Bacteroides propionicifaciens **sp. nov., isolated from rice-straw residue in a methanogenic reactor treating waste from cattle farms**

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ABSTRACT

Strictly anaerobic bacterial strains (SV434[T] and S562) were isolated from rice-straw residue in a methanogenic reactor treating waste from cattle farms in Japan. Both had identical 16S rRNA gene sequences and showed almost the same phenotypic properties. Cells of both strains were Gram-negative, non-motile, non-spore-forming rods. Extraordinarily long rods often occurred. Growth was remarkably stimulated by the addition of haemin and cobalamin (vitamin B_{12}) to the medium. The requirement for cobalamin and haemin could be replaced by autoclaved and clarified sludge fluid obtained from the reactor. Both strains utilized a range of growth substrates including arabinose, fructose, galactose, glucose, mannose,

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cellobiose, maltose, glycogen, starch, dextrin, amygdalin, lactate and pyruvate. Both strains produced acetate and propionate with a small amount of succinate from these substrates in the presence of haemin and cobalamin. Both strains were slightly alkaliphilic with pH optimum at about 7.9. Growth temperature range is 5-35°C with optimum at 30°C. NaCl concentration range for growth was 0-4% (w/v). Catalase activity was not detected in cells cultivated without haemin, while cells cultivated with it usually had the activity. Oxidase and nitrate-reducing activities were not detected. Aesculin was hydrolyzed, but not gelatin. Both strains were sensitive to bile acids. The major cellular fatty acids of both strains were anteiso-$C_{15:0}$ and iso-$C_{15:0}$. Menaquinones MK-8(H_0) and MK-9(H_0) were the major respiratory quinones and the genomic DNA G + C contents were 46.2-47.5%. Phylogenetic analysis based on 16S rRNA gene sequences placed both strains in the phylum *Bacteroidetes*. The most closely related species to both strains was *Bacteroides coprosuis* isolated from swine-manure storage pits with a 16S rRNA gene sequence similarity of 95.9%. Based on the phylogenetic, physiological and chemotaxonomic analyses of the novel strains, *Bacteroides propionicifaciens* sp. nov. is proposed to accommodate the strains. The type strain of the novel species is SV434^T (= JCM 14649^T = DSM 19291^T).

Molecular phylogenetic analyses of microbes in methanogenic reactors based on 16S rRNA gene sequences have revealed that a huge variety of *Bacteria* are present. Of the bacterial groups found, clones belonging to the phyla *Actinobacteria, Firmicutes* and *Bacteroidetes* (Garrity & Holt, 2001) seem to be the most dominant as they have been detected in many different reactors irrespective of waste treated in the reactors (Chouari *et al.*, 2005; Godon *et al.*, 1997; Levén et al., 2007). Amongst these dominant bacterial groups, the phylum *Bacteroidetes* contains species belonging to the *Bacteroides-Prevotella* group as major members, which

mainly consists of species derived from human fecal and oral sources as well as other specimens from mammalian bodies including the rumen (Holdeman *et al.*, 1984; Paster *et al.*, 1994). Bacterial clones affiliated with the group have often been readily detected as dominant components from methanogenic reactors (Chouari *et al.*, 2005; Godon *et al.*, 1997; Levén et al., 2007), however, few bacterial strains affiliated with the group have ever been isolated from there. Thus, exact properties of bacteria related to the *Bacteroides-Prevotella* group detected by the molecular techniques from methanogenic reactors remain to be clarified. In this study, we described a novel species in the genus *Bacteroides* based on phylogenetic, physiological, ecological and chemotaxonomic characteristics of two strains (SV434[T] and S562), which were isolated from a rice-straw residue sample in a methanogenic reactor treating waste from cattle farms. The novel strains were strictly anaerobic, slightly alkaliphilic, Gram-negative rods requiring haemin and cobalamin for growth and producing acetate and propionate from glucose.

Strains SV434[T] (= JCM 14649[T] = DSM 19291[T]) and S562 (= JCM 14650 = DSM 19346) were isolated from a rice-straw residue sample obtained from a methanogenic reactor treating waste collected from cattle farms (up to 1000 cattle as a total) in Betsukai-machi in Hokkaido, Japan. The reactor was a vertically-cylindrical type (1500 m^3) operated at 35°C. Rice-straw used as matting of the cattle farms was thrown into the reactor together with feces and urine of animals and treated as waste. Both strains were 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 in the presence or absence of the B-vitamin mixture (see below). 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

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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 (Kaku et al., 2000; Akasaka et al., 2003a). The homogenized samples were successively diluted anaerobically and used as inocula to the anaerobic roll tube agar for isolation of anaerobic fermentative bacteria (Akasaka *et al.*, 2003a, 2004). Colonies formed on agar during incubation for two weeks were picked at random and about 50 isolates were obtained from a sample. Two strains (SV434[T] and S562) were selected as representatives out of seven strains in the isolates, which were closely related each other based on 16S rRNA gene sequence data and showed similar phenotypic properties (GenBank 16S rRNA gene accession numbers of the isolates other than SV434[T] and S562; AB264621, AB264623, AB264626, AB264628 and AB264629). Strain SV434[T] was picked from the anaerobic roll tube agar inoculated with a 10^{-4} diluted sample using PY4S agar containing B-vitamin mixture (see below) and strain S562 was isolated from a 10^{-5} diluted sample with PY4S agar.

The strains were 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. (2006a). 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 strains in agar slants. PY liquid medium supplemented with haemin (at a final concentration of 5 mg l^{-1}) (Holdeman *et al.*, 1977) (PYH medium) and the B-vitamin mixture (10 ml l^{-1}) (PYHV medium) as well as 10 g glucose l^{-1} (PYHVG medium) was used for cultivation of the strains for various physiological tests and chemotaxonomic analyses of the cells unless otherwise stated (Ueki *et al.*, 2006b). Since both strains were slightly alkaliphilic as described below, the pH value of these liquid media was adjusted to pH 7.6-7.7

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using Bicine (N,N-Bis(2-hydroxyethyl)glycine) Good's buffer (Dotite, Japan) (20 mM). For the medium, KH_2PO_4 was replaced by K_2HPO_4 and Na_2CO_3 was omitted in the PY basal medium by using N_2 100% gas as the atmosphere. The composition of the B-vitamin mixture used was (100 ml^{-1}) 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 hydrochloride, 0.5 mg riboflavin and 1.5 mg pyridoxine hydrochloride (Akasaka *et al.*, 2004). Growth in liquid medium was monitored by changes in OD_{660}.

Growth of the strains under aerobic conditions was examined as described previously (Ueki *et al.*, 2006a). Spore formation was assessed by observation of cells after Gram-staining. Oxidase and nitrate-reducing activities were determined according to methods described by Akasaka *et al.* (2003b). Catalase activity was examined using cells cultivated in liquid media and collected by centrifugation. A small amount of H_2O_2 solution (3%, v/v) was mixed with the cell pellet and production of bubbles of O_2 was checked. Catalase activity was also examined by addition of the H_2O_2 solution to slant cultures of PY4S agar both before and after exposing them to air for more than 30 min (Wilkins *et al.*, 1978). Utilization of carbon sources was tested in PYHV liquid medium (pH 7.6-7.7) with each substrate added at 10 g l^{-1} (for sugars and sugar alcohols) or 30 mM (organic acids). Utilization of each substrate was determined by growth measured by OD_{660} as well as by determining changes to the pH of the medium after cultivation. Bile sensitivity was determined by the addition of bile salts (Oxioid) (0.1-0.5%, w/v) to PYHVG medium. Fermentation products were analyzed by GC or HPLC 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) and Ueki *et al.* (2006a,b).

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 (Shimidu, Japan) (Moore *et al.*, 1994). Isoprenoid quinones were extracted as described by Komagata & Suzuki (1987) and analyzed by using a mass spectrometer (JMS-SX102A; JEOL). 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).

PCR amplification of the V3 region of 16S rRNA gene for denaturing gradient gel electrophoresis (DGGE) was performed using 341f with a GC clamp and 534r as a primer set (Muyzer et al., 1993) using DNA extracted as described previously (Akasaka *et al.*, 2003b). DGGE was carried out using gels containing 10% (v/v) polyacrylamide gels with a urea-formamide gradient (30 to 60%) by using a DCode system (Bio-Rad). A 100% denaturing solution was defined as 7 M urea plus 40% formamide. The gels were run at 200 V for 4 hr at 58°C and stained with SYBER Gold. Almost full-length of 16S rRNA gene was PCR amplified using the primer set of 27f and 1492r or 8F and 1546r. The PCR-amplified 16S rRNA gene was sequenced by using a Thermo Sequenase Primer Cycle Sequencing kit (Amersham Biosciences) and a DNA sequencer (4000L; Li-COR) or 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

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sequences in GenBank were performed with the BLAST program (Altschul *et al.*, 1997). A phylogenetic tree was constructed with the neighbor-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.

Strains SV434[T] and S562 had almost the same properties. Of the characteristics described below, detailed data such as growth rates and amounts of end-products will be described mainly based on the data of strain SV434[T] as a representative strain.

Cells of the strains were Gram-negative rods, 0.7-0.8 μm in width and 1.3-2.1 μm in length with some longer (4-12 μm) cells (Fig. 1a, b). Extraordinarily long rods sometimes occurred depending on the cultures (Fig. 1c). Cells were non-motile as observed phase-contrast microscopy. Both strains grew very thinly as translucent colonies with smooth surface on PY4S agar. Both strains could not grow in air either on PY4S or nutrient agar. Spore formation was not observed.

Both strains grew very slowly in PYG liquid medium (without haemin and the B-vitamin mixture) at a growth rate (μ) of 0.092 h^{-1} and the addition of both haemin and the vitamin mixture or cobalamin to the medium greatly enhanced the growth (μ = 0.38-0.45 h^{-1}). The vitamin mixture and cobalamin showed the same effect on the growth rates of the strains and thus both strains appeared to require cobalamin for growth. Addition of the vitamin mixture or cobalamin alone slightly enhanced the growth, while addition of haemin did not. Further addition of vitamin K to the medium did not apparently affect the growth rate. Thus,

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physiological characteristics of the strains were usually determined in the presence of the vitamin mixture as well as haemin (PYHV medium). Cells for chemotaxonomic characterization were also cultivated in PYHVG medium. Addition (5%, v/v) of autoclaved and clarified sludge fluid, which was obtained from the methanogenic reactor used for isolation of both strains, to PYG medium showed almost the same effect on growth of the two strains as that of haemin and cobalamin, indicating the presence of enough amounts of haemin and cobalamin or alternatives of these compounds in the sludge.

Catalase activity was not detected in cells grown in PY4S agar slants or in PYG liquid medium, however, cells grown in the presence of haemin (PYHG or PHHVG medium) usually had the activity. Oxidase activity was not detected. Both strains utilized arabinose, fructose, galactose, glucose, mannose, cellobiose, maltose, glycogen, soluble starch, dextrin, amygdalin, lactate and pyruvate as growth substrates. Lactose, aesculin, and fumarate were utilized only weakly. Acids were produced from all these substrates. The strains did not use ribose, xylose, rhamnose, sorbose, melibiose, saccharose, trehalose, melezitose, raffinose, carboxymethylcellulose (CMC), cellulose powder, filter paper, xylan, pectin, inulin, salicin, glycerol, dulcitol, inositol, mannitol, sorbitol, ethanol, malate and succinate.

Acetate (2.7 mM), propionate (1.9 mM), lactate (13.9 mM) and a trace amount of succinate (0.4 mM) were produced as fermentation products in PYG medium after two weeks of cultivation, while acetate (9.7 mM) and propionate (15.4 mM) were produced with a trace amount of succinate (0.4 mM) in the presence of haemin and vitamin (PYHVG medium) after 48 hr of cultivation. From lactate and pyruvate, acetate (10.2 and 16.8 mM) and propionate (20.9 and 12.8 mM) were produced, respectively. Aesculin was hydrolyzed,

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but gelatin was not. Urease, hydrogen sulfide and indole were not produced. The strains did not reduce nitrate and could not grow in the presence of 0.1% (w/v) of bile salts.

pH optimum for growth was about 7.9 and both strains did not grow at initial pH lower than 5.7 and higher than 8.9. Growth rates (μ) at pH 7.9 and 6.6 are 0.41-0.45 and 0.21 h^{-1}, respectively. Final pH grown in PYHVG medium (initial pH 7.6) was about pH 5.1. Growth temperature range for growth was 5-35°C with optimum at 30°C ($\mu = 0.38$ h^{-1} at pH 7.6). Both strains grew at 35°C, but did not grow at 37°C. The growth rate at 10°C was relatively high ($\mu = 0.064$ h^{-1}). NaCl concentration range for growth was rather broad (0-4.0%, w/v) and the optimum growth occurred at 1.0% (w/v) NaCl in PYHVG medium.

The major CFAs of strain $SV434^T$ were anteiso-$C_{15:0}$ (41.2%), iso-$C_{17:0}$ (16.1%), anteiso-$C_{17:0}$ (14.0%) and iso-$C_{15:0}$ (6.3%) with lower amounts of $C_{15:0}$ (2.8%), $C_{16:0}$ (2.5%), iso-$C_{16:0}$ (2.3%), iso-$C_{12:0}$ (1.9%), anteiso-$C_{13:0}$ (1.7%) and $C_{17:0}$ (1.6%). Strain S562 had a somewhat different profile; anteiso-$C_{15:0}$ (42.8%), iso-$C_{15:0}$ (9.9%) and iso-3-OH $C_{17:0}$ (8.8%) were the major CFAs with lower amounts of $C_{14:0}$ (2.0%), $C_{15:0}$ (4.0%), $C_{16:0}$ (1.9%), iso-$C_{17:0}$ (2.8%), anteiso-$C_{17:0}$ (2.2%), and various hydoxy fatty acids such as 3-OH $C_{15:0}$ (3.8%), iso-3-OH $C_{15:0}$ (4.7%), anteiso-3-OH $C_{15:0}$ (1.2%), 3-OH $C_{16:0}$ (4.3%) and anteiso-3-OH-$C_{17:0}$ (3.0%). G + C contents of genomic DNA were 46.2% (strain $SV434^T$) and 47.5% (strain S562). The predominant respiratory quinones of both strains were menaquinones MK-8(H_0) and MK-9(H_0). Menaquinone MK-10(H_0) may be present as a trace component.

DGGE analysis of the V3 region of 16S rRNA gene for strains $SV434^T$ and S562 presented the same band

pattern with distinctly separate two bands (more than 7 mm distant each other under the condition shown above), indicating that both strains had at least two different copies in 16S rRNA gene sequences. Sequences of 16S rRNA gene determined for both strains (1428 bp for strain SV434^T and 1387 bp for strain S562) showed that they had the same 16S rRNA gene sequence. The nucleotide position at 467 (corresponding to the numbering of *Escherichia coli* sequence) was determined as "Y", that is, C or T, and the presence of two different sequences in the V3 region of 16S rRNA gene was confirmed.

Both strains were assigned to the phylum *Bacteroidetes* (Garrity & Holt, 2001) based on the 16S rRNA gene sequences determined. The most closely related described species of both strains was *Bacteroides coprosuis* CCUG 50528^T (Whitehead *et al.*, 2005) with a sequence similarity of 95.9% based on the sequence of strain SV434^T ("Y" shown above was replaced by T for calculation of similarity). The next closely related species were *Bacteroides intestinalis* (Bakir *et al.*, 2006) with a sequence similarity of 93.5% and *Bacteroides thetaiotaomicron* (93.2%) (Holdemen *et al.*, 1984). Strains SV434^T and S562 formed a separate branch from those of closely related *Bacteroides* species in the phylogenetic tree composed of related *Bacteroides* species (Fig. 2).

The genus *Bacteroides* consists of species almost exclusively from human fecal or other clinical sources and the rumen (Holdemen et al., 1984; Paster et al., 1994). Of the described *Bacteroides* species, *B. coprosuis* (Whitehead et al., 2005) and some cellulolytic or xylanolytic species including *Bacteroides cellulosolvens* (Murray *et al.*, 1984) and *Bacteroides xylanolyticus* (Scholten-Koerselman *et al.*, 1986) were derived from pig manure or sewage sludge. The latter two species, however, have been recognized to belong to the

phylum *Firmicutes* based on 16S rRNA gene sequences (Schwarz, 2001; GenBank accession numbers; L35517 and DQ497992, respectively). Thus, to our knowledge, *B. coprosuis* isolated from swine-manure storage pits and described recently (Whitehead *et al.*, 2005) may be the sole species validly affiliated with the genus *Bacteroides* derived from habitats other than animal bodies.

The characteristics of strains SV434[T] and S562 are significantly different from those of the closest relatives (Table 1). Optimum growth temperature of strains SV434[T] and S562 is 30°C, while all other three species have the optimum temperature at 37°C. G + C contents of our strains are 46-47%, while that of *B. coprosuis* is 36.4%, although the values of our novel strains are the same level with those of other related two species as well as other major *Bacteroides* species (Holdeman *et al.*, 1984; Shah & Collins, 1983). The composition of predominant respiratory quinones of our novel strains (MK-8 and MK-9) is different from that of *B. thetaiotaomicron* (MK-10 and MK-11), although it is reported that the latter also has MK-8 and MK-9 as minor components (Shah and Collins, 1980). Our strains are sensitive to bile acids, while other species are resistant. Our strains are also different from the three most closely related species in other properties such as indole production and acid production from various saccharides. It is reported that the major CFAs of species in the *Bacteroides-Prevotella* group are anteiso-$C_{15:0}$, iso-$C_{15:0}$, iso-3-OH $C_{17:0}$ and $C_{16:0}$ (Miyagawa *et al.*, 1979; Moore *et al.*, 1994). Although CFAs compositions of strains SV434[T] and S562 were somewhat different each other, the overall patterns representing anteiso-$C_{15:0}$ and iso-$C_{15:0}$ as major components seem to have a common feature with those of *Bacteroides* species reported.

Many species in the genus *Bacteroides* derived from mammalian bodies require haemin for growth

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(Holdeman *et al.*, 1984). Growth of strains SV434[T] and S562 was also strongly stimulated by the addition of haemin to the medium. We recently described three novel species in the phylum *Bacteroidetes* relating to the *Bacteroides-Prevotella* group isolated from plant residue from an irrigated rice field in Japan. Two of the three novel species also required haemin for growth (Ueki, et al., 2006a,b, 2007). These results strongly suggested that major bacterial species relating to the *Bacteroides-Prevotella* group living in natural habitats other than animal bodies might also require haemin for growth. Catalase activity is not usually detected in *Bacteroides* species, while it is known that catalase production in *B. thetaiotaomicron* is variable depending on the strains (Holdemen *et al.*, 1984) and the presence or absence of haemin in the culture medium affects the production of catalase of some species in the *Bacteroides-Prevotella* group (Wilkins *et al.*, 1978). Cells of strains SV434[T] and S562 also showed variable features for catalase production in relation to the presence of haemin in the medium.

Strains SV434[T] and S562 are isolated from a methanogenic reactor. Although both strains have some common features with those of *Bacteroides* species in such as haemin requirement, G + C content of genomic DNA and the CFAs profile, they showed various unique characteristics differentiating them from known *Bacteroides* species. The facts that the optimum growth temperature of the strains is 30°C and they do not grow at 37°C indicate that these strains adapted to the environments other than mammalian bodies, that is, the novel strains are not derived from cattle bodies. Sensitivity to bile acids and slightly alkaliphilic property of the strains seem to also support their ecological specificity differentiating them from *Bacteroides* species in mammalian bodies.

Most of *Bacteroides* species produce acetate and succinate as major products from glucose and species that produce propionate as a major end-product are less common. Strains SV434[T] and S562 require cobalamin in addition to haemin for growth and produce abundant amount of propionate. The methyl-malonyl CoA pathway, a major pathway involved in propionate production, is dependent on cobalamin, and thus it was indicated that our strains produce propionate through the pathway depending on exogenous supply of cobalamin. It is known that exogenous cobalamin is required for the propionate production from succinate in *Bacteroides fragilis* (Holdemen *et al.*, 1984). We recently described two novel species of anaerobic bacteria, which were isolated from plant residue in irrigated rice-field soil and produced propionate depending on exogenous cobalamin (Akasaka et al., 2003b; Ueki et al., 2006b). Cobalamin requirement of bacterial species producing propionate seems to be rather common in anaerobic bacteria irrespective of their habitats. The fact that cobalamin and haemin could be replaced by the clarified sludge fluid obtained from the methanogenic reactor indicates that the growth of these bacteria having complex nutritional requirements is readily supported by exogenous growth factors present in the environment.

In addition to the phylogenetic distance, these obvious physiological differences suggest that both strains should represent a novel species in the genus *Bacteroides*, having a significantly different ecological function from those of *Bacteroides* species living in mammalian bodies. Recently, species in the *Bacteroides-Prevotella* group are proposed to be a novel indicator of contamination of feces in the environment (Bernhard & Field 2000). Our result as well as other reports showing the presence of the group in various natural habitats indicates that distribution of the group in the environments other than animal bodies should be examined more carefully and extensively.

On the basis of the above-mentioned comprehensive analyses of the phylogenetic, phenotypic and chemotaxonomic characteristics as well as the ecological or functional properties, we propose here *Bacteroides propionicifaciens*, sp. nov. to accommodate strains SV434[T] and S562.

Description of *Bacteroides propionicifaciens* sp. nov.

Bacteroides propionicifaciens (pro.pi.on.i.ci.fa'ci.ens. N.L. n. *acidum propionicum* propionic acid; L. v. *facio* make; N.L. part. adj. *propionicifaciens* propionic acid-producing).

Cells are Gram-negative, non-motile, non-spore-forming rods (0.7-0.8 μm in width and 1.3-2.1 μm in length with some longer cells. Long, filamentous rods often occur. Growth is remarkably stimulated by the addition of haemin and cobalamin (vitamin B_{12}) to the medium. Utilizes arabinose, fructose, galactose, glucose, mannose, cellobiose, maltose, glycogen, starch, dextrin, amygdalin, lactate and pyruvate. Produces acetate and propionate with a small amount of succinate in the presence of haemin and cobalamin from the substrates used. Lactate is also produced in the absence of haemin. Utilizes lactose, aesculin and fumarate weakly. Does not use ribose, xylose, rhamnose, sorbose, melibiose, saccharose, trehalose, melezitose, raffinose, CMC, cellulose powder, filter paper, xylan, pectin, inulin, salicin, glycerol, dulcitol, inositol, mannitol, sorbitol, ethanol, malate and succinate. Slightly alkaliphilic; pH optimum at about 7.9. Growth temperature range is 5-35°C; optimum at 30°C. NaCl concentration range for growth is 0-4.0% (w/v); optimum at 1.0% (w/v) NaCl in PYHVG medium. Catalase activity is not usually detected in the cells cultivated without haemin, while cells cultivated with haemin usually have the activity. Does not have

oxidase, nitrate-reducing and urease activities. Does not produce hydrogen sulfide and indole. Hydrolyzes

aesculin but not gelatin. Sensitive to bile acids. The major cellular fatty acids are anteiso-$C_{15:0}$ and iso-$C_{15:0}$.

Menaquinone MK-8(H_0) and MK-9(H_0) are the major respiratory quinones and the genomic DNA G + C

content is 46.2-47.5%. The type strain of the novel species is SV434[T] (= JCM 14649[T] = DSM 19291[T])

isolated from rice-straw residue in a methanogenic reactor treating waste from cattle farms. Strain S562 (=

JCM 14650 = DSM 19346) as a reference strain is also isolated from the same reactor.

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Figure legends

Fig. 1. Phase-contrast photomicrographs of cells of strains SV434^T (a) and S562 (b) grown on agar slants of PY4S medium. Extraordinarily long rods of strain S562 grown in PYHVG liquid medium (c). Bar, 10 μm.

Fig. 2. Neighbour-joining tree, based on 16S rRNA gene sequences, showing the phylogenetic relationships of strain SV434^T and related species in the Genus *Bacteroides*. Bootstrap values shown at branch nodes are based on analysis of 1000 replicates. *Prevotella melaninogenica* ATCC 25845^T was used as the outgroup. Bar, estimated difference of 2% in nucleotide sequence positions.
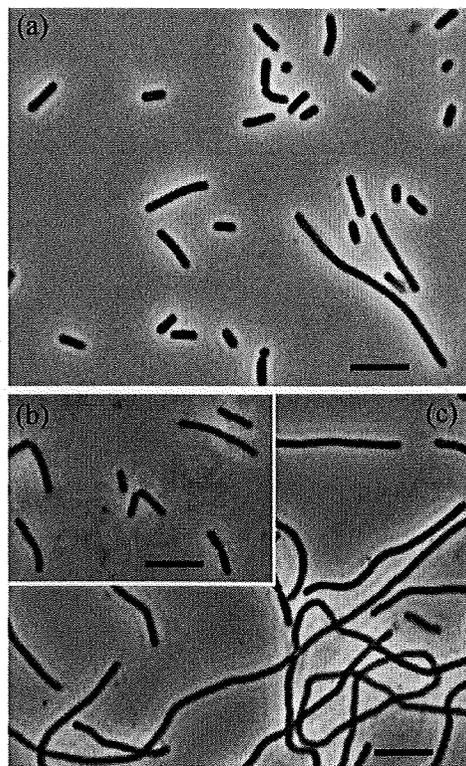
Fig. 1

Fig. 2

Bacteroides acidifaciens DSM 15896^T (AB021164)
628

Bacteroides caccae ATCC 43185^T (X83951)

978

Bacteroides finegoldii JCM 13345^T (AB222699)

995 Bacteroides thetaiotaomicron DSM 2079^T (AE015928)

883

1000 **Bacteroides propionicifaciens SV434^T (AB264625)**

732 **Bacteroides propionicifaciens S562 (AB264624)**

1000 Bacteroides coprosuis CCUG 50528^T (AF319778)

Bacteroides intestinalis JCM 13265^T (AB214328)

Bacteroides gallinarum JCM 13658^T (AB253732)

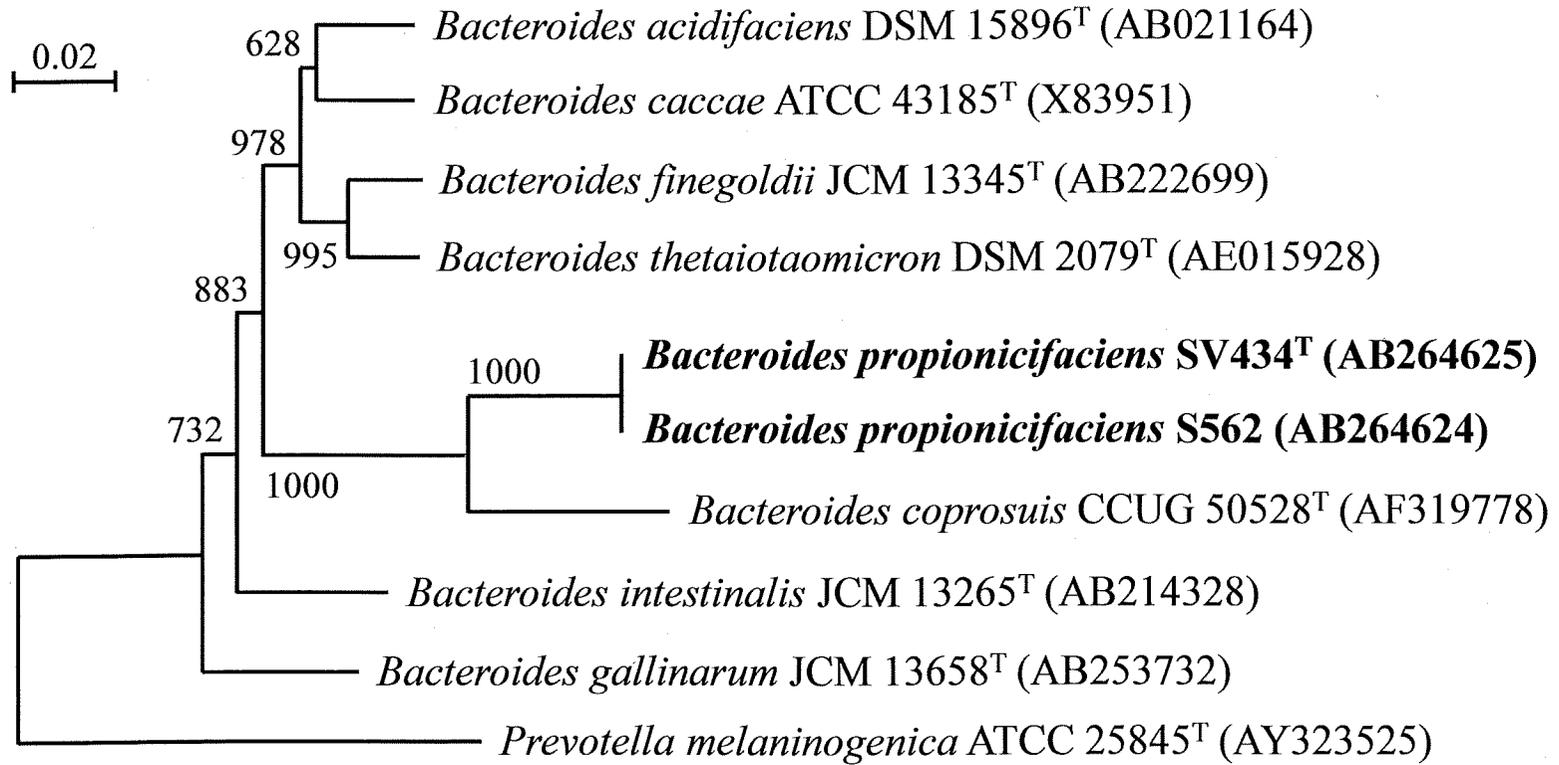Prevotella melaninogenica ATCC 25845^T (AY323525)

0.02

Table 1. Characteristics that differentiate strains SV434[T] and S562 from other related *Bacteroides* species.
Strains: 1, Strains SV434[T] and S562; 2, *B. coprosuis* CCUG 50528[T] (Whitehead *et al*., 2005); 3, *B*. *intestinalis*
JCM 13265[T] (Bakir *et al*., 2006); 4, *B*. *thetaiotaomicron* ATCC 29148[T] (Holdeman *et al*., 1984).
+, Positive; -, negative; ND, no data available. A, acetate; P/p, propionate; S/s, succinate; l, lactate.
Lower-case letters indicate minor products.

Characteristic	1	2	3	4
Habitat	Methanogenic reactor	Swine-manure storage pits	Human feces	Human feces
Optimum growth temp. (°C)	30	37	37	37
DNA G+C content (%)	46.2-47.5	36.4	44	40-43
Predominant quinones	MK-8, MK-9	ND	ND	MK-10, MK-11
Growth in bile	-	+	+	+
Indole production	-	-	+	+
Products from glucose	A, P, s, l	A, P, S	ND	A, S, p
Acid production from:				
Arabinose	+	-	+	+
Fructose	+	-	ND	+
Rhamnose	-	ND	+	+
Xylose	-	-	+	+
Cellobiose	+	-	+	+
Saccharose	-	ND	+	+
Trehalose	-	ND	-	+
Raffinose	-	ND	+	+