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-7</td>
<td>−4.0±2.2</td>
<td>−0.7±0.2</td>
<td>14.9±0.7</td>
<td>11.5±0.1</td>
<td>14.0±0.2</td>
</tr>
<tr>
<td>16-7</td>
<td>−3.9±1.3</td>
<td>−0.8±0.1</td>
<td>15.6±0.5</td>
<td>12.1±0.3</td>
<td>15.0±0.9</td>
</tr>
<tr>
<td>35-7T</td>
<td>−3.9±0.6</td>
<td>−0.8±0.2</td>
<td>15.1±1.7</td>
<td>11.3±0.5</td>
<td>14.1±0.6</td>
</tr>
<tr>
<td>50-7</td>
<td>−2.8±0.4</td>
<td>−0.8±0.01</td>
<td>14.7±0.9</td>
<td>12.7±0.1</td>
<td>15.1±1.2</td>
</tr>
</tbody>
</table>

sequence similarity with each other. Comparison of the 16S rRNA gene sequences with entries in public databases revealed the highest similarity with sequences of uncultured bacteria from human faeces (GenBank accession numbers DQ794058 and DQ793763; Ley et al., 2006), with sequence similarity values of 97.9 and 96.3 %, respectively. The cultured bacteria most closely related to strain 35-7T were two butyrate-producing bacteria from the human gut (strains SS2/1 and SSC/2; Louis et al., 2004), 94.3 % sequence similarity for both, and an unclassified *Anaerostipes* strain isolated from the rat gut (IE4; Gourgue-Jeannot et al., 2006) with 94.3 % sequence similarity.

After growth on solid M2GSC medium at pH 6 and 41 °C, cells of strain 35-7T were Gram-positive-staining, non-motile, anaerobic, large rods, 5–15 μm long and approximately 0.7 μm in diameter. Cells occurred in filaments containing oval spores which were visualized by staining with malachite green and light microscopy (Supplementary Fig. S1, available in IJSEM Online). Colonies after overnight incubation were 1.5–2.5 mm in diameter, circular, smooth, convex, white and shiny.

Growth of strain 35-7T at ten different temperatures (from 29 to 49 °C) and at nine different pH values (ranging from pH 4.5 to 8.5) was determined in brain heart infusion (BHI) broth supplemented with 1 mg cysteine hydrochloride ml⁻¹. The broth was adjusted to the different pH values, or adjusted to pH 6 for determination of the optimal temperature, and inoculated with an overnight-grown culture (500-fold diluted) of strain 35-7T. Growth rates were determined in triplicate by measuring the increase in OD₆₀₀ over time with a spectrophotometer. Growth occurred at pH 5.5–8 (optimum pH 6) and 35–47 °C (optimum 37–41 °C).

The biochemical properties of the novel isolates were characterized using API 20A, API Rapid ID 32A and API ZYM systems (bioMérieux), according to the manufacturer’s instructions except that the galleries were incubated at 41 °C instead of 36 ± 2 °C. All four isolates produced identical profiles. Aesculin was hydrolysed and acid was produced from cellobiose, glucose, maltose, mannitol, mannose, raffinose, salicin, sorbitol, sucrose and trehalose. Urease activity was not detected and hydrolysis of gelatin and fermentation of arabinose, glycerol, lactose, melizitose, raffinose, rhamnose and xylose were absent. Using the API ZYM system, positive reactions were obtained only for esterase (C4), acid phosphatase and z-glucosidase, with all other tests being negative. With the Rapid ID32A system, activity was detected for phenylalanine arylamidase, leucine arylamidase, tyrosine arylamidase, glycine arylamidase and histidine arylamidase.

The DNA G+C content of strain 35-7T was determined by using a Waters Breeze HPLC system and an Xbridge Shield RP18 column maintained at 37 °C, yielding a value of 44 mol%.

In conclusion, in the course of a study on the butyrate-producing caecal microbiota of healthy broiler chickens, four bacterial isolates represented a distinct taxon, with
Anaerostipes caccae L1-92T as the nearest neighbour with a validly published name. Although there is about 6% 16S rRNA gene sequence divergence between strain 35-7T and A. caccae L1-92T, they have similar G+C contents (44 and 45.5–46.0 mol%, respectively; Schwiertz et al., 2002). Strain 35-7T is also phenotypically compatible with the genus Anaerostipes in terms of colony morphology and end products of glucose metabolism: production of butyrate and utilization of acetate and lactate are observed when A. caccae and strain 35-7T are grown in PYG medium supplemented with DL-lactate and acetate. Strain 35-7T can be distinguished from A. caccae by the production of spores and, although most biochemical characteristics of strain 35-7T resemble those of A. caccae, the two can also be differentiated by acidification of cellobiose, raffinose and trehalose, activity of esterase (C4), production of α-glucosidase, arginine dihydrolase, α-galactosidase, phenylalanine arylamidase, leucine arylamidase, tyrosine ary lamidase, glycine arylamidase and histidine arylamidase, the reduction of nitrate and the production of indole. Therefore, we conclude that it is most appropriate to assign strain 35-7T to a novel species in the genus Anaerostipes, for which the name Anaerostipes butyraticus sp. nov. is proposed.

**Emended description of the genus Anaerostipes Schwiertz et al. 2002**

The description of the genus Anaerostipes is as given by Schwiertz et al. (2002) with the following amendments. Cells are spore-forming. Indole is produced. Arginine dihydrolase and α-galactosidase are not produced. Nitrate is not reduced. The DNA G+C content is 44–46 mol%.

**Description of Anaerostipes butyraticus sp. nov.**

Anaerostipes butyraticus (bu.ty.ra’ti.cus. N.L. n. butyras, -atis butyrate; L. masc. suff. -icus suffix used with the sense of pertaining to, related to; N.L. masc. adj. butyraticus related to butyrate, i.e. producing butyrate).

Gram-positive-staining, spore-forming, non-motile, anaerobic long rods (5–15 μm) that occur in filaments. On M2GSC agar, colonies are white, circular, smooth, convex, shiny and 1.5–2.5 mm in diameter after 24 h at 41 °C.
Colonies grown on M2GSC agar supplemented with 5% defibrinated sheep blood are non-haemolytic. The pH for growth is 5.5–8 (optimum pH 6). The temperature for growth is 35–47 °C (optimum 37–41 °C). Produces butyrate, H₂ and CO₂ and consumes small amounts of acetate and propionate in M2GSC broth. Does not produce urease or liquefy gelatin. Acid is produced from cellobiose, glucose, maltose, mannitol, mannose, raffinose, salicin, sorbitol, sucrose and trehalose. Activity for esterase (C4), acid phosphatase, z-glucosidase, phenylalanine arylamidase, leucine arylamidase, tyrosine arylamidase, glycine arylamidase and histidine arylamidase is observed. The G+C content of DNA of the type strain is 44 mol%.

The type strain is 35–7T (=LMG 24724T =DSM 22094T) and was isolated from the caecal content of a 4-week-old broiler chicken in Ghent, Belgium, in 2007.

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References


