Aminocella lysinolytica gen. nov., sp. nov., a L-lysine-degrading, strictly anaerobic bacterium in the class *Clostridia* isolated from a methanogenic reactor of cattle farms

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain WN037^T is AB298740.

Abstract A strictly anaerobic bacterial strain (WN037^T) was isolated from a methanogenic reactor. Cells were Gram-positive rods. Strain WN037^T was asaccharolytic. The strain fermented L-lysine in the presence of B-vitamin mixture or vitamin B_{12} and produced acetate and butyrate. L-arginine and casamino acids poorly supported the growth. Strain WN037^T used neither other amino acids nor organic acids examined. The strain had $C_{18:1}$ ω7c, $C_{16:0}$ and $C_{18:1}$ ω7c DMA as the predominant cellular fatty acids. The genomic DNA G + C content was 44.2 mol%. Phylogenetic analysis based on the 16S rRNA gene sequence placed strain WN037^T in the family *Eubacteriaceae* in the class *Clostridia*. The closest relative was *Eubacterium pyruvativorans* (sequence similarity, 92.8%). Based on the comprehensive analyses, the novel genus and species, *Aminocella lysinolytica* gen. nov. sp. nov., was proposed to accommodate the strain. The type strain is WN037^T (= JCM 19863^T = DSM 28287^T).

Key words *Aminocella lysinolytica*, butyrate, *Clostridia*, L-lysine, methanogenesis, vitamin B_{12}

Introduction

Degradation of protein is one of key processes in methanogenesis from waste containing wide variety of organic compounds including municipal sewage sludge and dairy waste. In the process of anaerobic degradation of organic matter, various amino acids are produced by hydrolysis of protein and the amino acids generated are then converted to volatile fatty acids (VFAs) such as acetate, propionate, butyrate and branched fatty acids (isobutyrate and isovalerate) as well as H_2 and CO_2 by diverse anaerobic bacterial populations. Each amino acid is degraded to different kinds of VFAs depending on the combinations of amino acid and bacterial species concerned (Buckel 1999; Ramsay and Pullammanappallil 2001; Russell, 2005; Smith and Macfarlane, 1997). VFAs other than acetate are then oxidized to acetate and H_2 by fatty acid-oxidizing bacterial groups to be used as substrates for methanogenic archaea. Ammonia released by deamination of amino acids should balance the decrease in pH with accumulation of VFAs in methanogenic sludge. Some groups of asaccharolytic, oral anaerobic Gram-positive rods have been reported to ferment amino acids and often predominate in human periodontal pockets (Uematsu and Hoshino 1996; Uematsu et al, 2003) and 'hyper-ammonia-producing', asaccharolytic amino acid-degrading bacteria have been thought to be partly responsible for the inefficiencies in ruminant nutrition to suppress their nitrogen retention (Paster et al, 1993; Wallace et al, 2003). However, ecology or physiology of protein- or amino aids-degrading anaerobic bacteria in the methanogenic process from protein-rich wastewater has not been studied in much detail except some studies (Díaz et al, 2007; Ganesan et al, 2008; Ramsay and Pullammanappallil, 2001; Ueki et al, 2009). In this study, a strictly anaerobic, Gram-positive, L-lysine-degrading bacterium is described. The strain was isolated from a methanogenic reactor treating waste from cattle farms and the strain fermented L-lysine as an almost sole energy and carbon source to produce acetate and butyrate depending on the presence of vitamin B_{12}.

Materials and Methods

Isolation of strain

Strain WN037^T is one of strains isolated from a methanogenic reactor treating waste collected from cattle farms (comprising up to 1,000 cattle in total) in Betsukai-machi, Hokkaido, Japan. The reactor was a vertical cylindrical type (1,500 m^3) operated at mesophilic temperatures. Rice-straw utilized for matting at the cattle farms was treated in the reactor together with feces and urine of the animals. Strain WN037^T was isolated by the anaerobic roll-tube method for isolation of anaerobic fermentative bacteria (Holdeman et al, 1977) from rice-straw residue samples collected from the reactor. The rice-straw samples were washed several times with sterile anoxic diluent and homogenized in a Waring blender (10,000 r.p.m. for 10 min.) under N_2 atmosphere (Satoh et al, 2002). The homogenate was successively diluted (10-fold) anaerobically and inoculated to the anaerobic roll-tube agar. Strain WN037^T was picked from a roll-tube inoculated with a 10^{-4} diluted sample by using PYV4S medium as shown below together with other isolates. The 16S rRNA gene sequences of the strains isolated from the same reactor during the investigation were determined according to the methods described below and deposited in the DDBJ database as the accession numbers AB264621 to AB264630, AB298723 to AB298778 and AB377175 to AB377179 (Abe et al, 2012; Nishiyama et al, 2009; Ueki et al, 2008, 2009). Strain WN037^T was placed in the order *Clostridiales* of the phylum *Firmicutes* with very low sequence similarities to related species. In addition, growth of the strain in general media for anaerobic bacteria was very poor (see below). These results promoted our further comprehensive studies to describe the strain as a novel species with unknown characteristics.

Cultivation and media

Peptone/yeast extract (PY) medium (Holdeman et al., 1977) was used for cultivation of the strain as the basal medium with oxygen-free mixed gas (N_2/CO_2, 95/5) as the headspace. PY medium contained (per liter) 0.3 g L-cysteine-HCl-$2H_2O$ (as a reducing agent) and 1 mg resazurin-Na (as a redox indicator) in addition to peptone (Trypticase)(10 g/l), yeast extract (5 g/l) and salt solutions (Satoh *et al*., 2002). PY medium supplemented with 0.25 g/l each of glucose, cellobiose, maltose and soluble starch as well as agar (Difco) (15 g/l) was designated as PY4S agar (Ueki et al, 2008). Since strain WN037^T essentially required vitamin B_{12} for growth as described below, PY medium was supplemented with the B-vitamin mixture (10 ml/l) (PYV medium) or vitamin B_{12} (PYB_{12} medium) for cultivation of the strain under various conditions unless otherwise stated. The composition of the B-vitamin mixture used was (per 100 ml) 0.1 mg biotin, 0.1 mg cyanocobalamin (vitamin B_{12}), 0.3 mg *p*-aminobenzoic acid, 0.5 mg folic acid, 0.5 mg thiamine-HCl, 0.5 mg riboflavin and 1.5 mg pyridoxine-HCl. PYB_{12} medium contained cyanocobalamin of the same concentration as that in the B-vitamin mixture as a sole vitamin (final concentration, 10 µg/l). Brain-heart infusion broth (BHI) (Eiken Co., Ltd) was modified by adding (per liter) 0.3 g L-cysteine-HCl-$2H_2O$ and 1 mg resazurin-Na as well as oxygen-free N_2 as the headspace. The strain was cultivated anaerobically at 30ºC unless otherwise stated. The pH of media was adjusted to around pH 7.0 (as verified after autoclaving) except for determination of the pH range for growth. For preparing sludge extract as a source of growth factors, sludge fluid obtained from the methanogenic reactor was autoclaved for 30 min. at 121°C. After cooling, the autoclaved fluid was centrifuged twice at 10,000 × *g* for 30 min. and the supernatant obtained was stored at –20°C until use as sludge extract for cultivation of the strain.

Characterization of the strain

Growth under the aerobic condition was examined as described previously (Akasaka et al., 2003). Spore formation was

assessed by observing cells after Gram staining or phase-contrast microscopy, and production of thermotolerant cells was examined by incubating heat-treated (80ºC for 10 min) cells in PYV broth containing L-lysine or on PYV4S agar slants as shown below. The motility of cells was examined using phase-contrast microscopy as well as observing growth in semisolid SIM medium (Holdeman et al., 1977). Utilization of carbon sources was usually tested in PYV liquid medium as basal medium, each substrate being added at (10 g/l) or 30 mM (for organic acids and amino acids). Utilization of each substrate was determined from measurement of growth (OD_{660}) as well as by determining fermentation products in the media after cultivation. When positive reactions were obtained for utilization of substrates, the strain was subcultured at least once at the same conditions. Optimum pH and temperature for growth was determined at pH 4.1, 5.2, 6.1, 6.8, 7.8 and 9.2 (as values verified after autoclaving) and at 10-45ºC at 5ºC intervals. Bicine [*N*,*N*-bis(2-hydroxyethyl)glycine] (Good's buffer; Dotite) (20mM) was used to adjust the pH higher than pH 7.8. Effect of the NaCl concentration on growth was determined in the presence of 0, 5, 10, 15, 20, 30, 40 4 and 50 g/l NaCl. Production of H_2S or indole was examined by using SIM medium. Nitrate-reducing activity was determined in PYV liquid medium according to the method described by Satoh et al., (2002). Characterization of strain WN037^T (such as production of catalase, oxidase, lipase and lecithinase, hydrolysis of esculin and starch, and digestion of milk and meat) was performed according to the methods described by Holdeman et al. (1977). Fermentation products were analyzed by gas-chromatography (GC) as described previously (Akasaka et al., 2003). All cultivation and experiments were carried out in duplicate and almost the same results were obtained for all examinations.

Chemotaxonomic analyses

Cell biomass for following chemotaxonomic analyses was obtained by cultivating strain WN037^T to the early stationary phase (about 48 h of cultivation) in PYV-lysine medium. Whole-cell fatty acids (CFAs) were converted to

methyl esters (Miller, 1982) and analyzed by GC (Hp6890; Hewlett-Packard or G-3000; Hitachi) equipped with a HP Ultra 2 column at TechnoSuruga Co., Ltd (Shimidu, Japan) based on the MIDI microbial identification system (Microbial ID) of MOORE (Moore et al., 1994) using equivalent chain-length (ECL) values for identification of peaks of GC analysis. The presence of diaminopimelic acid (DAP) isomers as the diagnostic component in the cell wall peptidoglycan was determined by TLC as described previously (Komagata and Suzuki, 1987; Akasaka et al, 2003). The presence of quinones (menaquinones and ubiquinones) was analyzed by TLC after extraction from cell biomass as described by Komagata and Suzuki (1987).

Phylogenetic analysis

Genomic DNA extracted from cell biomass was digested with P1 nuclease by using a Yamasa GC kit (Yamasa Shouyu) and its G + C content was measured by HPLC (HITACHI L-7400) equipped with a µBondapak C18 column (3.9 × 300 mm; Waters). Almost full-length of 16S rRNA gene was PCR-amplified using the primer set of 8f 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 references in GenBank were performed with the BLAST program (Altschul et al., 1997). All gaps and unidentified base positions in the alignments were excluded before sequence assembly. A phylogenetic tree was constructed by using the neighbor-joining method (Saitou and Nei, 1987) in the CLUSTAL W program (Thompson et al., 1994) and the maximum-likelihood method (DNAML) in the PHYLIP 3.66 package (Felsenstein, 2006). Sequence similarities between two strains were determined by pairwise comparisons.

Results and discussion

Cell morphology and colony

Growth of strain WN037^T in PY liquid medium, as the standard basal medium for cultivation of anaerobic fermentative bacteria, was scanty irrespective of the presence of various substrates including glucose. After examinations to find factors to support the growth, addition of B-vitamin mixture to PY medium (PYV medium) was confirmed to improve the growth, while other factors (such as vitamin K and hemin) did not affect the growth (Ueki et al., 2008). Strain WN037^T made translucent, thin and small colonies (about 0.5 mm after 3-4 days of cultivation) with smooth surface on PYV4S agar. Cells of strain WN037^T stained Gram-positive and most of the cells were straight to slightly curved rods (0.6-0.8 μm in width and 2.0-6.3 μm long) (Fig. 1). The strain did not grow in air. Spores were not observed, and heat-tolerant cells were not detected. Motility was not observed.

Physiological characteristics and substrate spectra

Strain WN037^T produced small amounts of acetate (3.5 mmol/l) and butyrate (5.7 mmol/l) after 3 days of cultivation in PYV liquid medium. Addition of glucose to PYV medium (PYVG) did not improve the growth as compared with that in PYV medium, and the concentrations of products were almost the same as those in PYV medium (Table 1). Strain WN037^T did not utilize any other carbohydrates and organic acids examined (arabinose, ribose, xylose, fructose, galactose, mannose, rhamnose, cellobiose, lactose, maltose, melibiose, saccharose, treharose, melezitose, raffinose, cellulose, pectin, soluble starch, xylan, inositol, mannitol, sorbitol, amygdalin, esculin, salicin, fumarate, lactate, malate, pyruvate and succinate) as growth substrates in PYV liquid medium.

Out of the amino acids examined, strain WN037^T grew actively in the presence of L-lysine (PYV-lysine medium) and produced acetate (24.3 mmol/l) and butyrate (39.4 mmol/l) together with a small amount of H_2 (0.8 mmol/l) and CO_2 (14.4 mmol/l). Cyanocobalamin (vitamin B_{12}) instead of the B-vitamin mixture supported the growth with

L-lysine almost similarly. The addition of sludge extract (50 ml/l) showed the same effect on growth of the strain as that in PYV-lysine medium. BHI containing L-lysine did not support the growth of the strain. Final pH in the medium containing L-lysine was usually pH 5.6-5.7. L-Arginine weakly supported the growth. Products were acetate (5.3 mmol/l) and butyrate (8.7 mmol/l) in PYV medium containing L-arginine. Final pH was higher (pH 7.9) than that with L-lysine, indicating active production of ammonia (Table 1). Other amino acids (L-alanine, L-aspartate, L-asparagine, L-citrulline, L-glutamate, L-glutamine, glycine, L-histidine L-isoleucine, L-leucine, L-methionine, L-ornithine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophane and L-valine) did not support the growth. Aminobutyrate was not utilized. The strain weakly used casamino acids and produced acetate (5.5 mmol/l) and butyrate (8.0 mmol/l) (Table 1).

The strain was negative for the production of catalase and oxidase activities. Neither starch nor esculin were hydrolyzed. The strain did not produce H_2S and indole in SIM medium. Nitrate was not reduced. Other characteristics determined will be presented in the species description.

Growth conditions

The optimum growth conditions were determined by using PYV-lysine medium. The pH range for growth was narrow (pH 5.2-7.8), with an optimum at around 6.5-7.0. The temperature range for growth was 15-35ºC, with the highest growth rate at 30ºC. The concentration range of NaCl for growth was 0-20 g/l. NaCl of 10 g/l did not significantly suppress the growth as compared with that without NaCl addition. The specific growth rate (μ) at the optimum condition (pH 7.0, 30ºC, without NaCl) was 0.157 h^{-1}.

Chemotaxonomic characteristics

The even-numbered components such as $C_{18:1}$ ω7c (42.0%), $C_{16:0}$ (17.7%), $C_{18:1}$ ω7c dimethylacetal (DMA) (14.6%), $C_{14:0}$ (9.6%) and $C_{16:1}$ ω7c (6.3%) were detected as the predominant compounds in the analysis of cellular fatty acids composition of strain WN037^T. Other compounds including $C_{16:1}$ ω5c (1.7%), $C_{16:0}$ DMA (1.3%) and $C_{17:2}$ (3.6%) were detected as minor components. Strain WN037^T has *meso*-DAP as a diagnostic amino acid of the cell wall components. The genomic DNA G + C content was 44.2 mol%. Neither menaquinones nor ubiquinones were detected.

Phylogenetic affiliation

Almost a full length of 16S rRNA gene sequence (1465 bp) of the strain was determined (the GenBank/EMBL/DDBJ accession number, AB298740). Species in the family *Eubacteriaceae* in the order *Clostridiales* of the phylum *Firmicutes* were placed as the most closely related species (Ludwig et al, 2009; Wade, 2009). The closest relatives were *Eubacterium pyruvativorans* ATCC BAA-574^T (AJ310135) (sequence similarity, 92.8%; sequence length compared, 1284 bp) (Wallace et al, 2003) and *Eubacterium minutum* (= *Eubacterium tardum*) ATCC 700079^T (AJ005636) (91.4%, 1432 bp) (Poco et al. 1996; Wade et al, 1999) and *Eubacterium nodatum* ATCC 33099^T (Z36274) (91.1%, 1344 bp) (Holdeman et al, 1980). The sequence similarity with *Eubacterium limosum* ATCC 8486^T (M59120) (the type species of the genus *Eubacterium*) (Wade, 2009) was 86.2% (1352 bp). Figure 2 shows the phylogenetic tree constructed by using the neighbor-joining method. When evaluated by using the maximum-likelihood method, the tree topology was almost the same as that shown in Fig. 2 (data not shown).

Conclusions

L-Lysine is fermented to acetate and butyrate by some species in the class *Clostridia*. In the pathway, L-lysine (2,6-diaminohexanoate) is converted to 3,6-diaminohexanoate by a vitamin B_{12}-independent intramolecular migration

of the amino group at the first step. In the following step, a vitamin B_{12}-dependent intramolecular migration of the amino group occurs to produce 3,5-diaminohexanoate. At the final step, 1 mol ATP is generated from 1 mol L-lysine by the reactions of phosphate acetyl transferase and acetate kinase accompanying with production of acetate and butyrate (Buckel 1999). The properties of strain WN037^T to degrade L-lysine depending on vitamin B_{12} and to produce acetate and butyrate are consistent with the pathway of clostridial species shown above. However, essential requirement of exogenous vitamin B_{12} for L-lysine-degradation of anaerobic bacteria has not been reported so far.

The two closely related species of strain WN037^T, *E. minutum* and *E. nodatum*, are oral human species isolated from human periodontal pockets (Table 2). These species are asaccharolytic. Although lysine-utilization was not described and hydrolysis of arginine was denied in the original description of *E. minutum* (Poco et al. 1996), the species was later reported to ferment both L-lysine and L-arginine to acetate and butyrate (Uematsu et al. 2003). *E. nodatum* was originally described to produce ammonia from peptone and probably from arginine (Holdeman et al. 1980), and later confirmed to degrade lysine (products: acetate, butyrate, ammonia) and arginine (products: butyrate and ammonia) (Uematsu and Hoshino, 1996). Other oral species, *Eubacterium infirmum, Eubacterium sulci* and *Eubacterium saphenum*, were also reported to utilize lysine and produce acetate, butyrate and ammonia (Uematsu et al. 2003). The utilization of lysine of these species was examined by using a nutrient-rich medium (BHI medium) as the basal medium and specific growth requirements of these species for growth were not described. Extract of sludge obtained from the methanogenic reactor supported the growth of strain WN037^T at 50 ml/l addition, suggesting that L-lysine should be degraded smoothly depending on vitamin B_{12} sufficiently present in the methanogenic sludge. Growth of the population relating to strain WN037^T depending on both L-lysine and vitamin B_{12} is very interesting to understand the ecology of protein-degrading anaerobic bacteria in methanogenesis from waste.

E. pyruvativorans, though remotely related, the phylogenetically closest species of strain WN037^T, did not ferment

carbohydrates, but grew using pyruvate or pancreatic casein hydrolysate (Trypticase). The species was designated as 'ammonia-hyperproducing' group of ruminal bacteria, since the species produced ammonia from Trypticase at a high rate. But utilization of each amino acid was not examined in detail (Wallace et al. 2003) (Table 2).

It has been suggested that large members of validly published species within the genus *Eubacterium* have historically been misclassified. Recent investigation suggested that the genus *Eubacterium* (*Eubacterium sensu stricto*) should be restricted to the type species *E. limosum* and its close relatives such as *Eubacterium aggregans, Eubacterium barkeri* and *Eubacterium callanderi* and novel genera could be created for the majority of the *Eubacterium* species on the basis of their phylogeny and phenotypes (Ludwig et al, 2009; Wade, 2009). Although the amino-acid degrading properties of strain WN037^T are common to the related *Eubacterium* species as shown above, their sequence similarities are too low to assign strain WN037^T to the same genus as these species. Thus, strain WN037^T should be accommodated to a novel genus other than the genus *Eubacterium*. Based on the comprehensive analyses, the novel genus and species, *Aminocella lysinolytica* gen. nov**.,** sp. nov. is proposed.

Description of *Aminocella* gen. nov.

Aminocella (a.mi.no.ce'lla. M.L. n. *aminum* amine; L. fem. n. *cella* a cell; N.L. fem. n. *Aminocella* amino acid-utilizing cell.

Cells are Gram-staining positive, non-motile rods. Non-spore-forming. Strictly anaerobic. Chemoorganotroph. Mesophilic. Catalase and oxidase are negative. Does not utilize carbohydrates and organic acids. Has $C_{18:1}$ ω7c as the major cellular fatty acid. Neither menaquinones nor ubiquinones are present. Has *meso*-diaminopimelic acid as a diagnostic amino acid of the cell wall components. On the basis of 16S rRNA gene sequence, the bacterium belongs to the class *Clostridia* of the phylum *Firmicutes.* The type species is *Aminocella lysinolytica*.

Description of *Aminocella lysinolytica* sp. nov.

Aminocella lysinolytica (ly.si.no.ly'ti.ca. N.L. n. *lysinum* lysine, an amino acid; Gr. adj. *lyticus* dissolving; N.L. fem. adj. *lysinolytica* lysine-dissolving)

Has the following characteristics in addition to those described for the genus.

Cells are straight to slightly curved rods (0.6-0.8 μm x 1.9-6.3 μm). Colonies on PY4S slants containing B-vitamin mixture are translucent, thin and small (0.4-0.5 mm). Grows in the medium containing L-lysine as a sole energy and carbon source in the presence of B-vitamin mixture or vitamin B_{12} (cyanocobalamin). Produces acetate and butyrate from L-lysine. L-Arginine and casamino acids poorly support the growth. The strain does not use carbohydrates (arabinose, ribose, xylose, fructose, galactose, mannose, rhamnose, cellobiose, lactose, maltose, melibiose, saccharose, treharose, melezitose, raffinose, cellulose, pectin, soluble starch, xylan, inositol, mannitol, sorbitol, amygdalin, esculin and salicin), organic acids (fumarate, lactate, malate, pyruvate, succinate and aminobutyrate) and amino acids other than L-lysine and L-arginine (L-alanine, L-aspartate, L-asparagine, L-citrulline, L-glutamate, L-glutamine, glycine, L-histidine, L-isoleucine, L-leucine, L-methionine, L-ornithine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophane and L-valine). Growth range of pH is 5.2-7.8; the optimum at 6.5-7.0. The temperature range for growth is 15-35ºC, the optimum at 30ºC. The NaCl concentration range for growth is 0-2.0 % (w/v). Does not have catalase, oxidase, lipase and lecithinase activities. Does not hydrolyze starch and esculin. Does not produce H_2S and indole in SIM medium. Does not reduce nitrate. Does not change milk and does not grow in cooked-meat medium. Has $C_{18:1}$ ω7c, $C_{16:0}$, $C_{18:1}$ ω7c DMA, $C_{14:0}$ and $C_{16:1}$ ω7c as the predominant cellular fatty acids. The genomic DNA G + C content is 44.2 mol%. The type strain is WN037^T (= JCM 19863^T = DSM 28287^T).

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Conflict of interest The authors declare that they have no conflict of interest.

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Figure captions

Fig. 1 Phase-contrast photomicrograph of cells of strain WN037^{T} on agar slant of PYV4S medium. *Bar* 5 μm

Fig. 2 Neighbor-joining tree showing the phylogenetic relationship of strain WN037^{T} with the related species in the family *Eubacteriaceae* based on the multiple alignments of 16S rRNA gene sequences of 1218 bp. Bootstrap values (expressed as percentages of 1000 replications) above 70% are shown at branch nodes. The sequence of *Escherichia coli* ATCC 11775^{T} (in the class *Gammaproteobacteria*) was used as the outgroup. The tree topology evaluated by using

the maximum-likelihood method was almost the same as that obtained with the neighbor-joining method (data not shown). Bar, 2% estimated difference in nucleotide sequence position

Fig. 1

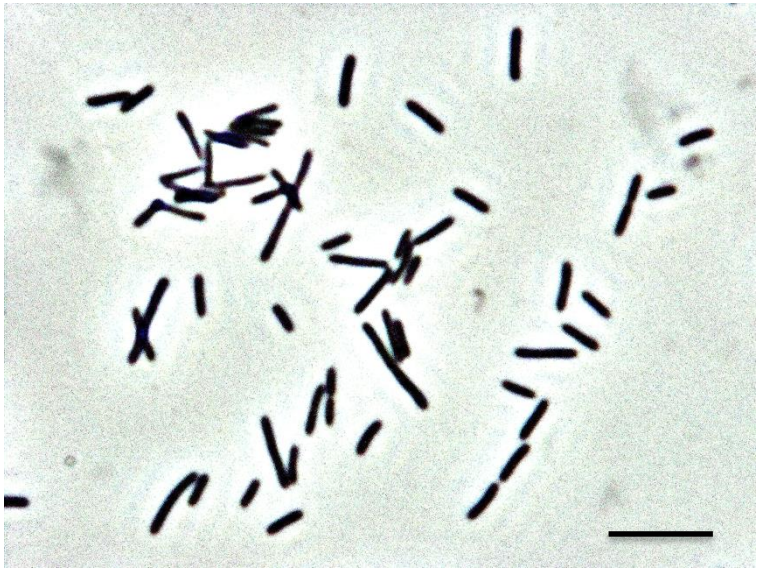

Fig. 2

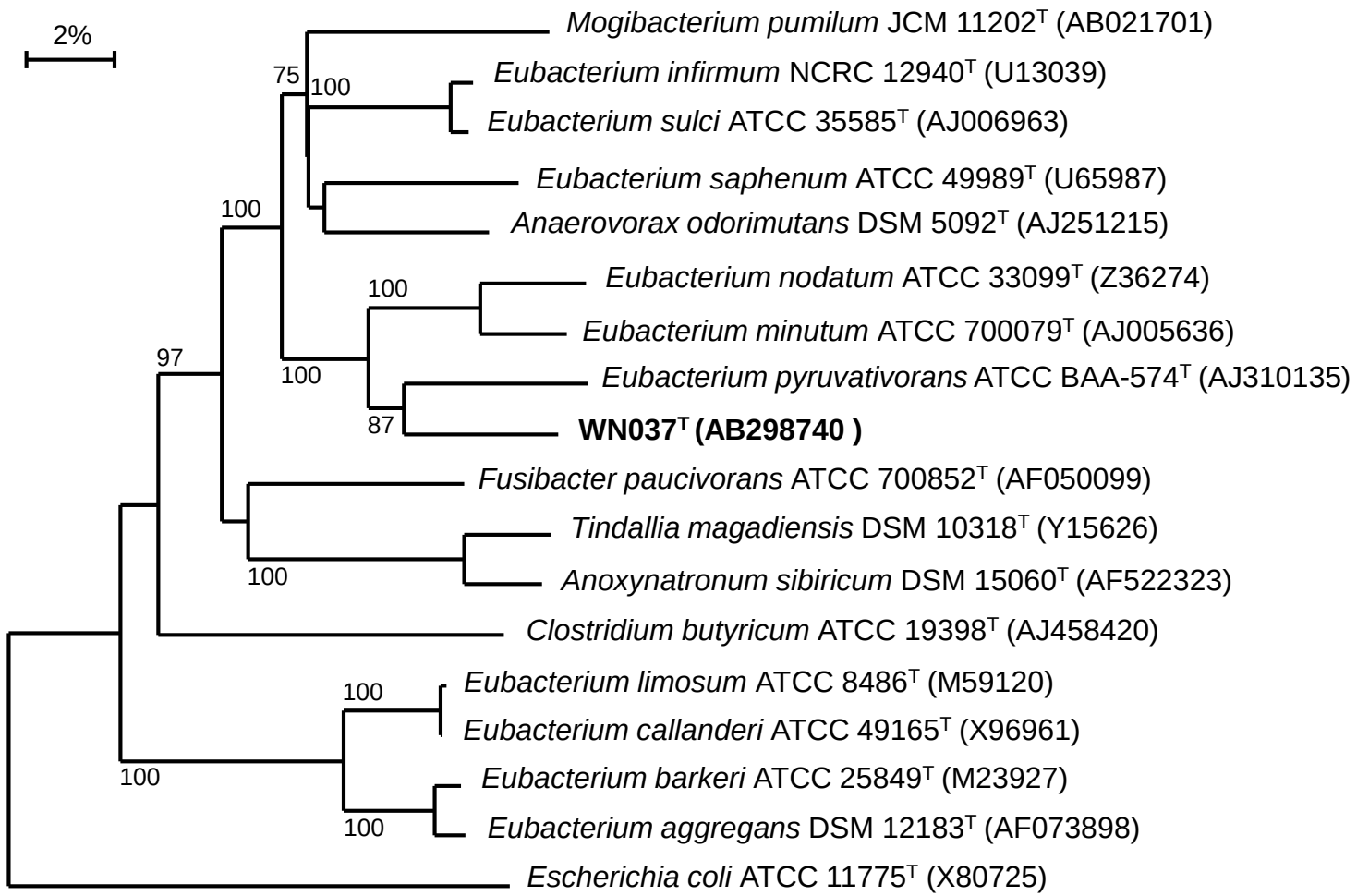
2%
Mogibacterium pumilum JCM 11202T (AB021701)
Eubacterium infirmum NCRC 12940T (U13039)
Eubacterium sulci ATCC 35585T (AJ006963)
Eubacterium saphenum ATCC 49989T (U65987)
Anaerovorax odorimutans DSM 5092T (AJ251215)
Eubacterium nodatum ATCC 33099T (Z36274)
Eubacterium minutum ATCC 700079T (AJ005636)
Eubacterium pyruvativorans ATCC BAA-574T (AJ310135)
WN037T (AB298740)
Fusibacter paucivorans ATCC 700852T (AF050099)
Tindallia magadiensis DSM 10318T (Y15626)
Anoxynatronum sibiricum DSM 15060T (AF522323)
Clostridium butyricum ATCC 19398T (AJ458420)
Eubacterium limosum ATCC 8486T (M59120)
Eubacterium callanderi ATCC 49165T (X96961)
Eubacterium barkeri ATCC 25849T (M23927)
Eubacterium aggregans DSM 12183T (AF073898)
Escherichia coli ATCC 11775T (X80725)
75
100
100
100
97
100
87
100
100
100
100

Table 1 Fermentation products of strain WN037^T in various conditions of growth m

Medium	Substrate	Maximum OD_{660}	Final pH	Product (mmol/l)	
				Acetate	Butyrate
PY	No addition	0.245	6.7	3.1	3.9
	L-Lysine	0.301	6.5	4.7	7.1
PY + vitamin mixture	No addition	0.478	6.7	3.5	5.7
	Glucose	0.444	6.5	3.4	5.2
	L-Lysine	2.388	5.7	24.3	39.4
	L-Arginine	1.256	7.9	5.3	8.7
	Casamino acids	0.806	6.6	5.5	8.0
PY + vitamin B_{12}	L-Lysine	1.742	5.6	25.6	35.8
PY + sludge extract	No addition	0.310	6.6	3.7	5.8
	L-Lysine	1.372	5.5	20.2	31.4

L-Lysine and L-arginine, 30 mM; glucose and Casamino acids,10 g/l

Table 2 Chracteristics of strain WN037^{T}and phylogenetically related species

Characteristic	Strain WN037^T	*Eubacterium pyruvativorans*	*Eubacterium minutum*	*Eubacterium nodatum*
Isolation source	Methanogenic reactor	Sheep ruminal fluid	Human periodontal pockets	Subgingival samples of periodontal disease
Gram staining	+	+	+	+
Cell shape	Straight to slightly curved rods	Straight or coccal rods	Short rods	Branched, somewhat filamentous rods
Cell size (μm)	0.6-0.8 x 1.9-6.3	0.3-0.5 x 1.0-1.5	0.5 x 1.0-1.5	0.5-0.9 x 2.0-12.0
Motility	-	-	-	-
Spore formation	-	-	-	-
Substrate utilization				
Carbohydrates	-	-	-	-
Amino acids	+ (Lysine, arginine)	w+	+ (Lysine, arginine)	+ (Lysine, arginine)
Pyruvate	-	+	*n.r.*	-
Products	Acetate, butyrate	Caproate, valerate	Acetate, butyrate	Acetate, butyrate
DNA G + C content (mol%)	44.2	56.8	38-40	36-38

Symbols and abbreviation: +, positive; w+, weekly positive; -, negative; *n.r.* , not reported

References: *E. pyruvativorans* , Wallace et al. 2003; *E. minutum* , Poco et al. 1996; Uematsu et al. 2003; *E. nodatum* , Holdeman et a
Hoshino 1996; *E. limosum,* Wade 2009