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***Anaerosphaera aminiphila* gen. nov., sp. nov., a novel, glutamate-degrading, Gram-positive anaerobic coccus isolated from a methanogenic reactor of cattle waste**

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Abbreviations: VFA, volatile fatty acid; GPAC, Gram-positive, anaerobic cocci; CFA, cellular fatty acid; DMA, dimethylacetal.

Short running title: *Anaerosphaera aminiphila* gen. nov., sp. nov.

Key words: Gram-positive anaerobic cocci, butyrate, glutamate, aminolytic bacteria, methanogenic reactor.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain WN036^T is AB298735.

Abstract

A strictly anaerobic, mesophilic and aminolytic strain (WN036^T) was isolated from a methanogenic reactor treating waste from cattle farms. Cells were Gram-positive cocci, often occurring in pairs, and non-motile. Although spore formation was not confirmed by microscopic observation of cells, the strain produced thermotolerant cells. The optimum temperature for growth was 35-37°C and the optimum pH was 6.7. Oxidase, catalase and nitrate-reducing activities were negative. The strain did not ferment carbohydrates and grew in PY medium without additional substrates. The strain utilized L-glutamate, L-glutamine, L-histidine and L-arginine as growth substrates. Major fermentation products were acetate and butyrate with a small amount of propionate. The genomic DNA G+C content was 32.5 mol%. The major cellular fatty acids were $C_{17:1}\omega8$, $C_{18:1}\omega7$ DMA and $C_{16:0}$. The diamino acid of the cell wall peptidoglycan was lysine, and glutamic acid, glycine, alanine and aspartic acid were also detected in the peptidoglycan. The most closely related species of strain WN036^T on the basis of 16S rRNA gene sequences were *Peptoniphilus asaccharolyticus* (89.8%) and *Peptoniphilus indolicus* (89.6%). Based on the differences in phenotypic and phylogenetic characteristics of strain WN036^T from those of closely related species, a novel genus and species, *Anaerosphaera aminiphila* gen. nov., sp. nov. was proposed to accommodate the strain. The type strain is WN036^T (=JCM 15094^T =DSM 21120^T).

MAIN TEXT

In anaerobic conditions, protein is hydrolyzed by proteolytic microbes, and then amino acids generated are fermented by aminolytic microbes mainly to volatile fatty acids (VFAs) such as propionate, butyrate, isobutyrate, valerate and isovalerate in addition to acetate (Holdeman *et al.*, 1977). Besides acetate, the major

VFAs propionate and butyrate greatly contribute to methanogenesis in the ecosystem through oxidation to acetate and H_2. Furthermore, ammonia produced by deamination of amino acids balances pH decrease with accumulation of VFAs in the system. Thus, degradation of protein and amino acids is one of key reactions for efficient methanogenesis in anaerobic treatment of waste containing organic matter. However, ecology or physiology of anaerobic bacteria involving in the degradation of proteinous compounds in the anaerobic waste treatment has not been studied so much. Members of Gram-positive anaerobic cocci (GPAC) are major anaerobic bacteria of the normal human flora and have been frequently recovered from human clinical specimens (Holdeman Moore *et al.*, 1986; Murdoch, 1998; Song *et al.*, 2003). Most of the members in GPAC are non-saccharolytic and utilize peptone for growth to produce mainly acetate or butyrate (Ezaki *et al.*, 2001; Holdeman Moore *et al.*, 1986). Almost all species in the GPAC group have been isolated from samples relating to human infections or animals. In this study, we described a novel bacterium belonging to the GPAC group isolated from a methanogenic reactor of cattle waste. The bacterium degraded a few kinds of amino acid including L-glutamate and produced acetate and butyrate as major products.

Strain WN036^T was isolated from a rice-straw residue sample obtained from a methanogenic reactor used to treat waste collected from cattle farms (comprising up to 1000 cattle in total) in Betsukai-machi in Hokkaido, Japan. The reactor was a vertical cylindrical type (1500 m^3) operated at mesophilic temperature. Rice-straw utilized for matting of the cattle farms was treated in the reactor together with faeces and urine of the animals (Nishiyama *et al.*, in press; Ueki *et al.*, 2008).

The strain was cultivated anaerobically at 30˚C unless otherwise stated by using peptone/yeast extract (PY)

medium as basal medium with oxygen-free mixed gas (95% N_2/ 5% CO_2) as the headspace as described by Ueki *et al.* (2006, 2007). PY medium supplemented with (l^{-1}) 0.25 g each of glucose, cellobiose, maltose and soluble starch as well as 15 g agar (Difco) was designated PY4S agar and used for maintenance of the strain in agar slants. Since addition of a B-vitamin mixture (Ueki *et al.*, 2008) to the medium was slightly stimulatory to the growth of the strain, the vitamin mixture solution (10 ml l^{-1}) was usually added to PY liquid medium (PYV medium) for cultivation of the strain. PYV liquid medium supplemented with 30 mM of sodium L-glutamate·H_2O (PYVGlu medium) was usually used for the cultivation of the strain for various physiological tests and chemotaxonomic analyses of the cells unless otherwise stated. Media were usually adjusted to pH 7.2 with 1M NaOH.

Strain WN036^T was isolated by the anaerobic roll tube method for enumeration of anaerobic fermentative bacteria by the colony-counting method (Hungate, 1966; Holdeman *et al.*, 1977) using PY4S agar medium supplemented with the B-vitamin mixture. Anaerobic sludge samples obtained from the reactor were filtrated through a mesh (2 mm of pore-size) and relatively large residue of rice-straw remained on the mesh was collected. The rice-straw samples obtained were washed several times with sterile anoxic diluent and homogenized by a Waring blender (10000 rpm, 10 min.) under N_2 gas. The homogenized samples were successively diluted anaerobically and used as inocula for isolation (Akasaka *et al.*, 2003a, b). Colonies formed on agar during incubation for two weeks were picked at random and about 50 isolates were obtained from the sample. One of the isolates, strain WN036^T, was selected for further studies based on the preliminary examinations for phylogeny and physiology of the isolates. Strain WN036^T was picked from the agar tube inoculated with a 10^{-5} diluted sample.

Growth of the strain under the aerobic condition was examined as described previously (Ueki *et al*., 2008). The motility of cells was examined by phase-contrast microscopy. Spore formation was assessed by observation of cells after Gram-staining as well as by phase-contrast microscopy. To examine formation of thermotolerant cells, cultures grown in PYVGlu medium or on agar slants were inoculated to fresh PYVGlu medium, and after exposure to heat (80°C for 10 min unless otherwise stated) of the whole tubes closed with butyl rubber stoppers, the tubes were incubated at 30°C to check the growth. Furthermore, thermotolerant cells in the culture were enumerated by the MPN method. Cultures grown to the early stationary phase in PYVGlu medium were 10-fold diluted anaerobically to extinction and inoculated to triplicate tubes containing fresh PYVGlu medium at each dilution. Each tube was incubated after heat treatment to check the growth as described above, and the MPN counts of thermotolerant cells were determined according to a statistical MPN table based on the number of positive tubes for growth. Catalase, oxidase and nitrate-reducing activities were determined according to the methods described by Akasaka *et al*. (2003b). Utilization of carbon sources was tested in PYV liquid medium with each substrate (monosaccharides, disaccharides, oligosaccharides and sugar alcohols) added at 10 g l^{-1}. Other substrates (polysaccharides and glycosides) were added at 5 g l^{-1}. Amino acids were added at 30 mM. Utilization of each substrate was determined by growth measured by OD_{660} as well as by measurement of fermentation products. Fermentation products were analyzed by GC as described previously (Ueki *et al*., 1986; Akasaka *et al*., 2003a). Other characterizations were performed according to the methods as described by Holdeman *et al*. (1977), Nishiyama *et al*. (in press) and Ueki *et al*. (2008).

Whole-cell fatty acids (CFAs) were converted to methyl esters according to the method of Miller (1982) and analyzed by GC (Hp6890; Hewlett-Packard or G-3000; Hitachi) equipped with a HP Ultra 2 column. CFAs were identified by equivalent chain-length (ECL) (Miyagawa *et al.*, 1979; Ueki & Suto, 1979) according to the protocol of TechnoSuruga Co., Ltd (Shimadu, Japan) (Moore *et al.*, 1994). Isoprenoid quinones were extracted and purified as described by Komagata & Suzuki (1987). Purification of cell wall was carried out according to the method described by Schleifer & Kandler (1972). The purified cell wall was hydrolyzed with 4 N HCl for 16 h at 100°C and the composition of amino acids were analyzed by thin layer chromatography (CelluloseTLC, Merck) as described by Harper & Davis (1979). Genomic DNA extracted according to the method as described by Akasaka *et al.* (2003b) 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) equipped with a μBondapack C18 column (3.9 × 300 mm; Waters).

DNA was extracted from cells as described previously (Akasaka *et al.*, 2003b). Almost full-length of 16S rRNA gene was PCR amplified using a primer set of 27f and 1546r. The PCR-amplified 16S rRNA gene was sequenced by using an ABI Prism BigDye Terminator Cycle Sequencing ready reaction kit and ABI Prism 3730 automatic DNA sequencer (Applied Biosystems). Multiple alignments of the sequences with reference sequences in GenBank were performed with the BLAST program (Altschul *et al.*, 1997). A phylogenetic tree was constructed with the neighbour-joining method (Saitou & Nei, 1987) by using the CLUSTAL W program (Thompson *et al.*, 1994). All gaps and unidentified base positions in the alignments were excluded before sequence assembly.

Cells of strain WN036^T were spherical; most of cells were approximately 0.7-0.9 μm in diameter. Cells occurred singly or in pairs, but not in chains (Fig. 1). Cells of strain WN036^T stained Gram-positive, and were non-motile as observed under phase-contrast microscopy. Colonies on PY4S agar after 2 days anaerobic incubation were translucent and dense with smooth surfaces. Cells of the strain did not grow in air. Although spore formation of the cells was not observed by microscopy (Fig. 1), cells cultivated in PYVGlu liquid medium and treated at 80°C for 10 min grew in the fresh medium after a relatively long lag period (3-4 days). Cultures treated at 85-90°C for 10 min still survived for most of the cases of the treatment. Thus, we concluded that the strain produced thermotolerant cells or spores. When the number of thermotolerant cells in cultures grown in PYVGlu liquid medium were enumerated by the MPN method, the thermotolerant cells accounted for 0.045-0.1% (about 0.9-2 x 10^6 cells ml^{-1}) of the total cells (2 x 10^9 cells ml^{-1}). Inoculation of one loop of cells from PY4S slant cultures to 10 ml of PYVGlu liquid medium was usually not enough for the certain growth after the heat treatment, while increase in the amount of cells for inoculation resulted in the growth after the treatment.

The strain grew in PY or PYV medium without additional substrates (final OD_{660}, 0.9-1.0; final pH, 7.1). The following substrates including carbohydrates and organic acids (arabinose, ribose, xylose, fructose, galactose, glucose, mannose, rhamnose, sorbose, cellobiose, lactose, maltose, melibiose, sucrose, trehalose, melezitose, raffinose, glycogen, cellulose, carboxymethylcellulose, inulin, dextrin, starch, xylan, pectin, dulcitol, glycerol, inositol, mannitol, sorbitol, amygdalin, aesculin, salicin, fumarate, lactate, malate, pyruvate, succinate and ethanol) were not utilized. A limited kinds of amino acid (sodium L-glutamate·H_2O, L-glutamine, L-histidine and L-arginine·HCl) supported rapid growth of the strain in PYV medium. In the

presence of these amino acids, final OD_{660} values ranged from 1.8 to 2.3 and final pH was around pH 7.0 except for L-arginine (pH 7.9). Other amino acids did not enhance the growth in PYV medium (L-threonine, sodium L-aspartate·H_2O, L-asparagine·H_2O, L-serine, L-alanine, glycine·HCl, L-methionine, L-lysine·HCl, L-phenylalanine, L-tryptophan, L-valine, L-leucine, L-isoleucine, L-tyrosine, L-proline and L-ornithine). Fermentation products in PY and PYV medium were almost the same; acetate (6.3 mmol l^{-1}), propionate (1.7 mmol l^{-1}) and butyrate (3.7 mmol l^{-1}). In the presence of L-glutamate (PYVGlu medium), acetate (30.8 mmol l^{-1}) and butyrate (25.0 mmol l^{-1}) were the dominant products with propionate as a minor product (1.6 mmol l^{-1}). H_2 was not produced. Almost the same amounts of products were detected from L-glutamine in PYV medium. The fermentation products with L-histidine were 35.5 mmol l^{-1} of acetate and 15.3 mmol l^{-1} of butyrate as well as 2.5 mmol l^{-1} of propionate. Products with L-arginine were not determined. Although apparent growth enhancement was not observed, the addition of L-threonine or L-methionine to PYV medium resulted in the slight increase in propionate production (4.0-4.5 mmol l^{-1}) of the strain.

Both catalase and oxidase activities were not detected. The strain did not reduce nitrate. Aesculin was hydrolyzed, while starch and gelatin were not. Hydrogen sulfide was produced. Indole, lecithinase, lipase and urease were not produced. The strain did not change milk and did not grow in chopped meat broth. The strain was sensitive to bile salts.

Temperature range for growth was 10-37°C with the highest growth rates at 35-37°C (μ = 0.27-0.29 h^{-1}). Growth rates at lower temperatures (10°C and 20°C) were relatively high (μ = 0.043 h^{-1} and μ = 0.13 h^{-1}, respectively). The strain did not grow at 5°C. Although a weak initial growth was observed at 40°C, the

growth stopped soon without increase in the turbidity of the culture. Thus, 37°C seemed to around the upper limit for growth of the strain. pH range for growth was 5.8-7.8 with an optimum at pH 6.7. The strain grew in PYVGlu liquid medium containing NaCl up to 4% (w/v), although the growth rate was the highest without addition of NaCl. The strain did not grow in the presence of 5% (w/v) of NaCl.

The G+C content of the genomic DNA was 32.5 mol%. Various compounds including aldehydes and dimethylacetals (DMAs) were detected by the analysis of CFAs of strain WN036^T. Compounds detected at relatively higher amounts were as follows; $C_{17:1}\omega8$, 14.3%; $C_{18:1}\omega7$ DMA, 12.2%; $C_{16:0}$, 9.4%; $C_{16:1}\omega9$, 7.4%; $C_{17:1}\omega9$, 7.1%; $C_{18:1}\omega7$, 6.9%; $C_{16:0}$ aldehyde, 6.8%; $C_{16:0}$ DMA, 6.5%; $C_{18:1}\omega9$ DMA, 6.4%; $C_{18:0}$ aldehyde, 4.4%. The following compounds were detected at about 2-3% as minor components; $C_{14:0}$, $C_{16:1}\omega7$, $C_{18:1}\omega9$, $C_{18:0}$ and $C_{18:0}$ DMA. The total of monounsaturated components of fatty acids and DMAs accounted for more than 60% of all the composition. Branched-chain fatty acids were not detected. Peptidoglycan of cell wall of strain WN036^T contained lysine as the diamino acid, and glutamic acid, glycine, alanine and aspartic acid were also detected. Respiratory quinones were not detected.

Analysis of the almost-complete 16S rRNA gene sequence (1472 bp) of strain WN036^T assigned the strain to the phylum *Firmicutes*. The strain had at least two copies of the 16S rRNA gene with different sequences (A or T) at position of 1128 (corresponding to the numbering of *Escherichia coli* sequence). Thus the sequence similarities of the strain with related species were calculated by using "A" for the position as the representative sequence. The most closely related species of strain WN036^T were members of the genus *Peptoniphilus* (Ezaki *et al.*, 2001) in the *Peptostreptococaceae* of cluster XIII consisting of GPAC in the

Clostridiales in the class *Clostridia* (Collins *et al.*, 1994). The closest relatives were *Peptoniphilus asaccharolyticus* ATCC 14963^T with 16S rRNA gene sequence similarity of 89.8% and *Peptoniphilus indolicus* ATCC 29427^T (89.6%). *Peptoniphilus ivorii* DSM 10022^T, *Peptoniphilus harei* DSM 10020^T and *Peptoniphilus lacrimalis* CCUG 31350^T were the next closely related species at similar levels of similarities (87.8%, 87.6% and 87.3%, respectively) (Ezaki *et al.*, 2001). Sequence similarities of strain WN036^T with other members of the GPAC group are still lower (85.4% for *Parvimonas micra* as the highest similarity) (Tindall & Euzéby, 2006) (Fig. 2).

Some characteristics of strain WN036^T and type species of each genus affiliated with the GPAC group are compared in Table 1. All closely related species of strain WN036^T in the genus *Peptoniphilus* shown above have common features as the same genus with those of the type species, *P. asaccharolyticus*. All *Peptoniphilus* species were isolated from samples relating to human infections (Ezaki *et al.*, 2001; Song *et al.*, 2007) and most of known species in the GPAC group were also derived from human clinical specimens (Murdoch, 1998; Song *et al.*, 2003). Since our novel bacterium was isolated from a methanogenic reactor treating waste from cattle farms, it is possible that the strain was derived from gastrointestinal tract of the animals. However, isolation of a novel member of GPAC from a sample other than human or animal specimens is of interest. Furthermore, all genera in the GPAC group shown in Table 1 were formerly classified as *Peptostreptococcus* species and they have been defined as non-spore-forming GPAC (Ezaki *et al.*, 1983, 2001; Finegold *et al.*, 2002; Holdeman Moore *et al.*, 1986; Murdoch & Shah, 1999; Murdoch *et al.*, 2000; Schiefer-Ullrich, 1985). Although we could not observe spores in the cultures of strain WN036^T by microscopy because of the low proportion (less than 0.1%) of thermotolerant cells, we confirmed formation

of thermotolerant cells of the strain by repetition of the heat treatment experiments. Demonstration of survival after heating for 10 min at 70°C or 80°C is generally considered to indicate the presence of spores in the cultures (Cato *et al.*, 1986). The formation of thermotolerant cells or spores of the strain does not fit the definition of the known GPAC species.

Strain WN036^T utilized L-glutamate, L-glutamine, L-histidine and L-arginine in PYV medium and produced abundant acetate and butyrate with a small amount of propionate. The strain did not produce H_2, while the most closely related species, *P. asaccharolyticus* and *P. indolicus*, produce abundant amounts of H_2 (Holdeman Moore *et al.*, 1986). Although the concentration was usually low, propionate was always detected in the cultures of strain WN036^T using PY or PYV basal medium. When L-threonine or L-methionine was used as a substrate, propionate formation increased slightly without apparent growth stimulation. These amino acids might weakly support growth and production of propionate of the strain. Most strains of *P. asaccharolyticus* and *P. indolicus* produce ammonia from glutamate as well as threonine and serine (Ezaki *et al.*, 1983).

The DNA G+C content of strain WN036^T was almost the same level of other GPAC members (Table 1). Lysine was detected as the peptidoglycan diamino acid of strain WN036^T, whereas that of the closest relatives, *Peptoniphilus* species, is ornithine. Species in other four genera except the genus *Gallicola* in the GPAC group (*Finegoldia*, *Parvimonas*, *Anaerococcus* and *Peptostreptococcus*) have lysine as the diamino acid, however, the characteristics of these species are different from those of strain WN036^T in the fermentation products (*Finegoldia*, *Parvimonas* and *Peptostreptococcus*) and utilization of sugars

(*Anaerococcus* and *Peptostreptococcus*), respectively (Ezaki *et al.*, 2001; Holdeman Moore *et al.*, 1986; Li *et al*, 1992). The major CFAs of *Peptoniphilus* species reported are $C_{18:1}$ (70-75%) (Ezaki *et al.*, 1983, 2001) or $C_{16:1}$ (about 30%) and $C_{18:0}$ (about 40%) (for *P. asaccharolyticus*; Lambert & Armfield, 1979). Both compositions are considerably different from that of strain WN036^T.

Thus, based on the cellular, physiological, chemotaxonomic, ecological and phylogenetic differences between strain WN036^T and the closely related species, we propose that strain WN036^T should be assigned to a novel genus and species of *Clostridium* cluster XIII as *Anaerosphaera aminiphila* gen. nov., sp. nov.

Description of *Anaerosphaera* gen. nov.

Anaerosphaera (A.na.e.ro.spha'e.ra. Gr. prep. *an* not; Gr. n. *aer* air; *anaero* not living in air; L. fem. n. *sphaera* a sphere; N.L. fem. n. *Anaerosphaera* sphere not living in air).

Strictly anaerobic, Gram-positive, non-motile cocci. Produces thermotolerant cells or spores. Chem-organotrophs. Does not utilize carbohydrates. Utilizes peptone or amino acids and produces acetate and butyrate as the major fermentation products. The cell wall peptidoglycan contains lysine as the diamino acid. The type species is *Anaerosphaera aminiphila.*

Description of *Anaerosphaera aminiphila* gen. nov., sp. nov.

Anaerosphaera aminiphila (a.mi.ni.phi'la. N.L. n. *aminum* amine; Gr. adj. *philos* loving; N.L. fem. adj. *aminiphila* amino acid-loving).

Has the following characteristics in addition to those given for the genus. Spherical cells are 0.7-0.9 μm in

diameter and often occurring in pairs, but not in chains. Oxidase and catalase activities are negative. Growth temperature range is 10-37°C; optimum is 35-37°C. Growth pH range is 5.8-7.8; optimum pH is 6.7. NaCl concentration range for growth is 0-4% (w/v); optimum is without addition of NaCl to PY medium. Grows in PY medium without additional substrates and produces acetate, butyrate and a small amount of propionate. Does not utilize carbohydrates and organic acids (arabinose, ribose, xylose, fructose, galactose, glucose, mannose, rhamnose, sorbose, cellobiose, lactose, maltose, melibiose, sucrose, trehalose, melezitose, raffinose, glycogen, cellulose, CMC, inulin, dextrin, starch, xylan, pectin, dulcitol, glycerol, inositol, mannitol, sorbitol, amygdalin, aesculin, salicin, fumarate, lactate, malate, pyruvate, succinate and ethanol). Utilizes L-glutamate, L-glutamine and L-histidine, and produces abundant amounts of acetate and butyrate. Also utilizes L-arginine as a growth substrate. Does not produce H_2. Does not utilize other amino acids; L-threonine, L-aspartate, L-asparagine, L-serine, L-alanine, glycine, L-methionine, L-lysine, L-phenylalanine, L-tryptophan, L-valine, L-leucine, L-isoleucine, L-tyrosine, L-proline and L-ornithine. Oxidase and catalase are negative. Hydrolyzes aesculin but not starch and gelatin. Produces hydrogen sulfide but not indole. Does not have nitrate-reducing, lecithinase, lipase and urease activities. Does not change milk and does not grow in chopped meat broth. Sensitive to bile salts. The genomic DNA G+C content is 32.5 mol%. The major cellular fatty acids are $C_{17:1}\omega8$, $C_{18:1}\omega7$ DMA and $C_{16:0}$. Monounsaturated components of fatty acids and DMAs constitute more than 60% of the total composition of CFAs. Glutamic acid, glycine, alanine and aspartic acid are present in the cell wall in addition to lysine as the diamino acid in the peptidoglycan. Does not have respiratory quinones. The type strain, WN036^T (=JCM 15094^T =DSM 21120^T), was isolated from rice-straw residue in a methanogenic reactor treating waste from cattle farms in Japan.

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FIGURE LEGENDS

Fig. 1. Phase-contrast photomicrograph of cells of strain WN036^T grown anaerobically on agar slant of PY4S medium. Bar, 5 μm.

Fig. 2. Neighbor-joining tree showing the phylogenetic relationship of strain WN036^T with the related species based on 16S rRNA gene sequences. Bootstrap values (expressed as percentages of 1000 replications) above 70% are shown at branch nodes. Bar, 2% estimated difference in nucleotide sequence position. The sequence of *Peptostreptococcus anaerobius* ATCC 27337^T was used as the outgroup.

Fig. 1

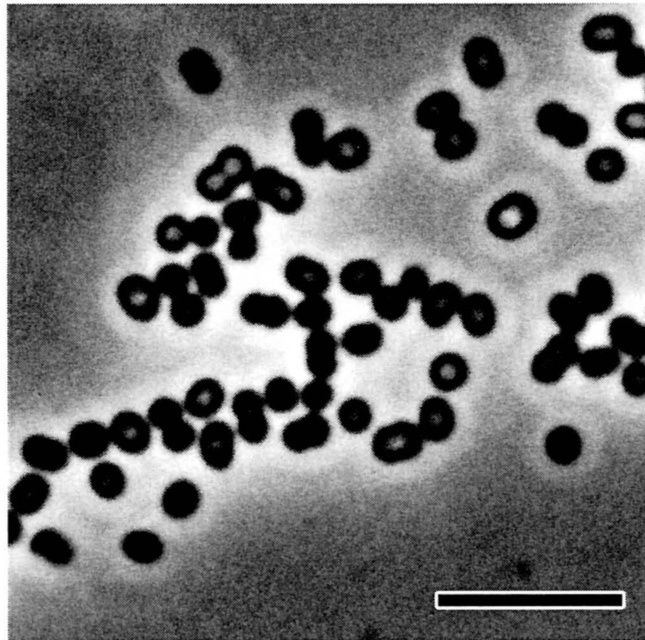

Fig. 2

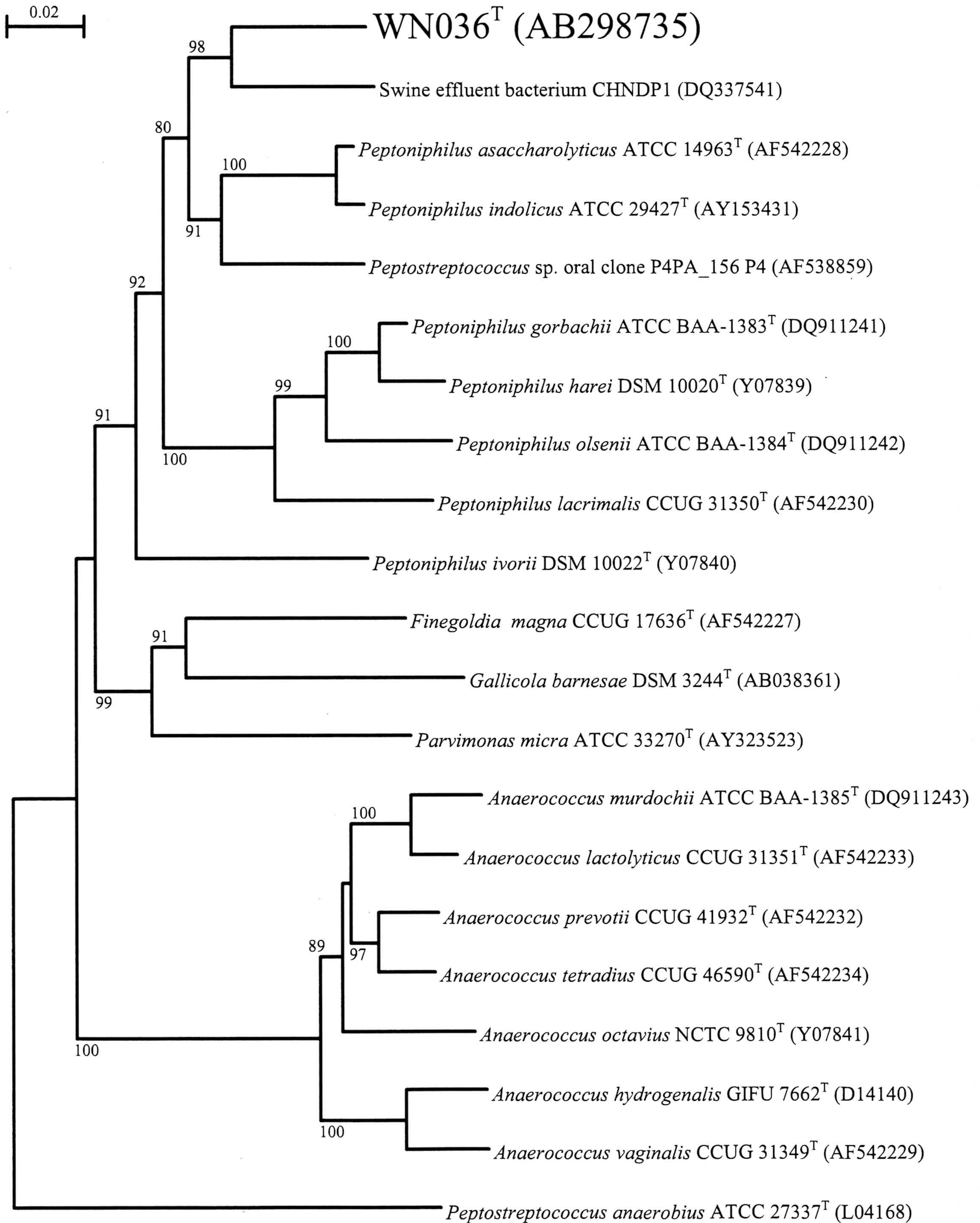

Table 1. Characteristics that differentiate strain WN036^{T} from members in the genera of the strictly anaerobic, Gram-positive cocci

4, *Gallicola barnesae* (data from Ezaki *et al*., 2001; Schiefer-Ullrich & Andreesen, 1985); 5, *Parvimonas micra* (data from Murdoch & Shah, 1999; Holdeman Moore *et al*., 1986);

6, *Anaerococcus prevotii* (data from Ezaki *et al*., 2001; Holdeman Moore *et al*., 1986); 7, *Peptostreptococcus anaerobius* (data from Murdoch *et al*., 2000; Holdeman Moore *et al*., 1986).

+, Positive; ^{W}+, weakly positive; -, negative.

Characteristics	1	2	3	4	5	6	7
Habitat	Methanogenic reactor	Clinical specimens	Human (intestine, skin, clinical specimens)	Chicken faeces	Human (mouth, intestine)	Human (vagina, purulent secretion)	Clinical specimens
Spore or thermotolerant cell	+	-	-	-	-	-	-
DNA G + C content (mol%)	32.5	31-32	32-34	32-34	28-30	29-33	34-36
Peptidoglycan	Lys	Orn	Lys	Orn	Lys	Lys	Lys
Utilization of peptone	+	+	+	^{W}+	+	+	^{W}+
Utilization of sugars	-	-	-	-	-	^{W}+	+
End products of fermentation	A, B, p	A, B, p, H_2	A, l, s, H_2^{*}	A, f	A, s	B, L, a, p, s	a, b, ib, iv, ic, H_2

A/a, Acetate; B/b, butyrate; f, formate; p, propionate; ib, isobutyrate; iv, isovalerate; ic, isocaproate; L/l, lactate; s, succinate. Lower-case letters and H_2^{*} indicate minor products.