INTERNATIONAL JOURNAL OF **SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY**

MICROBIOLOGY SOCIETY

# *Caecibacterium sporoformans* gen. nov., sp. nov., an anaerobic, butyrate-producing, spore-forming bacterium isolated from chicken caecum

Lonneke Onrust,[1] Diana Petzoldt,[2,3] Venessa Eeckhaut,[1] Celine De Maesschalck,[1] Freddy Haesebrouck,[1] Silke Rautenschlein,[3] Richard Ducatelle,[1] Filip Van Immerseel[1,*] and David Taras[2]

## Abstract

Strains of a Gram-stain-negative, rod-shaped and immotile bacterium were isolated from broiler chicken caecal content. The isolates required strict anaerobic conditions for growth, formed spores, were catalase-positive and oxidase-negative. They produced butyrate as the major metabolic end product in reinforced clostridial medium broth. The genomic DNA G+C content of the isolated strains was 32.5–34.6 mol%. The major cellular fatty acids were $C_{16:0\ \text{FAME}}$, $C_{14:0\ \text{FAME}}$, $C_{19:0\text{CYC}\ 9,10\text{DMA}}$ and $C_{16:0\text{DMA}}$. The fatty acid composition of the cell wall showed no similarity to any strain in the MIDI database. 16S rRNA gene sequence analysis showed that the nearest phylogenetic neighbours were *Anaerostipes hadrus* and *Clostridium populeti* (92 % sequence similarity) within *Clostridium* cluster XIVa of the phylum *Firmicutes*. Therefore, a novel genus is proposed, with the name *Caecibacterium sporoformans* gen. nov., sp. nov. The type strain of *Caecibacterium sporoformans* is LMG 27730[T]=DSM 26959[T].

A chicken's caecum harbours approximately $10^{10}$ to $10^{11}$ bacteria per gram content (wet weight) [1, 2], consisting of up to 640 species [3], of which up to 85 % are still unknown [4, 5]. The majority of the caecal microbiota are strict anaerobes [6, 7]. The abundance of spore-forming anaerobes in chicken faeces is estimated to be $10^4$ c.f.u. $g^{-1}$ [8]. In the past, the genus *Clostridium* was thought to consist of spore-forming, Gram-stain-positive, anaerobic rod-shaped bacteria within the class *Clostridia* in the phylum *Firmicutes*. Now the genus includes Gram-stain-negative bacteria, non-spore formers, cocci and non-anaerobes [9]. In 1994, Collins *et al.* demonstrated the marked phylogenetic incoherence of the genus *Clostridium* with distinct clusters[10]. Almost one-half of the clostridial species belonged to cluster I and the remaining clostridial species exhibited very considerable degrees of phylogenetic diversity, belonging to numerous clusters [10]. The need for extensive taxonomic revision has been recognized and only *Clostridia sensu stricto* has been retained in cluster I with *Clostridium butyricum* as type species. However, even after extensive reassignment in the past years, many organisms are still misplaced in the genus *Clostridium*, e.g. the Gram-stain-negative spore-formers.

Recently, the assignment of all cluster XIVa organisms that are still listed as *Clostridium* species to the new genus *Lachnoclostridium* was proposed [9]. However, this proposed genus would still consist of a very heterogeneous group of organisms.

The *Clostridium* cluster XVIa, or the family *Lachnospiraceae*, contains numerous butyrate-producing bacteria that colonize the distal intestinal tract of humans and animals including chickens [11]. Butyric acid plays an important role in the reduction of inflammation and pathogen colonization, and increased production of mucins and host antimicrobial peptides [12, 13]. Due to these properties, butyrate producers are interesting probiotic candidates. Several butyrate-producing strains have been isolated from chicken caecal content [14], however only a minority have been characterized and are spore-forming bacteria [15–17]. Utilization of spores as probiotics is advantageous because of their higher resistance to acidic conditions in the stomach, and the requirement of smaller numbers compared to probiotic vegetative cells [18]. In this study spore-forming butyrate-producing strains with 99 % 16S rRNA gene sequence similarity to each other, obtained from chicken

caecal content is characterized in more detail. Based on comprehensive phenotypic and phylogenetic analyses, a new genus is proposed to accommodate the novel isolates.

Two different approaches at two different locations were carried out to isolate bacteria from caecal content of broiler chickens. Eeckhaut et al. isolated butyrate-producing bacteria from the caeca of a 28-day-old healthy broiler chicken under anaerobic conditions (84 % $N_2$, 8 % $CO_2$ and 8 % $H_2$) at 38 $^\circ$C [14]. One of these isolates, strain LMG 27730[T], an anaerobic spore-forming butyric acid-producing bacteria was selected and characterized in more detail. Another isolation approach under anaerobic conditions (80 % $N_2$, 10 % $CO_2$ and 10 % $H_2$) at 37 $^\circ$C was carried out by Petzoldt et al. (unpublished) from pooled caecal content of three healthy broiler chickens of 35 days of age, targeting spore-forming bacteria by including a heating step of 65 $^\circ$C for 60 min. In this process, 17 hitherto-unknown isolates with >99 % rRNA gene sequence similarity to each other were recovered. One isolate, V19-240a1aT, an anaerobic spore-forming butyric acid-producing bacterium, was randomly selected for more detailed characterization together with strain LMG 27730[T]. Selectively, phenotypic tests were performed for strains V19-103b1T and V19-259a1T as well.

pH and temperature optima were determined in reinforced clostridial medium (RCM) broth (Sifin) in anaerobic Hungate tubes (Ochs Glasgerätebau; 80 % $N_2$, 10 % $CO_2$ and 10 % $H_2$). For determination of the optimum pH, Hungate tubes were incubated at 41 $^\circ$C on a shaking table (50 r.p.m.; Model 3005, GFL). For determination of optimum temperature, the Hungate tubes were incubated at a range of different temperatures on a shaking table. Bacterial growth was monitored spectrophotometrically at 600 nm (Tecan Sunrise). Potassium hydroxide, catalase and oxidase reactions were tested by standard methods [19] after anaerobic cultivation for 24 h at 37 $^\circ$C on RCM agar. Cell morphology was examined by light microscopy (Leica DMLB) and scanning electron microscopy (JEOL JSM 5600 LV, Jeol) after 1 day of anaerobic cultivation at 37 $^\circ$C in sporulation medium (Difco sporulation medium). The medium was centrifuged for 10 min at 5000 $g$, and the obtained pellet was fixated with 2.5 % glutaraldehyde in 0.1 M cacodylate buffer and dehydrated in a graded series of ethanol (10–95 % in water), followed by a graded series of acetone (10–100 % in ethanol). The sample was placed in 100 % acetone for incubation overnight, and afterwards dried with a Balzers CPD 030 critical-point dryer (Leica) and platinum-coated using a JEOL JFC-1300 Auto Fine Coater (Jeol).

Strains LMG 27730[T] and V19-240a1aT showed similar matrix-assisted laser desorption ionization–time-of-flight mass spectrometry spectra (data not shown). Both strains were obligate anaerobes, non-motile, produced endospores and were rod-shaped (Fig. 1). Cells stained Gram-negative and showed a positive potassium hydroxide reaction. Their size was 1.0–1.5×2.0–3.0 µm. The endospore position was variable. Growth occurred on gut microbiota medium (GMM) [20], RCM, Columbia agar with sheep blood
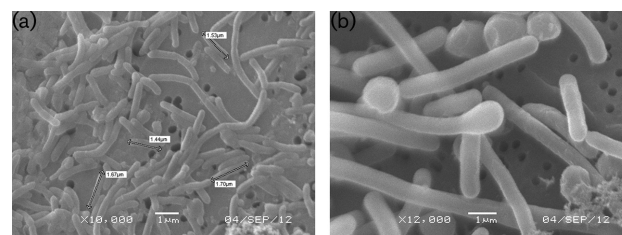


Fig. 1. Scanning electron micrograph of vegetative cells of strain LMG 27730[T] (a) 10000-fold magnification. (b) 12000-fold magnification.

(ColSB; Oxoid), standard 1 nutrient medium (Merck), CASO medium (Sifin), brain heart infusion medium (Oxoid) and Mueller–Hinton medium (Oxoid) at 25 to 48 $^\circ$C. The growth optima of both isolates was similar over a range from 37 to 45 $^\circ$C in two to five replications per individual temperature. Colonies were convex, rough and serrated in young colonies, and lobed in colonies older than 2 days. Colonies reached a diameter of 2–4 mm after 24 h incubation on GMM, RCM or ColSB. Colonies grown on colourless or yellowish media (RCM, standard 1 agar, GMM, CASO) appeared ivory, and light grey when grown on the Colsb. The isolates grew well anaerobically, but no growth occurred under aerobic or under micro-aerobic conditions generated with CampyGen (Oxoid) or in a candle jar. Growth of LMG 27730[T] and V19-240a1aT in RCM occurred at a pH range between 5 and 9. Fastest growth was observed at pH 6.0–8.0.

Fermentation products of LMG 27730[T] and V19-240a1aT were analysed using gas chromatography as described by De Weirdt et al. after growth in RCM for 24 h at 37 $^\circ$C under anaerobic conditions [21]. D-Lactate in RCM culture supernatants was determined enzymatically with a D-/L-Lactic Acid (Rapid) assay kit according to the manufacturer's instructions (Megazyme). Gas chromatographic analysis and UV-tests of RCM cultures of LMG 27730[T] and V19-240a1aT revealed butyrate as the major end product of fermentation (9.3–10.5 mM). Lactic acid and propionic acid were consumed (Table 1).

The metabolic characteristics of the novel isolate were determined using the API 20A, API ZYM and the Rapid ID 32A systems (bioMérieux), according to the manufacturer's instructions, except for the fact that incubation was performed anaerobically. Strains LMG 27730[T] and V19-

Table 1. Acid fermentation products in mM after 24 h incubation in RCM of strain LMG 27730[T] and V19-240a1aT

|  | LMG 22730[T] | V19-240a1aT |
| --- | --- | --- |
| Acetic acid | 3.60±1.07 | 2.71±0.94 |
| Propionic acid | −0.11±0.02 | −0.33±0.17 |
| Lactic acid | −1.94±0.56 | −2.35±0.016 |
| Butyric acid | 10.54±099 | 9.25±0.98 |

**Table 2.** Comparison of strain characteristics which allow differentiation of LMG 27730[T] and V19-240a1aT from related genera

Taxa: 1, LMG 27730[T]; 2, V19-240a1aT; 3, 3, *Anaerostipes hadrus* [33]; 4, *Clostridium populeti* [34]; 5, *Anaerocolumna aminovalericum* [35–37]; 6, *Clostridium phytofermentans* [38]. +, Positive; −, negative; w, weak; ND, no data available; A, acetate; B, butyrate; E, ethanol; F, formate; L, lactate; V, valerate.

| Test/Species | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Gram | − | − | + | − | + | − |
| Spores | + | + | − | + | + | + |
| G+C content | 32.5 | 36.4 | 37.0–42.0 | 28.0 | 33.0 | 36.0 |
| Acid end products | A, B | A, B | B, F, L* | A, B, L | A, P, V | A, E, F, L |
| Motility | − | − | − | + | + | + |
| Acid from | | | | | | |
| L-Arabinose | + | + | − | + | w | w |
| D-Fructose | + | + | + | + | + | w |
| D-Glucose | + | + | + | + | + | + |
| Lactose | + | + | ND | − | − | + |
| Maltose | + | + | + | + | + | + |
| D-Mannose | + | − | − | − | + | + |
| Raffinose | + | − | − | ND | − | ND |
| Sucrose | + | + | + | − | + | − |
| Trehalose | + | + | w* | − | + | − |
| D-Xylose | + | + | + | + | + | + |
| Nitrate reduction | − | − | − | ND | − | − |
| Production of indole | − | − | w | ND | − | ND |
| Urea hydrolysis | − | − | − | ND | − | − |
| Gelatin hydrolysis | − | − | −* | w | − | ND |
| Aesculin hydrolysis | + | + | ND | ND | + | − |
| pH range (optimum) | 5.0–9.0 (6.0–8.0) | 5.0–9.0 (6.0–8.0) | ND | 6.4–8.1 (7.0) | ND | 6.0–9.0 (8.0–8.5) |
| Temperature range (optimum) | 25–48 (37–45) | 25–48 (37–45) | ND | 20–40 (35) | ND (37) | 15–42 (35–37) |

*Data obtained from Bui *et al.* [39].

240a1aT showed hydrolysis of aesculin, but were oxidase- and indol-negative. Both isolates produced acid from D-glucose, lactose, D-sucrose, maltose, salicin, D-xylose and trehalose. LMG 27730[T] additionally fermented D-mannose (Table 2). L-Arabinose, cellobiose, D-mannitol, melezitose, raffinose, L-rhamnose and D-sorbitol were not fermented. No signs of urease activity or hydrolysis of gelatin were detected. In supplemented TYG$_S$ cultures, V19-103b1T, V19-240a1aT and V19-259a1T tested positive for fermentation of D(-)-fructose, galactose, inulin and D(-)-ribose (see Supplementary Material for method). Using the API ZYM and Rapid ID 32A system, strains LMG 27730[T] and V19-240a1aT were found to show activity for α- and β-galactosidase, β-glucosidase, α-arabinosidase, β-glucuronidase, α-glucosidase, alkaline phosphatase and leucine arylamidase. Only strain LMG 27730[T] fermented raffinose. No activity was detected in all tested isolates for urease, arginine dihydrolase, α-galactosidase, β-galactosidase-6-phosphate, β-N-acetyl-β-glucosamine, glutamine acid decarboxylase, esterase, esterase lipase, lipase, trypsin, α-chymotrypsin, acid phosphatase, α-fucosidase, α-mannosidase, glutamyl glutamin acid arylamidase, valine arylamidase, cysteine arylamidase and proline arylamidase.

For cellular fatty acid methyl ester (FAME) analysis, colonies were harvested from ColSB which had been incubated anaerobically for 24 h at 37 °C. Fatty acid preparation and gas chromatographic analysis were performed as described by Huang *et al.* [22]. The major components of the cellular fatty acid profiles of LMG 27730[T], V19-240a1aT, V19-103b1T and V19-259a1T were similar. The mean profiles (Table S1, available in the online Supplementary Material) are dominated by straight-chain fatty acids $C_{16:0}$ FAME (25.1–36.7 %) and $C_{14:0}$ FAME (7.4–17.5 %), followed by $C_{19:0}$ CYC 9, 10 DMA (6.9–8.1 %), $C_{18:1}$ CIS 9 DMA (3.4–9.9 %), $C_{18:1}$ FAME (4.6–6.9 %) and $C_{16:0}$ DMA (4.8–7.7 %). $C_{11:0}$ DMA, $C_{14:1}$ CIS 7 DMA, $C_{16:1}$ CIS 7 FAME, $C_{16:1}$ CIS 9 FAME, $C_{17:0}$ CYC FAME, $C_{18:2}$ CIS 9,12 FAME and $C_{19:0}$ CYC 9,10/:1 FAME were detected in less than 1.5 %, while $C_{12:0}$ FAME, $C_{16:0}$ ALDE, $C_{16:1}$ CIS 9 DMA, $C_{18:0}$ FAME, $C_{18:1}$ CIS 11 DMA and $C_{19:0}$ CYC 11, 12 DMA were present in portions of 0.5–3.8 %.

The G+C content (mol%) was determined by high-performance liquid chromatography (Shimadzu) using the method described in [23] at the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany). DNA purification was accomplished according to the procedure of [24]. The DNA base composition of

V19-240a1aT was 36.4 mol% G+C and the value of the G+C content for isolate LMG 27730[T] was 32.5 %.

DNA of strain LMG 27730[T] and V19-259a2aT was extracted via an alkaline lysis procedure [25]. Amplification of the DNA was done with the universal eubacterial primers fD1 and rD1 [26]. The purified amplicons were sequenced by GATC Biotech (European Genome and Diagnostics Centre, Konstanz, Germany) using the primers pD, Gamma*, 3 and O* on an ABI PRISM 310 Genetic Analyzer [27]. The sequences were compared to each other and compared with entries in the GenBank to search for the closest match using BLAST [28]. The 16S rRNA gene sequence of the novel caecal isolates were aligned with reference 16S rRNA gene sequences of their closest neighbours by using the MUSCLE program [29, 30]. The computer program MEGA 7 [31] was used to reconstruct a phylogenetic tree with maximum composite likelihood distances [32] (Fig. 2). Bootstrap support for internal branches was generated from 1000 replicates. It was demonstrated that the two isolates were highly related to each other (99–100 % gene sequence similarity).

Limiting the comparison to cultured type species, the closest relative with 92 % similarity were *Anaerostipes hadrus* DSM 3319 (NR_104799.1, NR 117138.1, 117139.1) [33] and *Clostridium populeti* 743A (NR_026103.1) [34].

Apart from the 16S rRNA gene sequence divergence, *A. hadrus* and *C. populeti* are well distinguished from the novel isolates by several features. Strain *A. hadrus* can be differentiated from the novel anaerobic strains by its higher G+C content, the lack of sporulation, Gram-positive staining, acetate production and a much higher abundance of $C_{12:0}$ fatty acids (>23 %). Differentiation of the new strain against *C. populeti* is possible by its lower G+C content and

its lack of utilization of lactose, sucrose and trehalose. An overview of the phenotypic properties that allow differentiation of the novel bacteria from the phylogenetic closest relatives are presented in Table 2.

In summary, sequence searches in GenBank and the phenotypic characterization revealed that strain LMG 27730[T], obtained from a study on butyrate-producing bacteria from the caecal content of a 4-week-old broiler chicken, and strain V19-240a1aT, obtained from a study on spore-forming bacteria from the caecal content of 5-week-old broiler chicken, belong to the same species. This species represents a previously unknown but solid and distinctly novel lineage within the *Clostridium* cluster XIVa group, but is not highly associated with any recognized species within this cluster (<93 % similarity in 16S rRNA gene sequence) [10]. Thus, based on the polyphasic evidence, a straightforward assignment to any existing genus is not reasonable. In general, the *Clostridium* cluster XIVa-group is a very heterogeneous group, which consists of approximately 20 genera of high heterogeneity, including a large number of probably misclassified species [10]. Historically, many anaerobic spore-forming bacteria would just have been (mis-)assigned to the genus *Clostridium*. However, placement of our novel species within the genus *Clostridium* is not advisable since it is widely agreed that the genus should be limited to its type strain, *Clostridium butyricum*, and a few additional very closely related *Clostridium* species of *Clostridium* cluster I according to Collins *et al.* [10]. Therefore, based on phenotypic and genotypic evidence, and to avoid additional misclassification, we propose placing our hitherto unknown strains as a novel species in a novel genus with strain LMG 27730[T] as its type species, *Caecibacterium sporoformans* gen. nov., sp. nov.
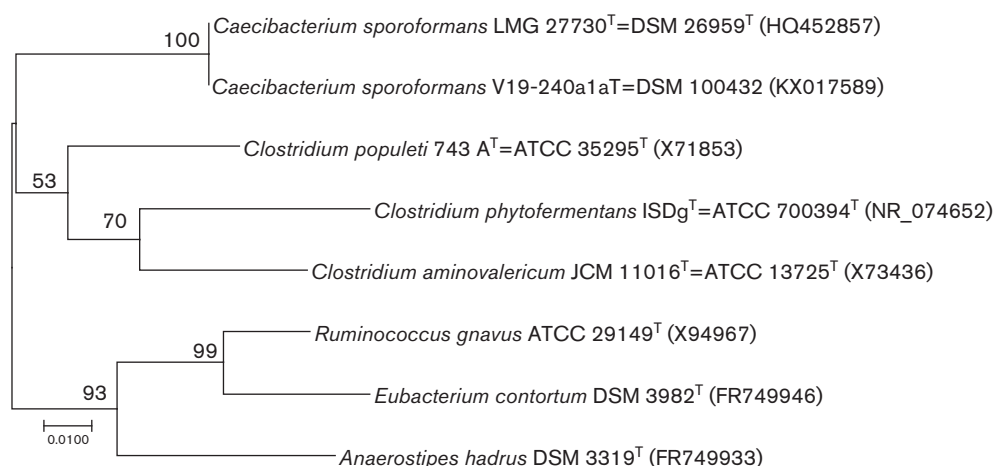


**Fig. 2.** Phylogenetic tree based on 16 s rRNA gene sequences showing the relationship of *Caecibacterium sporoformans* gen.nov., sp. nov. and its closest neighbours with a validly published name. Bootstrap support for internal branches was generated from 1000 replicates. The computer program MEGA 7 was used to reconstruct a phylogenetic tree with maximum composite likelihood distances and based on a comparison of 1356 nucleotides. Accession numbers of sequences are included in parentheses. Bar, 0.01 substitutions per nucleotide position.

## DESCRIPTION OF *CAECIBACTERIUM* GEN. NOV.

*Caecibacterium* (Cae.ci.bac.te′ri.um. N.L. neut. n. *caecum* (from L. adj. *caecus* blind) caecum; N.L. neut. n. *bacterium* a small rod; N.L. neut. n. *Caecibacterium* a rod from the caecum).

The members of this genus are Gram-negative staining rods, non-motile and spore-forming. Obligate anaerobic growth occurs at a mesophilic to thermophilic temperature range at a pH span from 6.0 to 9.0. Mono- and disaccharides are fermented. The strains produce butyrate and acetate, and consume propionate and lactate in RCM broth. The DNA G+C content is low at 32.5–36.4 %. This novel genus is classified in the phylum *Firmicutes*, class *Clostridia*. The type species is *Caecibacterium sporoformans.*

## DESCRIPTION OF *CAECIBACTERIUM SPOROFORMANS* SP. NOV.

*Caecibacterium sporoformans* (spo.ro.for′mans. Gr. n. *spora*, a seed; L. pres. part. *formans*, that gives shape, form; N.L. part. adj. *sporoformans*, sporeforming).

*Caecibacterium sporoformans* is a Gram-stain-negative, strict anaerobic, endospore-forming, rod-shaped bacterium. It metabolizes glucose, lactose, maltose, trehalose and xylose. It is negative for hydrolysis of urea and gelatin, negative for nitrate reduction and positive for aesculin hydrolysis. The strain produces butyrate and acetate and consumes propionate and lactate. The DNA G+C content is low at 32.5–36.4 %. The cell wall's fatty acid profile is dominated by $C_{16:0}$ FAME and $C_{14:0}$ FAME, followed by $C_{19:0\ \text{CYC}\ 9,\ 10}$ DMA, $C_{18:1\ \text{CIS}\ 9}$ DMA, $C_{18:1}$ FAME and $C_{16:0}$ DMA.

The type strain is LMG $27730^T$ (=DSM $26959^T$), isolated from the caecal content of a 4-week-old broiler chicken in Ghent (Belgium) in 2007.

**References**
1. **Salanitro JP, Fairchilds IG, Zgornicki YD.** Isolation, culture characteristics, and identification of anaerobic bacteria from the chicken cecum. *Appl Microbiol* 1974;27:678–687.
2. **Zhu XY, Zhong T, Pandya Y, Joerger RD.** 16S rRNA-based analysis of microbiota from the cecum of broiler chickens. *Appl Environ Microbiol* 2002;68:124–137.
3. **Apajalahti J, Kettunen A, Graham H.** Characteristics of the gastrointestinal microbial communities, with special reference to the chicken. *Worlds Poult Sci J* 2004;60:223–232.
4. **Bjerrum L, Pedersen AB, Engberg RM.** The influence of whole wheat feeding on *Salmonella* infection and gut flora composition in broilers. *Avian Dis* 2005;49:9–15.
5. **Lan PT, Hayashi H, Sakamoto M, Benno Y.** Phylogenetic analysis of cecal microbiota in chicken by the use of 16S rDNA clone libraries. *Microbiol Immunol* 2002;46:371–382.
6. **Barnes EM.** The avian intestinal flora with particular reference to the possible ecological significance of the cecal anaerobic bacteria. *Am J Clin Nutr Review* 1972;25:1475–1479.
7. **Fuller R.** Microbial activity in the alimentary tract of birds. *Proc Nutr Soc* 1984;43:55–61.
8. **Elam JF, Jacobs RL, Fowler J, Couch JR.** Effect of dietary *Clostridia* upon growth-promoting responses of penicillin. *Proc Soc Exp Biol Med* 1954;85:645–648.
9. **Yutin N, Galperin MY.** A genomic update on clostridial phylogeny: gram-negative spore formers and other misplaced clostridia. *Environ Microbiol* 2013;15:2631–2641.
10. **Collins MD, Lawson PA, Willems A, Cordoba JJ, Fernandez-Garayzabal J** *et al.* The phylogeny of the genus *Clostridium*: proposal of five new genera and eleven new species combinations. *Int J Syst Bacteriol* 1994;44:812–826.
11. **Meehan CJ, Beiko RG.** A phylogenomic view of ecological specialization in the *Lachnospiraceae*, a family of digestive tract-associated bacteria. *Genome Biol Evol* 2014;6:703–713.
12. **Barcelo A, Claustre J, Moro F, Chayvialle JA, Cuber JC** *et al.* Mucin secretion is modulated by luminal factors in the isolated vascularly perfused rat colon. *Gut* 2000;46:218–224.
13. **Van Immerseel F, Boyen F, Gantois I, Timbermont L, Bohez L** *et al.* Supplementation of coated butyric acid in the feed reduces colonization and shedding of *Salmonella* in poultry. *Poult Sci* 2005; 84:1851–1856.
14. **Eeckhaut V, Van Immerseel F, Croubels S, de Baere S, Haesebrouck F** *et al.* Butyrate production in phylogenetically diverse Firmicutes isolated from the chicken caecum. *Microb Biotechnol* 2011;4:503–512.
15. **De Maesschalck C, Van Immerseel F, Eeckhaut V, De Baere S, Cnockaert M** *et al.* *Faecalicoccus acidiformans* gen. nov., sp. nov., isolated from the chicken caecum, and reclassification of *Streptococcus pleomorphus* (Barnes *et al.* 1977), *Eubacterium biforme* (Eggerth 1935) and *Eubacterium cylindroides* (Cato *et al.* 1974) as *Faecalicoccus pleomorphus* comb. nov., *Holdemanella biformis* gen. nov., comb. nov. and *Faecalitalea cylindroides* gen. nov., comb. nov., respectively, within the family *Erysipelotrichaceae. Int J Syst Evol Microbiol* 2014;64:3877–3884.
16. **Eeckhaut V, Van Immerseel F, Pasmans F, De Brandt E, Haesebrouck F** *et al.* *Anaerostipes butyraticus* sp. nov., an anaerobic, butyrate-producing bacterium from Clostridium cluster XIVa isolated from broiler chicken caecal content, and emended description of the genus *Anaerostipes. Int J Syst Evol Microbiol* 2010;60:1108–1112.
17. **Eeckhaut V, Van Immerseel F, Teirlynck E, Pasmans F, Fievez V** *et al.* *Butyricicoccus pullicaecorum* gen. nov., sp. nov., an anaerobic, butyrate-producing bacterium isolated from the caecal content of a broiler chicken. *Int J Syst Evol Microbiol* 2008;58:2799–2802.
18. **Bader J, Albin A, Stahl U.** Spore-forming bacteria and their utilisation as probiotics. *Benef Microbes* 2012;3:67–75.
19. **Neumeister B, Geiss HK, Braun R, Kimmig P, Dahouk SA** *et al. Mikrobiologische Diagnostik: Bakteriologie - Mykologie - Virologie - Parasitologie*, 2nd ed. Stuttgart: Thieme; 2009.
20. **Goodman AL, Kallstrom G, Faith JJ, Reyes A, Moore A** *et al.* Extensive personal human gut microbiota culture collections characterized and manipulated in gnotobiotic mice. *Proc Natl Acad Sci USA* 2011;108:6252–6257.
21. **De Weirdt R, Possemiers S, Vermeulen G, Moerdijk-Poortvliet TC, Boschker HT** *et al.* Human faecal microbiota display variable

patterns of glycerol metabolism. *FEMS Microbiol Ecol* 2010;74:601–611.

22. **Huang Y, Ryll M, Walker C, Jung A, Runge M et al.** Fatty acid composition of *Yersinia ruckeri* isolates from aquaculture ponds in northwestern Germany. *Berl Munch Tierarztl Wochenschr* 2014;127:123–128.

23. **Mesbah M, Premachandran U, Whitman WB.** Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* 1989;39:159–167.

24. **Cashion P, Holder-Franklin MA, McCully J, Franklin M.** A rapid method for the base ratio determination of bacterial DNA. *Anal Biochem* 1977;81:461–466.

25. **Decat E, Cosyn J, De Bruyn H, Miremadi R, Saerens B et al.** Optimization of quantitative polymerase chain reactions for detection and quantification of eight periodontal bacterial pathogens. *BMC Res Notes* 2012;5:664.

26. **Weisburg WG, Barns SM, Pelletier DA, Lane DJ.** 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* 1991;173:697–703.

27. **Coenye T, Falsen E, Vancanneyt M, Hoste B, Govan JR et al.** Classification of *Alcaligenes faecalis*-like isolates from the environment and human clinical samples as *Ralstonia gilardii* sp. nov. *Int J Syst Bacteriol* 1999;49:405–413.

28. **Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z et al.** Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 1997;25:3389–3402.

29. **Edgar RC.** MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics* 2004;5:113.

30. **Edgar RC.** MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 2004;32:1792–1797.

31. **Kumar S, Stecher G, Tamura K.** MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* 2016;33:1870–1874.

32. **Kimura M.** A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 1980;16:111–120.

33. **Allen-Vercoe E, Daigneault M, White A, Panaccione R, Duncan SH et al.** *Anaerostipes hadrus* comb. nov., a dominant species within the human colonic microbiota; reclassification of *Eubacterium hadrum* Moore et al. 1976. *Anaerobe* 2012;18:523–529.

34. **Sleat R, Mah RA.** *Clostridium populeti* sp. nov., a cellulolytic species from a woody-biomass digestor. *Int J Syst Bacteriol* 1985;35:160–163.

35. **Hardman JK, Stadtman TC.** Metabolism of omega-acids. II. Fermentation of delta-aminovaleric acid by *Clostridium aminovalericum* n. sp. *J Bacteriol* 1960;79:549–552.

36. **Jeong H, Yi H, Sekiguchi Y, Muramatsu M, Kamagata Y et al.** *Clostridium jejuense* sp. nov., isolated from soil. *Int J Syst Evol Microbiol* 2004;54:1465–1468.

37. **Ueki A, Ohtaki Y, Kaku N, Ueki K.** Descriptions of *Anaerotaenia torta* gen. nov., sp. nov. and *Anaerocolumna cellulosilytica* gen. nov., sp. nov. isolated from a methanogenic reactor of cattle waste and reclassification of *Clostridium aminovalericum*, *Clostridium jejuense* and *Clostridium xylanovorans* as *Anaerocolumna* species. *Int J Syst Evol Microbiol* 2016;66:2936–2943.

38. **Warnick TA, Methé BA, Leschine SB.** *Clostridium phytofermentans* sp. nov., a cellulolytic mesophile from forest soil. *Int J Syst Evol Microbiol* 2002;52:1155–1160.

39. **Bui TP, de Vos WM, Plugge CM.** *Anaerostipes rhamnosivorans* sp. nov., a human intestinal, butyrate-forming bacterium. *Int J Syst Evol Microbiol* 2014;64:787–793.