Anaerocella delicata gen. nov., sp. nov., a strictly anaerobic bacterium in the phylum *Bacteroidetes* isolated from a methanogenic reactor of cattle farms

Full paper

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Names of authors, institutions and addresses:

Kunihiro Abe[1], Atsuko Ueki[1], Yoshimi Ohtaki[1], Nobuo Kaku[1], Kazuya Watanabe[2] and Katsuji Ueki[1]

[1]Faculty of Agriculture, Yamagata University, Wakaba-machi 1-23, Tsuruoka 997-8555, Japan

[2]Exploratory Research for Advanced Technology (ERATO), JST, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8656, Japan

Correspondent footnote: Atsuko Ueki. Mailing address: Faculty of Agriculture, Yamagata University, Wakaba-machi 1-23, Tsuruoka 997-8555, Japan

Phone: 0235-28-2866. Fax: 0235-28-2846.

E-mail: uatsuko@tds1.tr.yamagata-u.ac.jp

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Summary

A strictly anaerobic bacterial strain (WN081^T) was isolated from rice-straw residue in a methanogenic reactor treating waste from cattle farms in Japan. Cells were Gram-staining negative, non-motile, non-spore-forming straight rods. The strain grew rather well on PY agar slants supplemented with a B-vitamin mixture as well as sugars (PYV4S medium) and made translucent and glossy colonies. Growth in liquid medium with the same composition, however, was scanty, and growth was not improved in spite of various additives to the medium. Strain WN081^T produced small amounts of acetate, propionate, isobutyrate, butyrate, isovalerate and H_2 from PYV liquid medium. The strain did not use carbohydrates and organic acids. The pH range for growth was narrow (pH 6.8-8.2), having a pH optimum at 6.8-7.5. The temperature range for growth was 10-37^{o}C, the optimum being 25-30^{o}C. The strain was sensitive to bile, and did not have catalase and oxidase activities. Hydrogen sulfide was produced from L-cysteine and L-methionine as well as peptone. Indole was produced from L-tryptophan and peptone. The strain had iso-$C_{15:0}$ as the exclusively predominant cellular fatty acid (70%) together with some branched chain components (such as iso-$C_{15:0}$ DMA, iso-$C_{17:0}$ 3-OH and iso-$C_{15:0}$ aldehyde) as minor components. The genomic DNA G + C content was 32.3 mol%. Phylogenetic analysis based on the 16S rRNA gene sequence placed strain WN081^T in the phylum *Bacteroidetes* with rather low sequence similarities with the related species such as *Rikenella microfusus* (85.7% sequence similarity), *Alistipes putredinis* (85.5%) and *Alistipes finegoldii* (85.5%) in the family *Rikenellaceae*. Based on the phylogenetic, physiological and chemotaxonomic analyses, the novel genus and species *Anaerocella delicata* gen. nov**.,** sp. nov. is proposed to accommodate the strain. The type strain is WN081^T (= JCM 17049^T = DSM 23595^T).

Key words: *Alistipes, Anaerocella delicata*, *Bacteroidetes*, methanogenic reactor, *Rikenellaceae*

Introduction

The phylum *Bacteroidetes* is currently divided into four classes (Ludwig et al., 2008), which contains both strictly anaerobic (mainly in the order *"Bacteroidales"* in the class *"Bacteroidia"*) and aerobic (in the classes *Flavobacteria*, *"Sphingobacteria"* and *"Cytophagia"*) members. Most of strictly anaerobic species in the four families (*Bacteroidaceae*, *"Rikenellaceae"*, *"Porphyromonadaceae"* and *"Prevotellaceae"*) except *"Marinilabiliaceae"* (Suzuki et al., 1999) in the order *Bacteroidales* have been isolated from human or animal specimens, and they are the most dominant members in the bacterial community of human faeces (Holdeman et al., 1984; Paster et al., 1994). Only a few species within the four families of anaerobes have been isolated and described from habitats other than human or animal specimens (Nishiyama et al., 2009; Ueki et al. 2006, 2008, 2011; Whitehead et al., 2005). Out of the four families, *"Rikenellaceae"* currently includes two genera (*Rikenella* and *Alistipes*) (Ludwig et al., 2008). The genus *Rikenella* includes only one recognized species (*Rikenella microfusus*) and the genus *Alistipes* includes five species (*Alistipes putredinis, Alistipes finegoldii, Alistipes onderdonkii, Alistipes shahii and Alistipes indistinctus*) (Holdeman et al., 1984; Nagai et al., 2010; Rautio et al., 2003; Song et al., 2006). Members in the two genera have been also isolated mainly from human faeces as well as other human specimens including digestive systems, and none of the species is known from other habitats. In this study, we describe a novel genus and species in the *"Rikenellaceae"* based on the comprehensive characterization of strain WN081^T isolated from a methanogenic reactor of cattle farms in Japan.

Materials and Methods

Isolation of strain. Strain WN081^T is one of strains isolated from a methanogenic reactor treating waste collected from cattle farms (up to 1,000 cattle in total) in Betsukai-machi, Hokkaido, Japan. The reactor was a vertically-cylindrical type (1,500 m^3) operated at 35^{o}C. Rice straw used as matting at the cattle farms was thrown into the reactor together with cattle faeces and urine, and treated as waste. The 16S rRNA gene sequences of the strains isolated from the same reactor during the investigation were deposited in the DDBJ database as the accession numbers AB264621 to AB264630, AB298723 to AB298778 and AB377175 to AB377179.

Strain WN081^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 samples were washed several times with sterile anoxic diluent (Satoh et al., 2002) and homogenized in a Waring blender (10,000 r.p.m. for 10 min.) under N_2 atmosphere. The homogenate was successively diluted (10-fold) anaerobically and inoculated to the anaerobic roll-tube agar (Ueki et al., 2008). Colonies that formed on the agar were picked at random after incubation for two weeks at 30ºC and about 50 isolates were obtained from a homogenized sample. Strain WN081^T was picked from a roll-tube inoculated with a 10^{-4} diluted sample.

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. The strain was cultivated anaerobically at 30°C unless otherwise stated by using peptone/yeast extract (PY) as basal medium with oxygen-free mixed gas (95% N_2/ 5% CO_2) as the headspace. PY medium contained (l^{-1}) 10 g peptone (Trypticase, BBL), 5 g yeast extract, 0.2 g Na_2CO_3, 0.3 g L-cysteine-HCl-2H_2O (as a

reducing agent) and 1 mg resazurin-Na (as a redox indicator) as well as salt solutions (Satoh *et al.*, 2002). PY medium supplemented with (l^{1}) 0.25 g each of glucose, cellobiose, maltose and soluble starch as well as a B-vitamin mixture (10 ml l^{-1}) and 15 g l^{1} agar (Difco) was designated PYV4S agar (Satoh et al., 2002) and used for isolation and maintenance of the strain. The strain was cultivated anaerobically at 30ºC unless otherwise stated. PYV broth (Ueki et al., 2006, 2008) was used as a basal medium for cultivation for various physiological tests unless otherwise stated. The pH of media was adjusted to pH 7.4-7.5 (as verified after autoclaving) except for determination of the pH range for growth.

Characterization of the strain. Growth under aerobic conditions was examined as described previously (Ueki et al., 2006). Spore formation was assessed by observing cells after Gram staining, and production of thermotolerant cells was examined by incubating heat-treated (80ºC for 10 min) cells in PYVG broth or on PYV4S agar slants. The motility of cells was examined using phase-contrast microscopy. Utilization of carbon sources was usually tested in PYV liquid media, each substrate being added at 10 gl^{-1} (for sugars) or 30 mM (for organic acids and amino acids). Utilization of each substrate was determined from growth measurement (OD_{660}) as well as by determining fermentation products in the medium after cultivation. Since growth of the strain in liquid media was usually very poor and the turbidity after cultivation was slight as shown below, slant cultures of PYV4S agar were used for determination of growth ranges in various conditions, that is, on temperatures at 10-45ºC at 5ºC intervals with an exception at 37ºC, in the presence of 0, 0.5, 1, 1.5, 2, 3, 4 and 5% (w/v) NaCl, and at pH 5.9, 6.3, 6.5, 6.8, 7.1, 7.5, 7.8, 8.2 and 8.5 (as values verified after autoclaving). Bicine [*N*,*N*-bis(2-hydroxyethyl)glycine] (Good's buffer; Dotite) (20mM) was used to adjust the pH higher than 8.0 (Ueki *et al.*, 2008). Growth on slant cultures was checked visually. Bile sensitivity was determined by cultivating the strain on PYV4S agar slants supplemented with

0.1-2% (w/v) Oxgall (Difco). Production of H_2S from L-cysteine or L-methionine was examined in slant cultures of 1/10 PYV agar, which contained one-tenth of the concentrations for both Trypticase and yeast extract in PYV medium. In addition, each amino acid (10 mM) and ferric ammonium citrate (0.5 g l^{-1}) were supplemented to the medium. Black precipitation of FeS formed in the agar slants during the cultivation indicated H_2S production as well as decomposition of the amino acids. Production of indole from L-tryptophan was determined by adding the Ehrlich's reagent (Holdeman et al., 1977) to the medium after cultivating the strain in 1/10PYV agar containing L-tryptophan (10 mM). Production of H_2S or indole was also tested by using SIM medium (Holdeman et al., 1977). Nitrate-reducing activity was determined in PYV liquid medium according to the method described by Satoh et al., (2002). Fe(III)-reducing ability was examined by cultivation the strain in PYV agar slants containing triphenyltetrazolium chloride (TTC) (1 g l^{-1}) and ferric ammonium citrate (20 mM) as an Fe(III) source. Occurrence of red colonies on the slants with TTC reduction was considered to show Fe(III)-reduction. Production of catalase, oxidase, urease, lipase and lecithinase as well as hydrolysis of aesculin and gelatin was tested according to the methods described by Holdeman *et al.* (1977) and Ueki et al. (2006). Fermentation products were analyzed by GC or HPLC as described previously (Akasaka et al., 2003; Ueki et al., 1986).

Chemotaxonomic analyses. Cell biomass for chemotaxonomic analyses was collected by cultivating the strain on many slants of PYV4S agar (in 18 x 180 mm rubes). 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. CFAs were identified by equivalent chain-length (ECL) (Miyagawa et al., 1979) according to the protocol of TechnoSuruga Co., Ltd (Shimidu, Japan) based on the MIDI microbial identification system (Microbial ID) of MOORE (Moore et al., 1994). Genomic DNA extracted

was digested with Pi 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) (Suzuki et al., 2009).

Phylogenetic analysis. 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). The 16S rRNA gene was also amplified using a primer set, 27f and 1492r, and sequenced by using a Thermo Sequenase Primer Cycle Sequencing kit (Amersham Biosciences) and a model of 4000L DNA sequencer (Li-COR).

Multiple alignments of the sequences with references in GenBank were performed with the BLAST program (Altschul et al., 1997). A phylogenetic tree was constructed with the neighbor-joining method (Saitou and Nei, 1987) by using the CLUSTAL W program (Thompson et al., 1994) and with the maximum-likelihood program (DnaML) of the PHYLIP 3.66 package (Felsenstein, 2006). All gaps and unidentified base positions in the alignments were excluded before sequence assembly.

Results

Cell morphology and colony

Cells of strain WN081^T were Gram-staining negative. Most of the cells were short rods with slightly rounded ends (0.5-0.6 μm in width and 1.5-2.5 μm in length), however, much elongated cells often occurred (Fig. 1). Strain WN081^T grew poorly on PY4S agar slants, while it made translucent and viscous colonies with smooth surface on PY4S agar supplemented with the B-vitamin mixture (PYV4S). The cultures on the

slants glittered with clear rainbow-color under the light. Cell biomass collected from the slant cultures was fluffy, and it was difficult to get compact pellet by centrifugation of cell suspension. The strain did not grow in air. Spores were not observed, and the strain did not produce heat-tolerant cells. Although the strain showed ordinary growth on agar slants of PYV4S as shown above, growth in PYV liquid medium as a basal medium was scanty in spite of supplementation of growth factors (such as vitamin K, haemin or extract of digester fluid) (Ueki et al., 2008) irrespective of the presence or absence of various substrates including carbohydrates and organic acids as described below. Cultivation of the strain in semi-solid agar (0.2-0.5%, w/v) media did not improve the growth.

Physiological characteristics and substrate spectra

Strain WN081^T produced small amounts of fatty acids such as acetate (2.3 mmol l^{-1}), propionate (1.8 mmol l^{-1}), isobutyrate (1.0 mmol l^{-1}), butyrate (1.9 mmol l^{-1}) and isovalerate (2.3 mmol l^{-1}) together with H_2 (1.4 mmol l^{-1}) after about 7 days of incubation in PYV liquid medium. Addition of glucose to PYV medium (PYVG) did not improve the growth as compared with it in PYV, and the concentrations of products were almost the same as those from PYV medium. The strain did not produce succinate. The strain did not utilize following carbohydrates and organic acids in PYV liquid medium as growth substrates (arabinose, xylose, fructose, glucose, mannose, cellobiose, lactose, maltose, melibiose, cellulose, pectin, soluble starch, xylan, inositol, mannitol, citrate, fumarate, lactate, malate, pyruvate and succinate).

In the presence of L-cysteine (10 mM) added as a substrate, the strain showed a slightly better growth than in PYV medium and produced higher amounts of acetate (5.2 mmol L^{-1}) and butyrate (7.2 mmol L^{-1}) together with other products of similar amounts in PYV medium. Addition of L-serine slightly enhanced the

production of acetate (5.2 mmol L^{-1}). The presence of L-valine and L-leucine also slightly enhanced production of isobutyrate and isovalerate as compared with those in PYV medium, respectively. Other amino acids (L-alanine, L-aspartate, L-glutamate, glycine, L-isoleucine, L-lysine, L-phenylalanine and L-threonine) were not utilized. Addition of amino acids in pairs (L-serine and L-leucine, L-methionine and L-leucine, L-serine and L-methionine, etc.) did not stimulate the growth. The strain did not use casamino acids. Substrate utilization was also examined by using PYV or 1/10 PYV agar slants, however, it was difficult to compare the difference in growth depending on the substrates.

The strain weakly produced H_2S in PYV slants containing L-cysteine or L-methionine. Indole was produced from L-tryptophan in 1/10 PYV agar slants after incubation for about one month. The strain also produced H_2S and indole in SIM medium. The strain was sensitive to bile; the presence of 0.1% (w/v) Oxgall completely suppressed the growth. The strain weakly reduced Fe(III) (ferric ammonium citrate) to Fe (II). Nitrate was not reduced. Weak lecithinase activity was detected. The strain was negative for the production of catalase, oxidase and lipase activities. Gelatin and aesculin were not hydrolyzed.

Growth conditions

The optimum growth conditions were determined by using PYV4S slant cultures. The strain grew in the range of pH 6.3-8.2. The optimum and stable growth was obtained at pH 6.8-7.5, and growth at 6.3, 6.5 and 8.2 was rather weak. Final pH in various media was usually pH 6.5-6.6. Growth temperature range was 10-37°C; grew very weakly at 10 and 37°C. Optimum growth temperature was 25-30°C. The concentration range of NaCl for growth was 0-1.5% (w/v); the presence of 0.5% (w/v) NaCl did not apparently suppress the growth.

Chemotaxonomic characteristics

Strain WN081^T had iso-$C_{15:0}$ as the exclusively predominant cellular fatty acid (CFAs) (70.0%). Other major components of CFAs were iso-$C_{15:0}$ dimethylacetal (DMA) (9.2%), iso-$C_{17:0}$ 3-OH (ECL, 18.159) (9.2%) and iso-$C_{15:0}$ aldehyde (ECL, 13.560) (7.1%). CFAs such as $C_{14:0}$ (1.3%), $C_{16:0}$ (1.7%) and $C_{16:0}$ 3-OH (1.1%) were detected as minor components. The G + C content of genomic DNA was 32.3 mol%.

Phylogenetic affiliation

Almost a full length of 16S rRNA gene sequence (1451 bp) of the strain was determined. Sequence heterogeneities were confirmed at the two regions by repetition of the sequencing procedure, i.e., at No. 187-190 (RWTTWR) (*Escherichia coli* numbering with two gaps for the *E. coli* sequence) and No. 200-205 (WRAWTR). The strain was assigned to the phylum *Bacteroidetes* (Ludwig *et al.*, 2008), however, it was remotely related to any of recognized species. Species in the family *"Rikenellaceae"* were placed as the most closely related species at almost the same similarities (*Rikenella microfusus* ATCC 29728^T, 85.7%; *Alistipes putredinis* JCM 16772^T, 85.5%; *Alistipes finegoldii* JCM 16770^T, 85.5%; *Alistipes shahii* JCM 16773^T, 85.3% *Alistipes onderdonkii* JCM 16771^T, 85.2%). The similarity value between strain WN081^T and *"Cytophaga fermentans"* ATCC 19072^T, which has been proposed to be affiliated to a new genus (Ludwig et al., 2008; Suzuki et al., 1999), was 85.6%. Several species in the family *Sphingobacteriaceae* in the class "*Sphingobacteria"* followed at almost the same similarities (e.g., *Mucilaginibacter orizae* DSM 19975^T, 84.8%; *Sphingobacterium siyangense* KCTC 22131^T, 84.7%). Strain WN081^T formed a deep branch in the phylogenetic tree constructed by using the neighbour-joining method for representative species in the phylum

Bacteroidetes (Fig. 2). The tree topology evaluated by the maximum-likelihood method was essentially the same (data not shown).

Discussion

The closest relatives of strain WN081^T were the members in the genera *Rikenella* or *Alistipes* of strictly anaerobic rods, and strain WN081^T had some common features with these relatives. However, in addition to the large divergence in the 16S rRNA gene sequence, the strain had distinct different phenotypic characteristics from these relatives (Table 1). The most distinct difference of the strain from the relatives is the genomic DNA G + C content. The content of the strain was 32.3 mol%, while those of the members in the genera *Rikenella* and *Alistipes* are much higher (55-61%). The rather low genomic DNA G + C content level of the strain seems to be common with the members in the class *"Flavobacteria"* (Jooste & Hugo, 1999) in the same phylum. The *Rikenella* or *Alistipes* species usually produce succinate as the most dominant products from glucose, while the novel strain did not produce it at al. Although the relatives usually grow in the presence of bile or the growth is stimulated by the presence, the strain did not tolerate bile. The CFA profile of the strain was rather unique having iso-$C_{15:0}$ as an exclusively predominant CFA. Most of the members in the *Bacteroidales* have iso-$C_{15:0}$ as one of major CFAs, however, they usually contain other various CFAs at rather high percentages (Miyagawa et al., 1979; Moore et al., 1994). It is of interest that some other members in the phylum *Bacteroidetes* such as *Capynocytophaga* spp. in the class *"Flavobacteria"* have similar CFA profiles as that of strain WN081^T (Yamamoto et al., 1994; Vandamme et al., 1994).

Strain WN081^T showed various unique phenotypic properties. The strain was difficult to cultivate in liquid media irrespective of various additives, although it showed ordinary growth on agar slants containing B-vitamins. The results suggest that the strain adapts to proliferation on solid surface. The strain did not decompose carbohydrates, while some kinds of amino acid and peptone weakly supported the growth. The strain produced various fatty acids and H_2 as well as H_2S and indole from these substrates. The strain had a weak ability to reduce Fe(III) in the presence of peptone. These versatile properties suggest diverse physiological functions of the bacterium in anaerobic environments. Since the strain was isolated from plant residue in a methanogenic reactor, the bacterium may live in the environments by attaching on some solid surface and take a role in decomposition of substrates derived from decomposition of protein.

The significantly low similarity of 16S rRNA gene sequence with the recognized closest species clearly indicates that strain WN081^T should be affiliated to a novel taxon. Thus, based on the distinct differences of the cellular, physiological and chemotaxonomic characteristics of the novel strains from the closest species, *Anaerocella delicata* gen. nov., sp. nov. is proposed to accommodate the strains. The type strain of the novel species is WN081^T (= JCM 17049^T = DSM 23595^T).

Description of *Anaerocella* gen. nov.

Anaerocella (An.a.e.ro.ce'lla. Gr. pref. *an-*, not; Gr. n. *aer* air; L. fem. n. *cella* a cell; N.L. fem. n. *Anaerocella* an anaerobic cell).

Cells are Gram-staining negative, non-motile, straight rods. Non-spore-forming. Strictly anaerobic. Chemoorganotroph. Mesophilic. Catalase and oxidase are negative. Does not utilize carbohydrates and organic acids. Weakly utilizes peptone and some amino acids, and produces volatile fatty acids. Has

iso-$C_{15:0}$ as the major cellular fatty acid. Genomic DNA G + C content is 32.3 mol%. On the basis of 16S rRNA gene sequence, the bacterium belongs to the phylum *Bacteroidetes*. The genera *Rikenella* and *Alistipes* in the family *"Rikenellaceae"* are related most closely to this genus at about 86% sequence similarities. The type species is *Anaerocella delicata*.

Description of *Anaerocella delicata* sp. nov.

Anaerocella delicata (de.li.ca'ta. L. fem. adj. *delicata* delicate, i.e., growing delicately, referring to the scanty and unstable growth of the type strain)

Has the following characteristics in addition to those described for the genus. Cells are 0.5-0.6 μm in width and 1.5-2.5 μm in length. Elongated cells often occur. Grows well on agar slants containing peptone/yeast extract and B-vitamin mixture, while growth in liquid media is very scanty and unstable. Has a fermentative metabolism. Does not utilize carbohydrates and organic acids (arabinose, xylose, fructose, glucose, mannose, cellobiose, lactose, maltose, melibiose, cellulose, pectin, soluble starch, xylan, inositol, mannitol, citrate, fumarate, lactate, malate or pyruvate) as fermentative substrates. Produces small amounts of acetate, propionate, isobutyrate, butyrate, isovalerate and H_2 in PYV medium. Does not produce succinate. Weakly utilize L-cysteine, L-leucine, L-methionine, L-serine, L-tryptophan and L-valine as well as peptone. Does not utilize L-alanine, L-aspartate, L-glutamate, glycine, L-isoleucine, L-lysine, L-phenylalanine or L-threonine. Utilization of amino acids based on the Stickland reaction is not recognized. Growth temperature range on agar slants is 10-37°C with optimum at 25-30°C. pH range for growth is pH 6.3-8.2; optimum at 6.8-7.5. NaCl concentration range for growth is 0-1.0% (wt/vol). Does not grow in the presence of bile. Does not have urease and lipase activities. Weakly reduces Fe(III) to Fe(II) in the presence of peptone. Produces

hydrogen sulfide from L-cysteine, L-methionine and peptone. Produces indole from L-tryptophan and pepton. Gelatin and aesculin are not hydrolyzed. Nitrate is not reduced. Weak lecithinase activity is present. Has iso-$C_{15:0}$ as the exclusively dominant CFA. CFAs such as iso-$C_{15:0}$ dimethylacetal (DMA) and iso-$C_{17:0}$ 3-OH are present as minor components. The genomic DNA G + C content of the species is the same as that described for the genus. The type strain of the novel species is WN081^T (= JCM 17049^T = DSM 23595^T) isolated from a methanogenic reactor of cattle farms.

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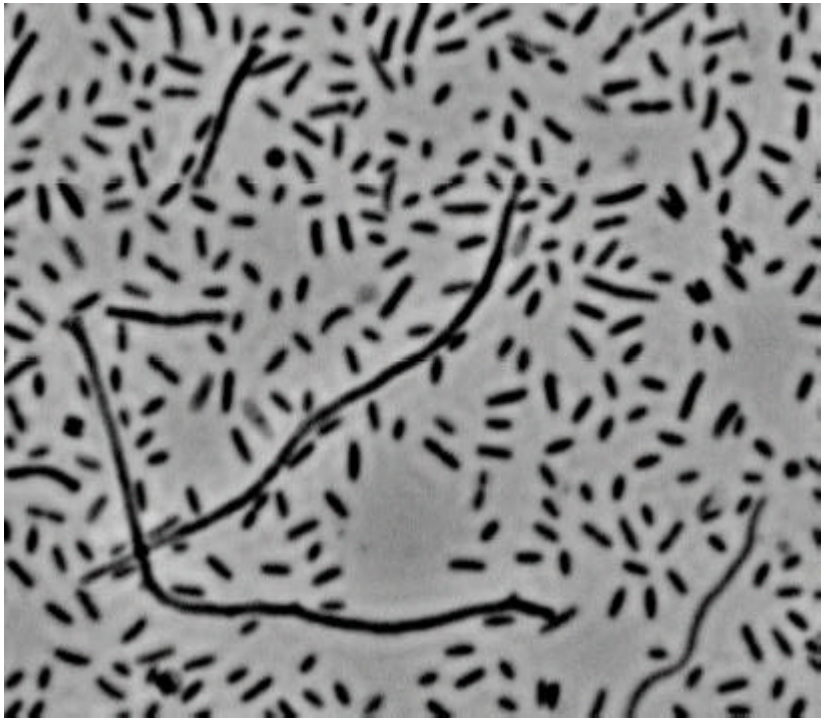
Legends for figures

Fig. 1. Phase-contrast photomicrograph of cells of strain WN081^{T} grown on PYV4S agar. Bar, 10 µm.

Fig. 2. Neighbor-joining tree showing the phylogenetic relationship of strain WN081^{T}, all species in the family *Rikenellaceae* and the type species of other families in the phylum *Bacteroidetes* based on 16S rRNA gene sequences. Bootstrap values (expressed as percentages of 1000 replications) above 70% are shown at branch nodes. The sequence of *Sphingobacterium siyangense* KCTC 22131^{T} was used as the outgroup. The tree topology evaluated by using the maximum-likelihood method was almost the same as that obtained with the neighbour-joining method. Bar, 2% estimated difference in nucleotide sequence position.



10 µm

Fig. 1

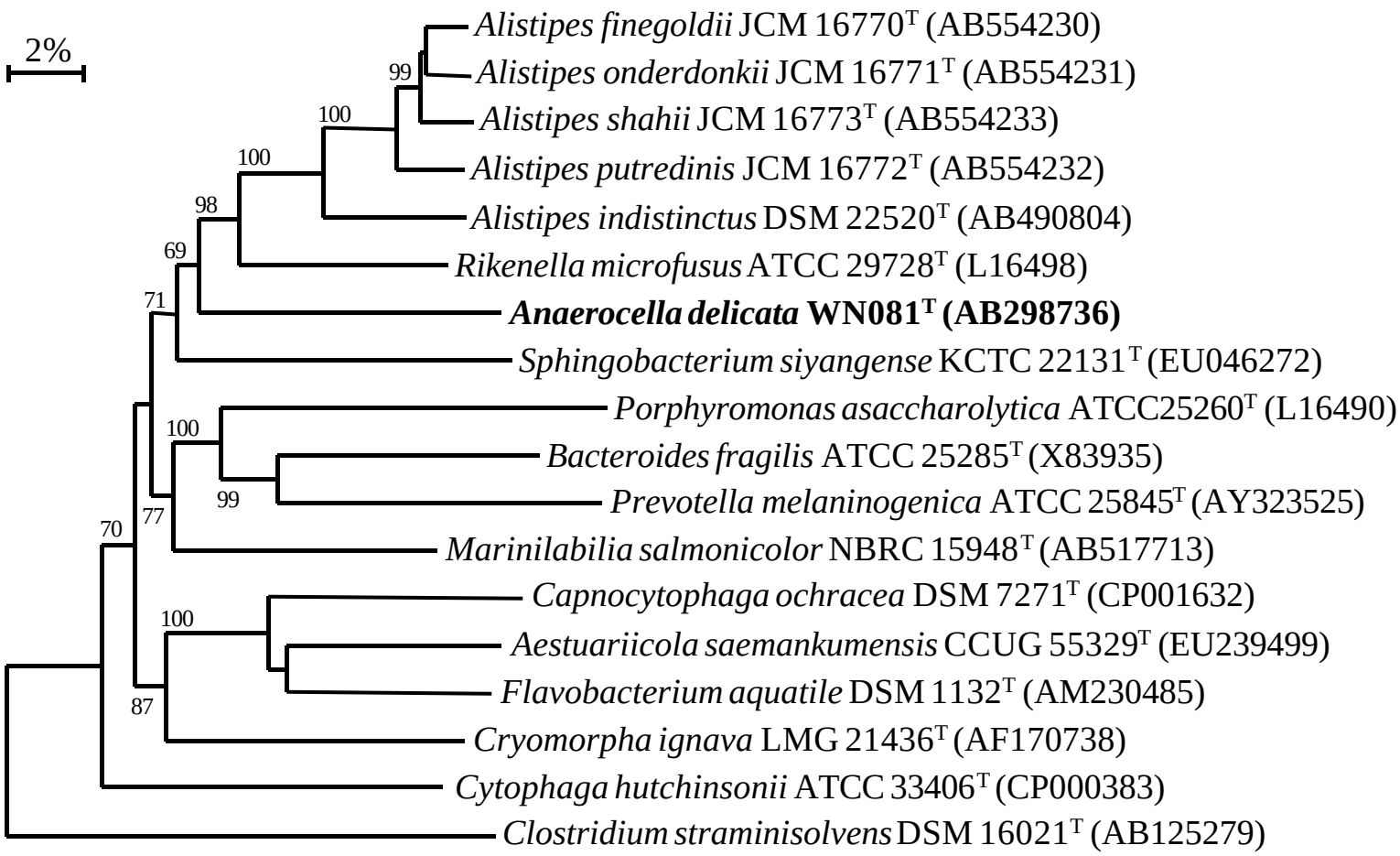

2%
Alistipes finegoldii JCM 16770T (AB554230)
Alistipes onderdonkii JCM 16771T (AB554231)
Alistipes shahii JCM 16773T (AB554233)
Alistipes putredinis JCM 16772T (AB554232)
Alistipes indistinctus DSM 22520T (AB490804)
Rikenella microfusus ATCC 29728T (L16498)
Anaerocella delicata WN081T (AB298736)
Sphingobacterium siyangense KCTC 22131T (EU046272)
Porphyromonas asaccharolytica ATCC25260T (L16490)
Bacteroides fragilis ATCC 25285T (X83935)
Prevotella melaninogenica ATCC 25845T (AY323525)
Marinilabilia salmonicolor NBRC 15948T (AB517713)
Capnocytophaga ochracea DSM 7271T (CP001632)
Aestuariicola saemankumensis CCUG 55329T (EU239499)
Flavobacterium aquatile DSM 1132T (AM230485)
Cryomorpha ignava LMG 21436T (AF170738)
Cytophaga hutchinsonii ATCC 33406T (CP000383)
Clostridium straminisolvens DSM 16021T (AB125279)
99
100
100
98
69
71
100
99
77
70
100
87

Fig. 2

Table 1. Some characteristics that differentiate strain WN081^T from other genera in the family *Rikenelaceae*.

Characteristic	WN081^T	*Rikenella*	*Alistipes*
Habitat	Methanogenic reactor	Faeces	Human faeces, intestine
Cell morphology	Straight rods with slightly rounded ends	Spindle-shaped rods	Slender, straight or slightly curved rods with rounded ends
Pigment production	-	-	+*
Indole production	+	-	+**
Optimum temperature (^oC)	25-30	37	37
Utilization of carbohydrates	-	+w	+
Fermentation products	a, p, ib, b, iv, H_2	A, S, p, H_2	S, a, p, iv
Growth in 20% bile	-	Stimulatory	+*
Major CFA	iso-$C_{15:0}$ (70%)	iso-$C_{15:0}$ (36%)	iso-$C_{15:0}$ (15-52%)
Genomic DNA G+C content (mol%	32.3	60-61	55-58

Data for the genus *Rikenella* are from Holdeman *et al*. (1984), and data for the genus *Alistipes* are from Nagai, *et al*. (2010) and Rautio *et al*. (2003). +, Positive; -, negative; +w, weakly positive. A or a, acetate; p, propionate; ib, isobutyrate; b, butyrate; iv, isovalerate; S, succinate. Lowercase letters indicate minor products. Out of five species in the genus *Alistipes*: *, negative for *A. putredinis*; **, negative for *A. indistinctus*.