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

Atsuko Ueki,1 Kunihiro Abe,1 Daisuke Suzuki,1 Nobuo Kaku,1 Kazuya Watanabe2 and Katsuji Ueki1

Correspondence
Atsuko Ueki
uatsuko@tds1.tr.yamagata-u.ac.jp

1Faculty of Agriculture, Yamagata University, Wakaba-machi 1-23, Tsuruoka, Yamagata 997-8555, Japan
2Exploratory Research for Advanced Technology (ERATO), JST, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8656, Japan

A strictly anaerobic, mesophilic and aminolytic strain (WN036T) was isolated from a methanogenic reactor treating waste from cattle farms. Cells were Gram-positive cocci, often occurred in pairs and were 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 novel 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 C17:1ω8, C18:1ω7 DMA and C16:0. The diagnostic diamino acid of the cell-wall peptidoglycan was lysine. Glutamic acid, glycine, alanine and aspartic acid were also detected in the cell-wall peptidoglycan. On the basis of 16S rRNA gene sequences, the most closely related species to strain WN036T were Peptoniphilus asaccharolyticus ATCC 14965T (89.8 %) and Peptoniphilus indolicus ATCC 29427T (89.6 %). Based on the differences in the phenotypic and phylogenetic characteristics of strain WN036T compared with those of closely related species, a novel genus and species, Anaerosphaera aminiphila gen. nov., sp. nov., is proposed. The type strain is WN036T (=JCM 15094T =DSM 21120T).

In anaerobic conditions, protein is hydrolysed by proteolytic microbes. The amino acids generated are then fermented by amino-olytic 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 H2. Furthermore, ammonia produced by deamination of amino acids balances the decrease in pH with the accumulation of VFAs in the system. Thus, degradation of protein and amino acids is one of the key reactions for efficient methanogenesis in the anaerobic treatment of waste containing organic matter. However, the ecology and physiology of the anaerobic bacteria involved in the degradation of proteinaceous compounds in anaerobic waste treatment has not been studied in much detail. Members of Gram-positive anaerobic cocci (GPAC) are major members of the 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 of the GPAC group are non-saccharolytic and utilize peptone for growth to produce mainly acetate or butyrate (Holdeman Moore et al., 1986; Ezaki et al., 2001). Almost all species in the GPAC group have been isolated from samples relating to human infections or animals. In this study, we describe a novel bacterium belonging to the GPAC group that was isolated from a methanogenic reactor treating cattle waste. The bacterium degraded several types of amino acid, including L-glutamate, and produced acetate and butyrate as major products.
Strain WN036$^T$ was isolated from a sample of rice-straw residue obtained from a methanogenic reactor used to treat waste collected from cattle farms (comprising up to 1000 cattle in total) in Betsukai-machi, Hokkaido, Japan. The reactor was a vertical cylindrical type (1500 m$^3$) operated at mesophilic temperatures. Rice-straw utilized for matting at the cattle farms was treated in the reactor together with the faeces and urine of the animals (Ueki et al., 2008; Nishiyama et al., 2009).

The strain was cultivated anaerobically at 30 °C unless otherwise stated by using peptone/yeast extract (PY) medium as the 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 (1 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 was used for the maintenance of the novel strain in agar slants. Since the 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 sodium L-glutamate. H$_2$O (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 1 M NaOH.

Strain WN036$^T$ was isolated by the anaerobic roll-tube method for the 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 filtered through a mesh (2 mm pore size) and the relatively large residue of rice-straw that remained on the mesh was collected. The rice-straw samples obtained were washed several times with sterile anoxic diluent and homogenized with a Waring blender (10000 r.p.m., 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 a preliminary examination of the 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 novel strain under aerobic conditions 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 the formation of thermostolerant cells, cultures grown in PYVGlumedium or on agar slants were inoculated into fresh PYVGlumedium, and after exposure to heat (80 °C for 10 min unless otherwise stated) of whole tubes closed with butyl rubber stoppers, the tubes were incubated at 30 °C to check for growth. Thermostolerant cells in the culture were enumerated by the most probable number (MPN) method. Cultures grown to the early stationary phase in PYVGlumedium were diluted anaerobically 10-fold to extinction and inoculated into triplicate tubes containing fresh PYVGlumedium at each dilution. Each tube was incubated after heat treatment to check for growth as described above and the MPN counts of thermostolerant cells were determined according to a statistical MPN table based on the number of tubes that were positive 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 a concentration of 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 the measurement of fermentation products. Fermentation products were analysed by GC as described previously (Ueki et al., 1986; Akasaka et al., 2003a). Other characterizations were performed according to the methods described by Holdeman et al. (1977), Ueki et al. (2008) and Nishiyama et al. (2009).

Whole-cell fatty acids (CFAs) were converted to methyl esters according to the method of Miller (1982) and analysed 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 (Moore et al., 1994). Isopenoid quinones were extracted and purified as described by Komagata & Suzuki (1987). Purification of the cell wall was conducted according to the method described by Schleifer & Kandler (1972). The purified cell wall was hydrolysed with 4 N HCl for 16 h at 100 °C and the composition of the amino acids was analysed by TLC (Cellulose TLC; Merck) as described by Harper & Davis (1979). Genomic DNA extracted using the method 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 (L-7400; Hitachi) equipped with a μBondapak C18 column (3.9 × 300 mm; Waters).

DNA was extracted from cells as described previously (Akasaka et al., 2003b). An almost full-length 16S rRNA gene was amplified by PCR using the primer set 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 from 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 WN036T were spherical; most of the 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 WN036T 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 was not observed by microscopy (Fig. 1), cells cultivated in PYVGlu liquid medium and treated at 80°C for 10 min grew in fresh medium after a relatively long lag period (3–4 days). Cultures treated at 85–90°C for 10 min still survived the treatment in most cases. Thus, we concluded that the strain produces thermostolerant cells or spores. When the number of thermostolerant cells present in cultures grown in PYVGlu liquid medium was enumerated by the MPN method, such cells accounted for 0.045–0.1% (about 0.9–2 × 10^6 cells ml^-1) of the total cells (2 × 10^6 cells ml^-1). Inoculation of one loop of cells from PY4S slant cultures to 10 ml PYVGlu liquid medium was usually not sufficient to guarantee growth after heat treatment. An increase in the number of cells used for inoculation resulted in growth after heat treatment.

The novel strain grew in PY or PY medium without additional substrates (final OD_{660} nm 0.9–1.0; final pH 7.1). The following substrates, including carbohydrates and organic acids, were not utilized: arabinose, ribose, xylose, fructose, galactose, glucose, mannose, rhamnose, sorbose, cellubiose, 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. A limited range of amino acids, sodium L-glutamate. H2O, L-glutamine, L-histidine and L-arginine HCl, supported rapid growth of the strain in PY medium. In the presence of these amino acids, the final OD_{660} values ranged from 1.8 to 2.3 and the final pH was around pH 7.0, except for with L-arginine (pH 7.9). Other amino acids did not enhance growth of the novel strain in PYV medium (L-threonine, sodium L-aspartate. H2O, L-asparagine. H2O, 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). Hydrogen 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 acetate and 15.3 mmol l^-1 butyrate, as well as 2.5 mmol l^-1 propionate. Fermentation products with L-arginine were not determined. Although apparent growth enhancement of the novel strain was not observed, the addition of L-threonine or L-methionine to PYV medium resulted in a slight increase in propionate production (4.0–4.5 mmol l^-1).

Neither catalase nor oxidase activities were detected. The novel strain did not reduce nitrate. Aesculin was hydrolysed, but starch and gelatin were not. Hydrogen sulphide 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 novel strain was sensitive to bile salts.

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

The G+C content of the genomic DNA of the novel strain was 32.5 mol%. Various compounds including aldehydes and dimethylacetals (DMAs) were detected by the analysis of the cellular fatty acids (CFAs) of strain WN036T. Compounds detected at relatively higher amounts were as

![Fig. 1. Phase-contrast photomicrograph of cells of strain WN036T grown anaerobically on an agar slant of PY4S medium.](http://ijs.sgmjournals.org)
follows: C17:0(9), 14.3 %; C18:1o7 DMA, 12.2 %; C16:0,
9.4 %; C16:1o9, 7.4 %; C17:1o9, 7.1 %; C18:1o8, 6.9 %;
C16:0 aldehyde, 6.8 %; C16:0 DMA, 6.5 %; C18:1o9 DMA,
6.4 %, and C18:0 aldehyde, 4.4 %. The following com-
ounds were detected at about 2–3 % as minor compo-
ents: C14:0, C16:1o7, C18:1o9, C18:0, and C18:0 DMA.
Monounsaturated fatty acid components and DMAs
accounted for > 60 % of the total CFA content.

Analysis of the almost-complete 16S rRNA gene sequence
(1472 bp) of strain WN036T led to the assignment of
the novel strain to the phylum *Firmicutes*. The novel strain had
at least two copies of the 16S rRNA gene with different
sequences (A or T) at position 1128 (numbering according
to the *Escherichia coli* gene sequence). Thus the sequence
similarities of the novel strain to related species were
calculated using the ‘A’ at this position as the represent-
ative sequence. The most closely related species to strain
WN036T were members of the genus *Peptoniphilus* (Ezaki
et al., 2001) in the family Peptostreptococcaceae of cluster
XIII consisting of GPAC in the order Clostridiales in the
class *Clostridia* (Collins et al., 1994). The closest relatives
were *Peptoniphilus asaccharolyticus* ATCC 14963T with a
16S rRNA gene sequence similarity of 89.8 % and
*Peptoniphilus indolicus* ATCC 29427T (89.6 %). The next
most closely related species were *Peptoniphilus ivorii* DSM
10022T, *Peptoniphilus harei* DSM 10020T and *Peptoniphilus
lacrimalis* CCUG 31350T with gene sequence similarity
levels of 87.8 %, 87.6 % and 87.3 %, respectively (Ezaki
et al., 2001). The sequence similarities of strain WN036T
with other members of the GPAC group were lower
(85.4 % for *Parvimonas micra* ATCC 33270T, the species from
this group with the highest gene sequence similarity) (Tindall & Euzeby, 2006) (Fig. 2).

Some characteristics of strain WN036T and the type species
of each genus affiliated with the GPAC group are compared
in Table 1. All species in the genus *Peptoniphilus*, as shown
above, that were closely related to strain WN036T had
features that were common to the type species of the genus,
*Peptoniphilus asaccharolyticus*. All species of the genus
*Peptoniphilus* have been isolated from samples relating to
human infections (Ezaki et al., 2001; Song et al., 2007) and
most of the recognized species in the GPAC group have
also been derived from human clinical specimens
(Murdoch, 1998; Song et al., 2003). Since the novel strain
was isolated from a methanogenic reactor treating waste
from cattle farms, it is possible that the strain was derived
from the gastrointestinal tract of the animals. However,
the isolation of a new member of the GPAC group from a
sample other than a human or animal specimen is of
interest. Furthermore, all of the genera in the GPAC group
shown in Table 1 were formerly classified as species of the
genus *Peptostreptococcus* and they have been defined as
non-spore-forming GPAC (Ezaki et al., 1983, 2001;
Schiefer-Ullrich & Andreessen, 1985; Holdeman Moore
et al., 1986; Murdoch & Shah, 1999; Murdoch et al., 2000;
Finegold et al., 2002). Although spores were not observed
in cultures of strain WN036T by microscopy in this study
due to the low proportion (<0.1 %) of thermostolerant
cells, the formation of thermostolerant cells was confirmed
by the repetition of the heat treatment experiments.

Strain WN036T 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 novel strain did not produce H2, while the
most closely related species, *Peptoniphilus asaccharolyticus*
and *Peptoniphilus indolicus*, produce abundant amounts of
H2 (Holdeman Moore et al., 1986). Although the
concentration was usually low, propionate was always
detected in cultures of strain WN036T using PY or PYV
basal medium. When L-threonine or L-methionine were
used as a substrate, propionate formation increased slightly
without apparent growth stimulation. These amino acids
might weakly support growth and the production of
propionate by the strain. Most strains of *Peptoniphilus
asaccharolyticus* and *Peptoniphilus indolicus* produce
ammonia from glutamate as well as threonine and serine
(Ezaki et al., 1983).

The DNA G+C content of strain WN036T was almost at
the same level as that of other GPAC members (Table 1).
Lysine was detected as the diagnostic diamino acid of the
cell-wall peptidoglycan of strain WN036T, whereas the
diagnostic diamino acid of its closest relatives, species of
the genus *Peptoniphilus*, is ornithine. Species in four other
genera of the GPAC group, but not the genus *Gallicola*,
have lysine as the diagnostic diamino acid (Finegoldia, *Parvimonas*, *Anaerococcus* and *Peptostreptococcus*).
However, the characteristics of these species are different
from those of strain WN036T as regards the fermentation
products (Finegoldia, *Parvimonas* and *Peptostreptococcus*)
and in the utilization of sugars (*Anaerococcus* and *Peptostreptococcus*), respectively (Holdeman Moore et al.,
1986; Li et al., 1992; Ezaki et al., 2001). The major CFAs of
species of the genus *Peptoniphilus* reported so far are C18:1
(70–75 %) (Ezaki et al., 1983, 2001) or C16:1 (about 30 %)
and C18:0 (about 40 %) (for *Peptoniphilus asaccharolyticus*;
Lambert & Armfield, 1979). Both of these compositions are
considerably different from that of strain WN036T.

Thus, based on the cellular, physiological, chemotaxo-
nomic, ecological and phylogenetic differences between
strain WN036T and the closely related species, we propose
that strain WN036T 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’era. Gr. prep. an not; Gr. n. aer air; anaero not living in air; L. fem. n. *sphaera* a sphere; N.L. fem. n. *Anaerosphaera* a sphere not living in air).

Strictly anaerobic, Gram-positive, non-motile cocci. Produce thermotolerant cells or spores. Chem-organo-trophs. Do not utilize carbohydrates. Utilize peptone or amino acids and produce acetate and butyrate as the major fermentation products. The cell-wall peptidoglycan contains lysine as the diagnostic diamino acid. The type species is *Anaerosphaera aminiphila*.

**Description of Anaerosphaera aminiphila 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 occur 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 growth occurs with no 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 or 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, maltate, pyruvate, succinate or 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 H₂. Does not utilize other amino acids such as 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...
Table 1. Characteristics that differentiate strain WN036<sup>T</sup> from the type species of the genera of the strictly anaerobic, Gram-positive cocci

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Habitat</td>
<td>Methanogenic reactor</td>
<td>Clinical specimens</td>
<td>Human (intestine, skin, clinical specimens)</td>
<td>Chicken faeces</td>
<td>Human (mouth, intestine)</td>
<td>Human (vagina, purulent secretion)</td>
<td>Clinical specimens</td>
</tr>
<tr>
<td>Spore or thermotolerant cell</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>32.5</td>
<td>31–32</td>
<td>32–34</td>
<td>32–34</td>
<td>28–30</td>
<td>29–33</td>
<td>34–36</td>
</tr>
<tr>
<td>Peptidoglycan</td>
<td>Lys</td>
<td>Orn</td>
<td>Lys</td>
<td>Orn</td>
<td>Lys</td>
<td>Lys</td>
<td>Lys</td>
</tr>
<tr>
<td>Utilization of:</td>
<td>Peptide</td>
<td>Orn</td>
<td>Orn</td>
<td>Orn</td>
<td>Orn</td>
<td>Orn</td>
<td>Orn</td>
</tr>
<tr>
<td>Sugars</td>
<td>Glucose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>End products of fermentation</td>
<td>A, B, p, H&lt;sub&gt;2&lt;/sub&gt;</td>
<td>A, l, s, (H&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>A, f</td>
<td>A, s</td>
<td>B, L, a, p, s</td>
<td>a, b, ib, iv, ic, H&lt;sub&gt;2&lt;/sub&gt;</td>
<td></td>
</tr>
</tbody>
</table>

L-ornithine. Oxidase and catalase activities are negative. Hydrolyses aesculin but not starch or gelatin. Produces hydrogen sulfide but not indole. Does not have nitrate-reducing, lecinthenase, lipase or urease activities. Does not change milk and does not grow in chopped meat broth. Sensitive to bile salts. The major cellular fatty acids are C<sub>17</sub>:<sub>1</sub>ω9<sup>C</sup>, C<sub>18</sub>:<sub>1</sub>ω7<sup>C</sup> DMA and C<sub>16</sub>:0. Monounsaturated components of fatty acids and DMAs constitute >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 diagnostic diamino acid in the peptidoglycan. Does not possess respiratory quinones.

The type strain, WN036<sup>T</sup> (=JCM 15094<sup>T</sup>=DSM 21120<sup>T</sup>), was isolated from rice-straw residue in a methanogenic reactor treating waste from cattle farms in Japan. The genomic DNA G+C content of the type strain is 32.5 mol%.

Acknowledgements

This work was partly supported by a Grant-in-Aid from the Institute for Fermentation, Osaka, and also by the Project for Development of Technology for Analysing and Controlling the Mechanism of Biodegrading and Processing supported by the New Energy and Industrial Technology Development Organization (NEDO). We are grateful to Y. Ohtaki for the technical assistance and Dr T. Hoaki of Taiei Corporation for the sampling of sludge in the methanogenic reactor.

References


Reclassification of


Edited by P. H. A. Sneath. Baltimore: Williams & Wilkins.

H. barnesae sp. nov., a gram-positive, anaerobic, obligately purine utilizing coccus from chicken feces. Arch Microbiol 143, 26–31.


