Description of Anaerotignum aminivorans gen. nov., sp. nov., a strictly anaerobic, amino-acid-decomposing bacterium isolated from a methanogenic reactor, and reclassification of Clostridium propionicum, Clostridium neopropionicum and Clostridium lactatifermentans as species of the genus Anaerotignum

Atsuko Ueki,* Kazushi Goto, Yoshimi Ohtaki, Nobuo Kaku and Katsuji Ueki

Abstract
A strictly anaerobic bacterial strain (SH021T) was isolated from a methanogenic reactor. Cells were Gram-stain-positive, motile, straight or slightly curved rods. The optimum temperature for growth was 35°C, and the optimum pH was 6.1–7.7. The strain was asaccharolytic and utilized amino acids as growth substrates. The strain produced acetate and propionate from L-alanine and L-serine, and propionate and butyrate from L-threonine. Branched-chain amino acids (L-isoleucine, L-leucine and L-valine) were utilized weakly, and isovalerate or isobutyrate was produced. Strain SH021T utilized pyruvate and lactate, and converted them to acetate and propionate. The genomic DNA G+C content was 38.2 mol%. Compounds related to iso-C₁₅₀ were detected as major components in the cellular fatty acids analysis. The diagnostic diamino acid of the cell-wall peptidoglycan was meso-diaminopimelic acid. On the basis of 16S rRNA gene sequences, the most closely related known species were Clostridium propionicum, Clostridium neopropionicum and Clostridium lactatifermentans in cluster XIVb of the class Clostridia. Based on the phylogenetic and phenotypic data, Anaerotignum aminivorans gen. nov., sp. nov. is proposed to accommodate strain SH021T (=JCM 31556T=DSM 103575T). For the three related species of the genus Clostridium, Anaerotignum propionicum comb. nov. (type strain DSM 1682T=JCM 1430T=ATCC 25522T=CCUG 9280T=NCIMB 10656T=VPI 5303T), Anaerotignum neopropionicum comb. nov. (type strain X4T=DSM 3847T=KCTC 15564T) and Anaerotignum lactatifermentans comb. nov. (type strain G17T=DSM 14214T=LMG 20954T) are proposed with emended descriptions of these species.

Many members of the order Clostridiales within the phylum Firmicutes are asaccharolytic and decompose proteins or amino acids under anaerobic circumstances [1–7]. In this study, we describe a strictly anaerobic, amino-acid-decomposing bacterium (strain SH021T) isolated from a methanogenic reactor. The strain was assigned to clostridial cluster XIVb in the order Clostridiales [8] based on the 16S rRNA gene sequence. The strain decomposes several kinds of amino acids as well as pyruvate and lactate, and produces mainly acetate and propionate. Species in clostridial cluster XIVb are classified in the family Lachnospiraceae together with many species in cluster XIVa [2]. Species with validly published names affiliated with cluster XIVb are currently Clostridium propionicum [9], Clostridium neopropionicum [10], Clostridium lactatifermentans [11], Cellulosilyticum ruminicola, Cellulosilyticum lentocellum (=Clostridium lentocellum) [12] and Clostridium colinum [13]. Three of these species, Clostridium propionicum, Clostridium neopropionicum and Clostridium lactatifermentans, were the most closely related species to strain SH021T with 16S rRNA gene sequence similarities of about 95%. Strain SH021T had many common phenotypic characteristics with these closely related species of the genus Clostridium, and formed a distinct cluster together with these species in the phylogenetic tree reconstructed based on the 16S rRNA gene sequences. According to the proposal for restriction of the genus Clostridium to the species in clostridial cluster I (Clostridium sensu stricto; Clostridium butyricum and

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Abbreviations: CFA, cellular fatty acid; DAP, diaminopimelic acid; DMA, dimethylacetal; ECL, equivalent chain length; VFA, volatile fatty acid.
Two supplementary tables and two supplementary figures are available with the online Supplementary Material.
related species) [2, 14], a novel genus in the family Lachnospiraceae within the order Clostridiales is proposed to accommodate strain SH021\textsuperscript{T} as well as the three closely related species of the genus Clostridium with emended descriptions of these species.

Strain SH021\textsuperscript{T} was isolated from a methanogenic reactor treating waste collected from cattle farms (comprising up to 1000 cattle in total) in Betsukai-machi in Hokkaido, Japan [15]. The strain was cultivated anaerobically at 30 °C unless otherwise stated by using peptone/yeast extract (PY) as a basal medium with oxygen-free mixed gas (95 % N\textsubscript{2}/5 % CO\textsubscript{2}) as the headspace as described previously [5, 6]. PY medium contained (l\textsuperscript{-1}) 10 g trypticase (BBL), 5 g yeast extract, 0.2 g Na\textsubscript{2}CO\textsubscript{3}, 0.3 g l-cysteine hydrochloride dihydrate (as a reducing agent) and 1 mg resazurin sodium (as a redox indicator) as well as salt solutions [16]. PY medium supplemented with (l\textsuperscript{-1}) 0.25 g each of glucose, cellobiose, maltose and soluble starch together with 15 g agar was designated PY\textsubscript{4}S agar and used for isolation and maintenance of the strain in agar slants. All media were usually adjusted to pH 7.2–7.4 (pH 6.8–7.0 after autoclaving) with 1 M NaOH.

Growth of the novel strain under aerobic conditions was examined as described previously [15]. The motility of cells was examined by phase-contrast microscopy. Flagella staining was carried out according to the method of Blenden and Goldberg [17]. Spore formation was assessed by phase-contrast microscopy and observation of cells after Gram staining. To examine formation of thermotolerant cells, cultures grown in PY-serine medium (see below) or on agar slants were inoculated to fresh PY-serine medium and the culture tubes were treated at 80 °C for 10 min before cultivation at 30 °C.

Cells of strain SH021\textsuperscript{T} were straight to slightly curved rods; most of the cells were approximately 2.5–8.0 μm in length and 0.7 μm in diameter (Table 1). Cells usually occurred singly (Fig. S1a, available with the online Supplementary Material). Cells of strain SH021\textsuperscript{T} were Gram-stain-positive and motile by a tumbling motion as observed by phase-contrast microscopy. Two to four peritrichous flagella were observed by flagella staining (Fig. S2). Surface colonies on PY\textsubscript{4}S agar were greyish white and 0.7 mm in diameter, greyish white and translucent with slightly irregular surface. Cells of the strain did not grow in air. Spore formation was not observed by microscopy, but strain SH021\textsuperscript{T} grew after the heat treatment of cells, indicating the presence of spores or thermotolerant cells.

Utilization of carbon sources was tested in PY liquid medium with each substrate (monosaccharides, disaccharides, oligosaccharides and sugar alcohols) added at 10 g l\textsuperscript{-1}. Other substrates (polysaccharides, glycosides and Casamino acids) were added at 5 g l\textsuperscript{-1}. To determine utilization of organic acids, amino acids or ethanol, each compound was added at 30 mM as the final concentration in PY medium. Utilization of each substrate was determined by growth monitored by optical density at 660 nm (OD\textsubscript{660}) as well as by measurement of fermentation products after cultivation. Fermentation products including volatile fatty acids (VFAs), H\textsubscript{2} and CO\textsubscript{2} were analysed by gas chromatography, and the amounts of products in the presence of substrates were compared with those in PY medium without added substrates. All tests were carried out in duplicate, and reproducibility was checked (Table 1). The novel strain grew in PY medium without additional substrates and produced propionate (5.6 mmol l\textsuperscript{-1}) and isovalerate (5.9 mmol l\textsuperscript{-1}) as well as trace amounts of acetate (0.4 mmol l\textsuperscript{-1}), isobutyrate (2.2 mmol l\textsuperscript{-1}) and butyrate (2.4 mmol l\textsuperscript{-1}). Carbohydrates including glucose and xylose did not improve the growth and production of VFAs. Ethanol was not utilized. Pyruvate and lactate promoted growth, and were converted to acetate (13.4 and 6.4 mmol l\textsuperscript{-1}, respectively) and propionate (16.7 and 19.5 mmol l\textsuperscript{-1}, respectively). Other organic acids were not utilized. The strain utilized several kinds of amino acids and produced substantial amounts of VFAs. l-Serine supported good growth to produce acetate (14.6 mmol l\textsuperscript{-1}) and propionate (15.7 mmol l\textsuperscript{-1}), and the highest amount of propionate was produced from l-alanine (27.2 mmol l\textsuperscript{-1}) together with acetate (9.3 mmol l\textsuperscript{-1}). Substantial amounts of propionate (17.4 mmol l\textsuperscript{-1}) and butyrate (9.5 mmol l\textsuperscript{-1}) were produced from l-threonine. Branched-chain amino acids enhanced production of branched-chain VFAs; isovalerate from l-isoleucine and l-leucine and isobutyrate from l-valine (Table S1). Other amino acids did not affect growth or products. Casamino acids increased production of isovalerate. H\textsubscript{2} was not produced. The final pH after growth in PY medium with or without amino acids utilized was pH 6.3–6.6. Growth and production of VFAs in PY medium without additional substrates indicated that proteinous compounds in the medium including the amino acids shown above must support growth of the strain. B vitamin requirement was not recognized. The substrates tested but not utilized are given in the species description.

The optimum growth conditions for strain SH021\textsuperscript{T} were tested in PY medium containing serine (30 mM; PY-serine) at temperatures of 10–45 °C (at 5 °C intervals), at pH 4.1, 5.1, 6.1, 6.8, 7.7, 8.2, 9.4 and 10.3 (as values verified after autoclaving), and by the addition of 0–50 g NaCl l\textsuperscript{-1} (at 10 g l\textsuperscript{-1} intervals with the exception of 5 g l\textsuperscript{-1}) in the medium. The temperature range for growth was 15–40 °C with the highest growth rate at 35 °C. The strain grew at pH 6.1–8.2; optimum growth was at pH 6.1–7.7. The strain grew in medium containing NaCl up to 20 g l\textsuperscript{-1}; the highest growth rate was obtained without NaCl addition. The specific growth rate (µ) under the optimum conditions (35 °C and pH 6.7 in the absence of added NaCl) was about 0.13 h\textsuperscript{-1}.

Other physiological characterization was carried out according to the methods described by Holdeman et al. [18] and Ueki et al. [5]. Catalase, oxidase and nitrate-reducing activities were not detected. The novel strain produced hydrogen.
sulfide and hydrolysed ascenin. Other physiological characteristics are presented in the species description.

Genomic DNA extracted according to the method described by Akasaka et al. [19] was digested with P1 nuclease by using a YAMASA GC kit (Yamasa shoyu) and its G+C content was measured by HPLC (HITACHI L-7400) with a µBondapack C18 column (3.9 × 300 mm; Waters). Whole-cellular fatty acids (CFAs) were converted to methyl esters according to the method of Miller [20] and analysed by gas chromatography with a HP Ultra 2 column. CFAs were identified by equivalent chain length (ECL) [21] at Techno-Suruga (Shizuoka, Japan). The cell-wall diagnostic diamino acid was analysed according to the method described by Akasaka et al. [19]. Respiratory isoprenoid quinones were extracted and analysed according to the method of Komagata and Suzuki [22] for the presence of menaquinones and ubiquinones. The G+C content of the genomic DNA was 38.2 mol%. Components such as iso-C₁₅₋₀ dimethylacetal (DMA) (19.9 %), iso-C₁₅₋₀ aldehyde (as summed feature 3) (17.3 %) and iso-C₁₅₋₀ (7.4 %) were detected by the analysis of CFAs, with many minor compounds of C₁₃₋₁₈ (Table 2).

Peptidoglycan of the cell wall contained meso-diaminopimelic acid (DAP) as the diagnostic diamino acid. Neither menaquinones nor ubiquinones were detected.

DNA was extracted from cells as described previously [19]. Almost the full-length of the 16S rRNA gene was PCR-amplified using a primer set of 27f and 1546r [23]. The PCR-amplified 16S rRNA gene was sequenced by using an ABI Prism BigDye Terminator Cycle Sequencing ready reaction kit and an ABI Prism 3730 automatic DNA sequencer (Applied Biosystems). Multiple alignments of the sequence with reference sequences in GenBank were performed with the BLAST program [24]. A phylogenetic tree was reconstructed with the neighbour-joining method [25] by using the CLUSTAL W program [26]. All gaps and unidentified base positions in the alignments were excluded before sequence assembly.

Analysis of the almost-complete 16S rRNA gene sequence (1471 bp) of strain SH021ᵀ assigned the strain to the phylum Firmicutes. The most closely related known species to strain SH021ᵀ were members of clostridial 16S rRNA cluster XIVb [2, 8], with similar levels of sequence similarities; Clostridium propionicum DSM 1682ᵀ (sequence similarity, 95.6 %); Clostridium neopropionicum DSM 3847ᵀ (95.3 %); Clostridium lactatifermentans DSM 14214ᵀ (95.0 %). The phylogenetic tree comprising these relatives as well as representative species in clostridial clusters XIVa and XIVb showed that strain SH021ᵀ and the three closely related species of the genus Clostridium formed a distinct cluster from any of the species in clostridial clusters XIVa and XIVb, now regarded as belonging to the family Lachnospiraceae (Fig. 1).

Phenotypic properties of strain SH021ᵀ were compared with those of the three related species by cultivating each type strain under the same conditions as for strain SH021ᵀ. The results obtained matched with the original descriptions of each species with a few exceptions (Table 1). Cells of the
strains except strain DSM 14214\textsuperscript{T} were motile, regular rods; however, cells of strain DSM 14214\textsuperscript{T} were non-motile and usually occurred as spindle-shaped short rods in chains (Fig. S1). The presence of spores or thermotolerant cells for the strains except strain DSM 14214\textsuperscript{T} was confirmed by observation of cells by microscopy or their growth after heat treatment (80 °C for 10 min) of cells. As for strain DSM 14214\textsuperscript{T}, we could not observe formation of spores by ant cells by using cells grown in PY-serine as well as in PYG, but cells from neither culture grew after the heat treatment. Thus, we concluded that the strain produces neither cells nor thermotolerant cells as described in the original description. For the substrate utilization, however, all strains including \textit{Clostridium lactatifermentans} DSM 14214\textsuperscript{T} showed similar properties to strain SH021\textsuperscript{T} (Table 1). The three reference type strains utilized the same amino acids as those utilized by strain SH021\textsuperscript{T}, and produced the same kinds of VFAs from each amino acid with the same combination of VFAs. All utilized pyruvate and lactate, and all produced acetate and propionate, although \textit{Clostridium propionicum} DSM 1682\textsuperscript{T} and \textit{Clostridium neopropionicum} DSM 3847\textsuperscript{T} produced lower amounts of acetate from lactate as compared with those of other two strains. \textit{Clostridium propionicum} DSM 1682\textsuperscript{T} utilized neither glucose nor xylose in a similar way as strain SH021\textsuperscript{T}, whereas \textit{Clostridium neopropionicum} DSM 3847\textsuperscript{T} utilized xylose and produced acetate and propionate with a minor amount of butyrate. \textit{Clostridium lactatifermentans} DSM 14214\textsuperscript{T} also utilized xylose as well as glucose as described in the original description [2, 10] and produced mainly propionate with lower amounts of acetate from both substrates (Table S1). \textit{Clostridium propionicum} X4\textsuperscript{T} (=DSM 3847\textsuperscript{T}) was originally reported to use both glucose and xylose [10], whereas the strain could not utilize these carbohydrates in the re-examination by van der Wielen et al. [11]. The authors described that the difference in carbohydrates utilization could be related to the use of different media for growth incubation. In our present study, utilization of xylose by \textit{Clostridium neopropionicum} DSM 3847\textsuperscript{T} was clearly confirmed, but utilization of glucose was not verified. Ethanol was not utilized by strain SH021\textsuperscript{T} as well as \textit{Clostridium lactatifermentans} DSM 14214\textsuperscript{T}, while the remaining two strains utilized it. Thus, \textit{Clostridium propionicum} DSM 1682\textsuperscript{T} and \textit{Clostridium neopropionicum} DSM 3847\textsuperscript{T} had similar phenotypic properties including utilization of amino acids and ethanol [2], but the two strains had significant differences in their cell morphologies (Fig. S1b, c) and utilization of carbohydrates (at least for xylose). \textit{Clostridium lactatifermentans} DSM 14214\textsuperscript{T} showed characteristics for substrate utilization intermediate among these strains.

CFA compositions were also determined for the three related type strains in this study (Table 2). All strains including strain SH021\textsuperscript{T} had similar compounds related to branched-chain C\textsubscript{15:0} (iso-C\textsubscript{15:0} or anteiso-C\textsubscript{15:0}) including DMA and aldehyde as the major components, although the percentages of each component were different according to the strain. For the three strains except \textit{Clostridium lactatifermentans} DSM 14214\textsuperscript{T}, iso-C\textsubscript{15:0} compounds such as iso-C\textsubscript{15:0}, iso-C\textsubscript{15:0} DMA and iso-C\textsubscript{15:0} aldehyde (as summed feature 3) were the major constituents, whereas strain DSM 14214\textsuperscript{T} had anteiso-C\textsubscript{15:0} and anteiso-C\textsubscript{15:0} DMA (as summed feature 13) as the two major components. The presence of iso-C\textsubscript{15:0} aldehyde (summed feature 3) as a major component and less than 10% iso-C\textsubscript{15:0} was a distinctive feature of CFAs for strain SH021\textsuperscript{T}. The three reference type strains also had unique whole profiles of CFAs showing many minor C\textsubscript{13–18} compounds (fatty acids, DMA, saturated and unsaturated). We analysed CFA compositions of strain SH021\textsuperscript{T} and \textit{Clostridium propionicum} DSM 1682\textsuperscript{T} three times by using cell biomass cultured at

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*Summed features: 1, C\textsubscript{13:1}/C\textsubscript{14:0} aldehyde; 3, iso-C\textsubscript{15:0} aldehyde/unknown; 4, C\textsubscript{15:1} /ω\textsubscript{8c}/C\textsubscript{15:2} /ω\textsubscript{8c}/C\textsubscript{17:2}; 8, C\textsubscript{17:1} /ω\textsubscript{8c}/C\textsubscript{17:2}; 13, anteiso-C\textsubscript{15:0} DMA/ C\textsubscript{14:0} 2-OH.
different times independently and obtained almost the same results with many minor peaks in the gas chromatograms for all samples, thus supporting that the many minor components detected were constituents of cells of these strains. Rainey et al. [2] pointed out that a wide variety of lipid fatty acids, both straight- and branched-chain, saturated and unsaturated, are present in cells of the type strain of *Clostridium propionicum*. The results obtained in this study showed that the presence of such a wide variety of CFAs is a common feature for the species in this group. All strains had meso-DAP in peptidoglycan of cell walls as the diagnostic diamino acid.

The genomic DNA G+C content of *Clostridium lactatifermentans* DSM 14214\textsuperscript{T} (44.6 % in the original description) is higher than those of the other three strains, and this was confirmed in this study (46.0 % in our analysis). However, strain DSM 14214\textsuperscript{T} had very similar physiological characteristics to those of the related strains examined in this study, especially for amino acids utilization and end-products from the substrates tested. The 16S rRNA gene sequence similarities between SH021\textsuperscript{T} and each of the related three species of the genus *Clostridium* shown above (95.0–95.6 %) are slightly higher than the threshold value for defining bacterial genera (94.5–95.0 %) recommended by Yarza et al. [27]. But the four related strains examined in this study formed a distinct cluster in the phylogenetic tree from any of the species in clostridial clusters XIVb and XIVa as shown above (Fig. 1). Furthermore, *Clostridium colinum* and the remaining two species in cluster XIVb,
**Cellulosilyticum ruminicola** and **Cellulosilyticum lentocellum**, are saccharolytic and produce acetate, formate or ethanol as major products. These phenotypic properties are basically different from those of strain SH021\(^T\) as well as its three closely related species (Table S2). Thus, we concluded that strain SH021\(^T\) and these three species of the genus *Clostridium* should be affiliated with the same genus in the family *Lachnospiraceae*. 'Tyzzerella' gen. nov. was proposed to reclassify *Clostridium nexile*, *Clostridium propionicum*, *Clostridium neopropionicum*, *Clostridium lactifermantans* and *Clostridium colinum* [28]. However, these descriptions do not comply with the Bacteriological Code and, therefore, the names could not be validly published in the *International Journal of Systematic and Evolutionary Microbiology* and therefore have no standing in the literature.

It has been proposed that the genus *Clostridium* should be restricted to species belonging to clostridial cluster I (*Clostridium sensu stricto*) (*Clostridium butyricum* and related species) [2, 14]. Thus, based on the data obtained in this study we propose the novel genus *Anaerotignum* gen. nov. in the family *Lachnospiraceae* in the order *Clostridiales* to accommodate strain SH021\(^T\) in *Anaerotignum aminivorans* gen. nov., sp. nov. In addition, we also propose that the three closely related species (*Clostridium propionicum*, *Clostridium neopropionicum* and *Clostridium lactifermantans*) should be reclassified in the novel genus as the new combinations *Anaerotignum propionicum* comb. nov. (type strain DSM 1682\(^T\)), *Anaerotignum neopropionicum* comb. nov. (type strain X4\(^T\)) and *Anaerotignum lactifermantans* comb. nov. (type strain G17\(^T\)).

### DESCRIPTION OF *ANAEROTIGNUM* GEN. NOV.

*Anaerotignum* (Ana.e.ro.tig’num. Gr. prep. anot; Gr. n. aer air; L. neut. n. tignum a small log or a stick of timber; N. L. neut. n. *Anaerotignum* a small log or timber not living in air, pertaining to the cell shape resembling a small log).

Strictly anaerobic rods. Have Gram-positive-type cell walls. Chemo-organotrophs. Utilize amino acids (L-alanine, L-serine and L-threonine) and produce mainly acetate, propionate and butyrate. Utilize L-isoleucine, L-leucine and L-valine weakly and produce branched-chain fatty acids (isovalerate or isobutyrate). Ferment pyruvate and lactate to acetate and propionate. Odd-numbered branched-chain compounds such as iso-C\(_{15:0}\) , iso-C\(_{17:0}\) DMA or anteiso-C\(_{15:0}\) are major components of CFAs with a wide variety of minor C\(_{13-18}\) compounds. Cell wall peptidoglycan contains meso-DAP as a diagnostic amino acid. The genus belongs to the family *Lachnospiraceae* within the order *Clostridiales*. The type species is *Anaerotignum aminivorans*.

### DESCRIPTION OF *ANAEROTIGNUM AMINIVORANS* SP. NOV.

*Anaerotignum aminivorans* (ami.ni.vo’rans. N.L. n. am. num amine; L. v. vorare devour, to eat; N.L. part. adj. aminivorans amino acid-consuming bacterium).

Cells are Gram-stain-positive, straight to slightly curved rods, 2.5–8.0 \(\mu\)m in length and 0.7 \(\mu\)m in diameter. Motile with 2–4 peritrichous flagella. Spore formation is not observed, but thermotolerant cells are present. Colonies on PY445 agar are 0.5–0.7 mm in diameter, greyish white and translucent with slightly irregular surface. B vitamins are not required for growth. Grows at 15–40 °C (optimum 35 °C), pH 6.1–8.2 (optimum pH 6.1–7.7, \(\mu\)=0.13 h\(^{-1}\)) and with 0–20 g NaCl l\(^{-1}\) (optimum 0 g l\(^{-1}\)). Grows in PY medium and produces propionate, isobutyrate, butyrate and isovalerate with a trace amount of acetate. Does not utilize carbohydrates (arabinose, ribose, xylose, fructose, galactose, glucose, mannose, rhamnose, cellobiose, lactose, maltose, melibiose, sucrose, trehalose, melezitose, raffinose, glycerogen, cellulose, carboxymethylcellulose, dextrin, inulin, starch, xylan, pectin, mannitol, inositol, sorbitol, amygdalin, aesculin or salicin). Does not utilize ethanol or glycerol. Utilizes pyruvate and lactate. Does not utilize other acids (acrylate, aminobutyrate, crotonate, fumarate, malate or succinate). Utilizes L-alanine, L-serine and L-threonine. Utilizes Casamino acids, L-isoleucine, L-leucine and L-valine weakly. Produces mainly acetate and propionate from L-alanine and L-serine, and propionate and butyrate from L-threonine. Produces isovalerate from L-isoleucine and L-leucine, and isobutyrate from L-valine. Does not utilize other amino acids (L-arginine, L-asparagine, L-aspartate, L-glutamate, L-glutamine, glycine, L-histidine, L-lysine, L-methionine, L-phenylalanine, L-proline or L-tryptophan). Converts pyruvate and lactate to acetate and propionate. Final pH after growth with L-alanine and L-serine is pH 6.3–6.6. Oxidase, catalase and nitrate-reducing activities are negative. Produces hydrogen sulfide. Hydrolyses aesculin, but not starch or gelatin. Indole, lecinthinase and lipase are not produced. Milk is unchanged, and no growth occurs in chopped meat broth. Major CFA components are iso-C\(_{15:0}\) DMA, iso-C\(_{15:0}\) aldehyde and iso-C\(_{15:0}\). Respiratory quinones are not detected.

The type strain, SH021\(^T\) (=JCM 31556\(^T\)=DSM 103575\(^T\)), was isolated from a methanogenic reactor treating waste from cattle farms in Japan. The genomic DNA G+C content of the type strain is 38.2 mol%.

### DESCRIPTION OF *ANAEROTIGNUM PROPIONICUM* COMB. NOV.

*Anaerotignum propionicum* (pro.pi.o’ni.cum. N.L. neut. n. acidum propionicum propionic acid; N.L. neut. adj. propionicum pertaining to production of propionic acid).

Basonym: *Clostridium propionicum* Cardon and Barker 1946 [9, 29]

In addition to the original description of *Clostridium propionicum* [2, 9] as well as the genus description of *Anaerotignum*, the species has the following properties. iso-C\(_{15:0}\) is the most dominant CFA with many minor compounds.

The type strain is DSM 1682\(^T\) (=JCM 1430\(^T\)=ATCC 25522\(^T\)=CCUG 9280\(^T\)=NCIMB 10656\(^T\)=VPI 5303\(^T\)).
DESCRIPTION OF ANAEROGITNUM NEOPROPIONICUM COMB. NOV.

Anaerotignum neopropionicum (ne.o.pro.pi.o’ni.cum. Gr. adj. neos new; N.L. neut. n. acidum propionicum propionic acid; N.L. neut. adj. neopropionicum a new propionic acid-producing bacterium).

Basionym: Clostridium neopropionicum Tholozan et al. 1995 [30]

In addition to the original description of Clostridium neopropionicum [30] as well as the genus description of Anaerotignum, the species has the following properties. The major CFAs are iso-C₁₅:₀ and iso-C₁₅:₀ DMA with many minor compounds.

The type strain is X₄ᵀ (=DSM 3847ᵀ=KCTC 15564ᵀ).

DESCRIPTION OF ANAEROGITNNUM LACTATIFERMENTANS COMB. NOV.

Anaerotignum lactatifermentans (lac.ta.ti.fer.men’tans N.L. n. lactas -atis n. lactate; L. pres. part. fermentans fermenting; N.L. part. adj. lactatifermentans fermentating lactate).

Basionym: Clostridium lactatifermentans van der Wielen et al. 2002 [11]

In addition to the original description of Clostridium lactatifermentans [11] as well as the genus description of Anaerotignum, the species has the following properties. iso-C₁₅:₀, anteiso-C₁₅:₀ and anteiso-C₁₅:₀ DMA are major components of CFAs with many minor compounds.

The type strain is G₁₇ᵀ (=DSM 14214ᵀ=LMG 20954ᵀ).

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References


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