Prevotella paludivivens sp. nov., a novel strictly-anaerobic, Gram-negative, hemicellulose-decomposing bacterium isolated from plant residue and rice roots in irrigated rice-field soil

Atsuko Ueki[1], Hiroshi Akasaka[1,2], Atsuya Satoh[1,3], Daisuke Suzuki[1] and Katsuji Ueki[1]

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

[2]Present address: Creative Research Initiative 'Sousei' (CRIS), Hokkaido University, Kita 21, Nishi 10, Kita-ku, Sapporo, 001-0021, Japan

[3]Present address: Graduate School of Agriculture, Hokkaido University, Kita 9, Nishi 9, Kita-ku, Sapporo, 060-8589, Japan

Author for correspondence: Atsuko Ueki. Tel: +81 235 28 2846. Fax: +81 235 28 2846.

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

Abbreviations: CMC, carboxymethylcellulose; CFA, whole-cell fatty acid.

Key words: *Bacteroidetes*, *Prevotella*, anaerobic Gram-negative rods, haemin, xylan

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains KB7^T and A42 are AB078827 and AB081578, respectively.

SAMMARY

Two strictly anaerobic bacterial strains, KB7^T and A42, were isolated from rice plant residue and living rice roots, respectively, from irrigated rice-field soil in Japan. These two strains were closely related each other with 16S rRNA gene sequence similarity of 99.8%. Both strains showed almost the same physiological properties. Cells were Gram-negative, non-motile, non-spore-forming rods. Growth was remarkably stimulated by the addition of haemin to the medium. The strains utilized various saccharides including xylan,

xylose, pectin and carboxymethylcellulose and produced acetate and succinate with small amounts of formate and malate. The strains grew at 10-40ºC; optimum growth was observed at 30ºC and pH 5.7-6.7. Oxidase, catalase and nitrate-reducing activities were not detected. Aesculin was hydrolyzed. The major cellular fatty acids were anteiso-$C_{15:0}$, iso-$C_{15:0}$, $C_{15:0}$ and iso-$C_{17:0}$ 3-OH. Menaquinones MK-11 and MK-11(H_2) were the major respiratory quinones and the genomic DNA G + C content was 39.2 mol%. Phylogenetic analysis based on 16S rRNA gene sequences placed both strains in the phylum *Bacteroidetes*. 16S rRNA gene sequences showed that the most related species of both strains was *Prevotella oulorum* (92.8-92.9% similarity). *Prevotella veroralis* and *Prevotella melaninogenica* were the next most closely related known species with sequence similarities of 91.9-92.4%. Based on differences in the phylogenetic, ecological, physiological and chemotaxonomic characteristics between the two isolates and related species, it is proposed that strain KB7^T and A42 represent a novel species, *Prevotella paludivivens* sp. nov. This is the first described *Prevotella* species derived from a natural habitat; all other *Prevotella* species are from mammalian sources. The type strain of the novel species is KB7^T (= JCM 13650^T = DSM 17968^T).

MAIN TEXT

In Japan, rice is widely cultivated as the principal food product in irrigated fields, the soil of which develops a highly reduced condition during the flooding period (Takai, 1970; Wassmann *et al*., 2000). Diverse fermentative bacterial groups play a key role in decomposition of organic matter, including plant residue such as rice straw, stubble and roots ploughed into the soil in the anoxic rice-field soil, thus producing methanogenic substrates such as acetate, formate and H_2. Living plant roots also provide growth substrates for microbes either by secreting organic matter such as saccharides, amino acids and organic acids or from

cellular material peeling-off the root epidermis and cortex (Kaku *et al*., 2000). Methane produced from decomposition of these substrates is emitted to the atmosphere as one of the major greenhouse gases (Takai, 1970; Seiler *et al*., 1984; Boone, 2000; Khalil, 2000; Wassmann *et al*., 2000).

Various fermentative anaerobes from samples of rice plant residue and living rice roots from irrigated rice-field soil have been isolated during the course of our investigation for microbes in anoxic rice-field soil (Satoh *et al*. 2002; Akasaka *et al*., 2003a; Akasaka *et al*., 2004). Of the isolates from rice plant residue, three novel propionate-producing species in the phyla *Actinobacteria* and *Bacteroidetes* have been described recently (Akasaka *et al*., 2003b; Ueki *et al*., 2006a,b). In this study, two other strains that are phylogenetically distant from any related recognized species were characterized. The isolates were strictly anaerobic, xylanolytic bacteria consisting of Gram-negative, non-motile rod-like cells. Although all recognized species in the genus *Prevotella* have been isolated from mammalian sources such as human oral, urogenital or other clinical sources and the rumen, it is proposed that the two strains isolated here (KB7^T and A42) should be accommodated in the genus *Prevotella* as a novel species based on the comprehensive characterization carried out.

Strain KB7^T (= JCM 13650^T = DSM 17968^T) was isolated from a plant residue sample (rice stubble and roots) collected from the irrigated rice-field soil in the Shonai Branch of the Yamagata Agricultural Experimental Station (Tsuruoka, Yamagata, Japan) during the flooding period of the field in June 1994 (Akasaka *et al*., 2003a). Strain A42 (= JCM 13651 = DSM 17969) was isolated from roots of living rice plants in the same field in August 1993 during the period of intermittent irrigation (Satoh *et al*., 2002).

Cultivation practices for rice plants and other field conditions have been described previously (Ueki *et al*., 2000). Samples homogenized by a Waring blender (10000 rpm, 10 min.) under N_2 gas were used for isolation (Satoh *et al*., 2002; Akasaka *et al*., 2003a, 2004). Strains were isolated by the anaerobic roll tube method for enumeration of anaerobic fermentative bacteria using the colony-counting method (Hungate, 1966; Holdeman *et al*., 1977).

The strains were cultivated anaerobically at 30ºC unless otherwise stated by using peptone/yeast extract (PY) medium as basal medium with O_2-free, 95% N_2/ 5% CO_2 mixed gas in the headspace as described by Akasaka *et al*. (2003a,b). 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 isolation and maintenance of the strain 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) were used for the cultivation of the strains for various physiological tests and chemotaxonomic analyses of the cells unless otherwise stated (Ueki *et al*., 2006b). 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 by plate culture on nutrient agar (Nissui Pharmacy) and PY4S agar modified to exclude Na_2CO_3, cysteine-HCl-H_2O and sodium resazurine in the PY

basal medium. Spore formation was assessed by observation of cells after Gram-staining and by growth of cells exposed to 80ºC for 10 min. Oxidase, catalase and nitrate-reducing activities were determined according to methods described by Satoh *et al*. (2002) and Akasaka *et al*. (2003a, b). Utilization of carbon sources was tested in PYHV liquid medium with each substrate added at 10 g l^{-1} (for sugars and sugar alcohols) or 30 mM (organic acids). Bile sensitivity was determined by the addition of bile salts (Oxioid) (0.1-0.5%, w/v) to PYHVG medium as well as PYG medium (Lawson *et al*., 2002). 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 methods 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). Methyl esters of CFAs were 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), based on the MIDI Microbial Identification System (Microbial ID; Moore *et al*., 1994). The TSBA40 microbial identification system was also used to confirm the identification. Isoprenoid quinones were extracted as described by Komagata & Suzuki (1987) and analyzed by MS (JMS-SX102A; JEOL). Genomic DNA was extracted according to the method as described by Kamagata & Mikami (1991). Extracted DNA was digested with P1 nuclease by using a YAMASA GC kit (Yamasa shoyu) and its G + C content was measured by HPLC (HITACHI L-7400) equipped with a μBondapack C18 column (3.9 × 300 mm; Waters).

DNA extraction and PCR amplification were performed according to the method described by Akasaka *et al*. (2003a). The 16S rRNA gene was PCR-amplified using the 27f and 1492r primer set and sequenced by using a Thermo Sequenase Primer Cycle Sequencing kit (Amersham Biosciences) and a DNA sequencer (4000L; Li-COR). Multiple alignments of the sequence with reference sequences in GenBank were performed with the program BLAST (Altschul *et al*., 1997). A phylogenetic tree was constructed with the neighbor-joining method (Saitou & Nei, 1987) by using the program CLUSTAL W (Thompson *et al*., 1994). All gaps and unidentified base positions in the alignments were excluded before sequence assembly.

Cellular and various physiological characteristics were determined for strains KB7^T and A42 and they showed almost the same phenotypic properties. Of the physiological characteristics of both strains shown below, detailed descriptions such as growth rates and amounts of products were based mainly on the data from strain KB7^T as the representative strain.

Cells of both 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. 1). Cells were non-motile as observed phase-contrast microscopy. Colonies on PY4S agar were translucent and thin with a smooth surface. Neither strain could grow in air either on PY4S or nutrient agar. Spore formation was not observed and cells treated at 80ºC for 10 min did not grow. Catalase and oxidase activities were not detected.

The strains grew very slowly in PYG liquid medium without haemin at a growth rate (μ) of 0.092 h^{-1} at 30ºC and the addition of haemin to the medium greatly enhanced growth (μ = 0.40-0.45 h^{-1}). The addition of

haemin did not significantly affect the morphology of the cells (Ueki *et al*., 2006b). Further addition of vitamin K to the medium did not seem to affect the growth rate. Although addition of B-vitamin mixture did not stimulate the growth, it seemed to bring about a stable growth behavior of the strains. Thus, physiological characteristics of the strains were usually determined in the presence of B-vitamin mixture plus haemin (PYHV medium). Cells for chemotaxonomic characterization were also cultivated in the presence of these growth factors (PYHVG medium).

Both strains utilized arabinose, xylose, fructose, galactose, glucose, mannose, rhamnose, cellobiose, lactose, maltose, sucrose, carboxymethylcellulose (CMC), soluble starch, xylan, pectin, and salicin as growth substrates. Ribose, pyruvate and fumarate were utilized weakly. Acids were produced from all these substrates utilized, but gas was not. The strains did not use sorbose, trehalose, melezitose, cellulose powder, filter paper, inulin, glycerol, inositol, mannitol, lactate, malate or succinate.

Fermentation products in PYG medium (without haemin and vitamin) were acetate (2.6 mM), succinate (4.1 mM) and malate (5.9 mM), whereas in the presence of haemin and vitamin (PYHVG medium), both strains produced acetate (9.2 mM) and succinate (15.9 mM) as major acids and lower amounts of formate (2.6 mM) and malate (0.4 mM). Almost the same amounts of acetate and succinate were produced from other substrates tested including xylan and pectin. Acids production from CMC was rather weak (3.4 mM acetate and 6.1 mM succinate). Products from fumarate were acetate (3.9 mM), succinate (6.6 mM) and malate (8.8 mM) with a trace amount of formate; products from pyruvate were formate (3.9 mM), acetate (8.9 mM), succinate (4.1 mM) and a trace amount of malate. Aesculin was hydrolyzed, but gelatin was not. The strain

was negative for the production of urease, hydrogen sulfide and indole. They did not reduce nitrate and could not grow in the presence of 0.1% (w/v) of bile salts.

The strain grew at pH 4.7-7.6, with optimum growth at 5.7-6.7, a rather wide pH range for optimum growth. The final pH of the PYHVG medium was about pH 4.5. The temperature range for growth was 10-40^{o}C, with optimum growth at 30^{o}C. The growth rate at 37^{o}C ($\mu = 0.35\ h^{-1}$) was rather high, but it dropped sharply to a much lower level at 40^{o}C ($\mu = 0.071\ h^{-1}$). The growth rate at 10^{o}C was 0.019 h^{-1}. The NaCl concentration range for growth was 0-1.0% (w/v).

The major CFAs of strain KB7^{T} were anteiso-$C_{15:0}$ (27.3%), iso-$C_{15:0}$ (11.9%), $C_{15:0}$ (10.1%), iso-$C_{17:0}$ 3-OH (9.1%), iso-$C_{14:0}$ (7.9%) and $C_{16:0}$ 3-OH (6.8%) with lower amounts of iso-$C_{13:0}$ (4.4%), $C_{14:0}$ (3.9%), iso-$C_{16:0}$ 3-OH (3.9%) and $C_{16:0}$ (3.3%). Unsaturated fatty acids were not detected. The predominant respiratory quinones of strain KB7^{T} were menaquinones MK-11 and MK-11(H_2). The genomic DNA G + C content o was 39.2 mol%. These chemotaxonomic analyses were not carried out for strain A42.

The similarity between the 16S rRNA gene sequences of strains KB7^{T} and A42 was 99.8% and both strains were assigned to the phylum *Bacteroidetes* (Garrity & Holt, 2001). The closest relative of both strains in the GenBank database was *Prevotella* sp. HY-36-2 (AY581270) with sequence similarity of 98.7-99.0%. Of species with validly described names, the species most closely related to both strains all belonged to the genus *Prevotella*, i.e. *Prevotella oulorum* ATCC 43324^{T} (Shah *et al*., 1985; 92.8-92.9% similarity), *Prevotella veroralis* ATCC 33779^{T} (Watabe *et al*., 1983; 92.0-92.4%) and *Prevotella melaninogenica* ATCC

25845^T (Holdeman *et al*., 1984; 91.9-92.0%). The next most closely related species were *Prevotella corporis* and *Prevotella bryantii,* (Holdeman *et al*., 1984; Avgustin *et al*., 1997; 91.1-92.0%)). Strains KB7^T and A42 formed a separate branch from related *Prevotella* species in the phylogenetic tree composed of all recognized *Prevotella* species (Fig. 2).

The genus *Prevotella* consists of species almost exclusively isolated from human oral, urogenital or other clinical sources (Shah & Collins, 1989, 1990; Paster *et al*., 1994) including recently described species (Sakamoto *et al*., 2004, 2005a, b; Berger *et al*., 2005; Downes *et al*., 2005, 2006) with the exception of species from the rumen (Avgustin *et al*., 1997). Thus, the habitats of strains KB7^T and A42 are extremely different, i.e. rice plant residue in flooded rice field soil and living rice roots, from those of currently known *Prevotella* species. The optimum growth temperature of the strains is 30^{o}C and they grow only weakly at 40^{o}C; thus, the temperature of mammals at around 37^{o}C is near the upper limit for growth of both strains.

Many species in the genus *Prevotella* have a requirement for haemin for growth, and the closest relatives of strains KB7^T and A42 also require haemin for growth or they are usually cultivated in the presence of haemin in the medium (Holdeman *et al*., 1984; Shah *et al*., 1985). Growth of our strains was also strongly stimulated by the addition of haemin to the medium. We have reported that *Xylanibacter oryzae* strain KB3^T isolated from plant residue in the same rice field as strain KB7^T, also requires haemin for growth (Ueki *et al*., 2006b).

Some characteristics of strain KB7^T, as the representative strain, were compared with those of the three

most closely related known species (*P. oulorum, P. veroralis* and *P. melaninogenica*) (Table 1). Substrates that can be utilized by these related species are relatively limited; these species are not able to utilize pentoses (arabinose and xylose), rhamnose or salicin, which are utilized by strain KB7^T and A42. Furthermore, the two isolates utilized polysaccharides such as xylan, pectin and CMC, whereas the related species do not utilize these polymers except for xylan-utilizing *P. veroralis*. In addition, KB7^T, A42 and *P. veroralis* utilized cellobiose, whereas the other two species do not.

Strains KB7^T and A42 produced acetate and succinate as major acids from glucose and other closely related species also produce these acids from carbohydrates. Thus, both strains have a common feature with respect to the fermentation products with the related species (Holdeman *et al*., 1984; Shah *et al*., 1985).

The G + C content of genomic DNA of strain KB7^T (39.2%) is close to those of *P. veroralis* (42.1%) and *P. melaninogenica* (36-40%) and slightly lower than that of *P. oulorum* (45-46%) (Watabe *et al*., 1983; Holdeman *et al*., 1984; Shah *et al*., 1985). Many *Prevotella* species has menaquinone MK-11 as one of predominant menaquinones (Shah & Collins, 1980) and strain KB7^T possesses menaquinones MK-11 and MK-11(H_2). Although the presence of hydrogenated menaquinone, MK-11(H_2), is rather rare in *Prevotella* species (Shah & Collins, 1980; Sakamoto *et al*., 2005a), strain KB7^T and related *Prevotella* species do share a common menaquinone.

It has been reported that the major CFAs in species of the two genera of *Bacteroides* and *Prevotella* are anteiso-$C_{15:0}$, iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH and $C_{16:0}$ (Miyagawa *et al*., 1979; Moore *et al*., 1994). Although $C_{16:0}$

is only a minor component of CFAs of strain KB7^T, the overall pattern of the CFAs composition with anteiso-$C_{15:0}$ and iso-$C_{17:0}$ 3-OH as major components seems to have a common profile to those reported for *Bacteroides* and *Prevotella* species. However, the CFAs composition of strain KB7^T is actually rather different from that of *P. oulorum* in the levels of some CFAs such as $C_{15:0}$ and iso-$C_{17:0}$ (Shah *et al*., 1985). Furthermore, although *P. veroralis* and *P. melaninogenica* have an unsaturated fatty acid ($C_{18:1}$) as one of the most dominant CFAs (17.2 and 17.9%, respectively) (Sakamoto *et al*., 2004), strain KB7^T does not contain unsaturated fatty acids (Table 2).

Strains KB7^T was isolated from rice plant residue (plant stubble and roots) together with three other strains (strains KB1, KB9 and KB12) (Akasaka *et al*., 2003a) and strain A42 was isolated from living rice roots as one of predominant culturable anaerobic bacteria (Satoh *et al*., 2002). Hemicellulose together with cellulose and lignin makes up the major polymeric constituents of plant cell walls. Hemicellulose is heteropolysaccharides composed of various sugars such as xylose, arabinose, glucose, galactose and mannose. Xylan is composed of xylose and constitutes the major component of hemicellulose (Collins *et al*., 2005). Pectin also occurs in the primary cell walls and intercellular regions of plants. It is known that pectin, which is a complex polysaccharide mainly composed of galacturonic acid, usually contains rhamnose residues as important components. Strains KB7^T and A42 both utilized these plant polymers (xylan, pectin and CMC) as well as the major components of these polymeric constituents. Both strains also utilize cellobiose, which is generated by hydrolysis of cellulose. Thus, it was strongly suggested that the bacterial group related to strains KB7^T and A42 should take an important part in decomposition of the plant cell wall, especially hemicellulose and pectin, including intermediate substrates derived from the decomposition of

plant cell walls. The isolation of strain A42 from living rice roots indicates that the bacterial group colonizes rice roots before harvesting of rice and take an important role in the decay of rice roots in rice-field soil.

Strain KB7^T as well as strain A42 has some features in common with those of related *Prevotella* species as shown above, although these related species are all isolated from human clinical specimens and all known species in the genus *Prevotella* are derived from mammalian sources (Fig. 2). In addition to low 16S rRNA gene sequence similarities, the obvious ecological difference suggests that strains KB7^T and A42 should represent a new species in the genus *Prevotella*, having a significantly different ecological function from those of *Prevotella* species living in mammals.

On the basis of above-mentioned comprehensive analyses of the phylogenetic, phenotypic and chemotaxonomic characteristics as well as the ecological or functional properties, the novel species *Prevotella paludivivens*, sp. nov. is proposed to accommodate strains KB7^T and A42.

Description of *Prevotella paludivivens* sp. nov.

Prevotella paludivivens (pa.lu.di'vi.vens. L. n. *palus, -udis* swamp, marsh; L. v. *vivo* to live; N.L. part. adj. *paludivivens* living in swamps).

Cells are Gram-negative, non-spore-forming, non-motile, short rods. Strictly anaerobic. Chemo-organotroph. Haemin significantly stimulates growth. pH optimum for growth is 5.7-6.7. Temperature range for growth is 10-40^{o}C, with optimum at 30^{o}C. Growth at 37^{o}C is delayed compared that at 30^{o}C. NaCl concentration range for growth is 0-1.0% (w/v) in PYG medium. Oxidase, catalase, and nitrate-reducing activities are not

detected. Utilizes arabinose, ribose, xylose, fructose, galactose, glucose, mannose, rhamnose, cellobiose, lactose, maltose, sucrose, CMC, soluble starch, xylan, pectin, salicin as growth substrates and produces acetate and succinate as major fermentation end products. Gas is not produced. Pyruvate and fumarate are also utilized. Does not use sorbose, trehalose, melezitose, cellulose powder, filter paper, inulin, glycerol, inositol, mannitol, lactate, malate, and succinate. Hydrolyzes aesculin, but not gelatin. Urease-negative. Hydrogen sulfide and indole is not produced. Does not grow in the presence of bile salts. The major cellular fatty acids are anteiso-$C_{15:0}$, iso-$C_{15:0}$, $C_{15:0}$, and iso-$C_{17:0}$ 3-OH. Major respiratory quinones are MK-11 and MK-11(H_2).

The type strain is KB7^T (= JCM 13650^T = DSM 17968^T) isolated from rice plant residue in flooded rice-field soil; the genomic DNA G + C content of strain KB7^T is 39.2%. Strain A42 (= JCM 13651 = DSM 17969) is a reference strain derived from rice roots of living rice plants.

ACKNOWLEDGEMENTS

This work was partly supported by a Grant-in-Aid from the Institute for Fermentation, Osaka. We are grateful to Yoshimi Ohtaki for her technical assistance on physiological examinations of the strain.

REFERENCNES

Akasaka, H., Izawa, T., Ueki, K. & Ueki, A. (2003a). Phylogeny of numerically abundant culturable anaerobic bacteria associated with degradation of rice plant residue in Japanese paddy field soil. *FEMS Microbiol Ecol* **43**, 149-161.

Akasaka, H., Ueki, A., Hanada, S., Kamagata, Y. & Ueki, K. (2003b). *Propionicimonas paludicola* gen. nov., sp. nov., a novel facultatively anaerobic, Gram-positive, propionate-producing bacterium isolated from plant residue in irrigated rice-field soil. *Int J Syst Environ Microbiol* **53**, 1991-1998.

Akasaka, H., Ueki, K. & Ueki, A. (2004). Effects of plant residue extract and cobalamin on growth and propionate production of *Propionicimonas paludicola* isolated from plant residue in irrigated rice field soil. *Microbes Environ* **19**, 112-119.

Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**, 3389-3402.

Avgustin, G., Wallace, R. J. & Flint, H. (1997). Phenotypic diversity among ruminal isolates of *Prevotella ruminicola*: Proposal of *Prevotella brevis* sp. nov., *Prevotella bryantii* sp. nov., and *Prevotella albensis* sp. nov. and redefinition of *Prevotella ruminicola*. *Int J Syst Bacteriol* **47**, 284-288.

Berger, P., Adékambi, T., Mallet, M-N. & Drancourt, M. (2005). *Prevotella massiliensis* sp. nov. isolated from human blood. *Res Microbiol* **156**, 967-973.

Boone, R. D. (2000). Biological formation and consumption of methane. In *Atmospheric methane*. pp. 42-62.

Edited by M. A. K. Khalil. Berlin: Springer.

Collins, T., Gerday, C. & Feller, G. (2005). Xylanases, xylanase families and extremophilic xylanases. *FEMS Microbiol Reviews* **29**, 3-23.

Downes, J., Sutcliffe, I. C., Tanner, A. C. R. & Wade, W. G. (2005). *Prevotella marshii* sp. nov. and *Prevotella baroniae* sp. nov., isolated from the human oral cavity. *Int J Syst Bacteriol* **55**, 1551-1555.

Downes, J., Sutcliffe, I. C., Hofstad, T. & Wade, W. G. (2006). *Prevotella bergensis* sp. nov., isolated from human infections. *Int J Syst Bacteriol* **56**, 609-612.

Garrity, G. M. & Holt, J. G. (2001). The road map to the manual. In *Bergey's Manual of Systematic Bacteriology Second Edition*, Vol. 1, pp. 119-166. Edited by D. R. Boone & G. M. Garrity. NY: Springer.

Holdeman, L. V., Cato, E. P. & Moore, W. E. C. (1977). *Anaerobe Laboratory Manual*, 4th edn. Blacksburg, VA: Virginia Polytechnic Institute and State University.

Holdeman, L. V., Kelly, R. W.. & Moore, W. E. C. (1984). Genus I. *Bacteroides* Castellani and Chalmers 1919, 959. In *Bergey's Manual of Systematic Bacterology*, Vol. 1, pp. 604-631. Edited by Krieg, N. R. & Holt, J. G. Baltimore: Williams & Willkins.

Hungate, R. E. (1966). *The Rumen and Its Microbes*. NY: Academic Press.

Kamagata, Y. & Mikami, E. (1991). Isolation and characterization of a novel thermophilic *Methanosaeta* strain. *Int J Syst Bacteriol* **41**, 191-196.

Kaku, N., Ueki, A., Fujii, H. & Ueki, K. (2000). Methanogenic activities on rice roots and plant residue and their contributions to methanogenesis in wetland rice field soil. *Soil Biol. Biochem.* **32,** 2001-2010.

Khalil, M. A. K. (2000). *Atmospheric Methane*. Berlin: Springer.

Komagata, K. & Suzuki, K. (1987). Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19,** 161-207.

Lawson, P. A., Falsen, E., Inganas, E., Weyant, R. S. & Collins, M. D. (2002). *Dysgonomonas mossi* sp. nov., from human sources. *Syst Appl Microbiol* **25**, 194-197.

Miller, L. T. (1982). Single derivatization method for routine analysis of bacterial whole-cell fatty acid methyl esters, including hydroxyl acids. *J Clin Microbiol* **16**, 584-586.

Miyagawa, E., Azuma, R. & Suto, E. (1979). Cellular fatty acid composition in Gram-negative obligately anaerobic rods. *J Gen Appl Microbiol* **25**, 41-51.

Moore, L. V. H., Bourne, D. M. & Moore, W. E. C. (1994). Comparative distribution and taxonomic value of cellular fatty acids in thirty-three genera of anaerobic Gram-negative bacilli. *Int J Syst Bacteriol* **44**, 338-347.

Paster, B. J., Dewhirst, F. E., Olsen, I. & Fraser, G. J. (1994). Phylogeny of *Bacteroides*, *Prevotella*, and *Porphyromonas* spp. and related species. *J Bacteriol* **176**, 725-732.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406-425.

Sakamoto, M., Suzuki, M., Huang, Y., Umeda, M., Ishikawa, I. & Benno, Y. (2004). *Prevotella shahii* sp. nov. and *Prevotella salivae* sp. nov., isolated from the human oral cavity. *Int J Syst Environ Microbiol* **54**, 877-883.

Sakamoto, M., Huang, Y., Umeda, M., Ishikawa, I. & Benno, Y. (2005a). *Prevotella multiformis* sp. nov., isolated form human subgingival plaque. *Int J Syst Environ Microbiol* **55**, 815-819.

Sakamoto, M., Umeda, M., Ishikawa, I. & Benno, Y. (2005b). *Prevotella multisaccharivorax* sp. nov., isolated form human subgingival plaque. *Int J Syst Environ Microbiol* **55**, 1839-1843.

Satoh, A., Watanabe, M., Ueki, A. & Ueki, K. (2002). Physiological properties and phylogenetic affiliations of anaerobic bacteria isolated from roots of rice plants cultivated on a paddy field. *Anaerobe* **8**, 233-246.

Seiler, W., Holzapfel-Pschorn, A., Conrad, R. & Scharffe, D. (1984). Methane emission from rice paddies. *J Atoms Chem* **1**, 241-268.

Shah, H. N. & Collins, D. M. (1980). Fatty acid and isoprenoid quinone composition in the classification of *Bacteroides melaninogenicus* and related taxa. *J Appl Bacteriol* **48**, 75-87.

Shah, H. N. & Collins, D. M. (1989). Proposal to restrict the genus *Bacteroides* (Castellani and Chalmers) to *Bacteroides fragilis* and closely related species. *Int J Syst Bacteriol* **39**, 85-87.

Shah, H. N. & Collins, D. M. (1990). *Prevotella*, a new genus to include *Bacteroides melaninogenicus* and related species formerly classified in the genus *Bacteroides*. *Int J Syst Bacteriol* **40**, 205-208.

Shah, H. N., Collins, D. M., Watabe, J. & Mitsuoka, T. (1985). *Bacteroides oulorum* sp. nov., a non-pigmented saccharolytic species from the oral cavity. *Int J Syst Bacteriol* **35**, 193-197.

Takai, Y. (1970). The mechanism of methane fermentation in flooded paddy soil. *Soil Sci Plant Nutr* **6**, 238-244.

Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673-4680.

Ueki, A. & Suto, T. (1979). Cellular fatty acid composition of sulfate-reducing bacteria. *J Gen Appl Microbiol* **25**, 185-196.

Ueki, A., Matsuda, K. & Ohtsuki, C. (1986). Sulfate reduction in the anaerobic digestion of animal waste. *J Gen Appl Microbiol* **32**, 111-123.

Ueki, A., Kainuma, Y., Fujii, H. & Ueki, K. (2000). Seasonal variations in vertical distribution of methanogenic activity and Fe(II) content and relationship between them in wetland rice field soil. *Soil Sci Plant Nutr* **46**, 401-415.

Ueki, A., Akasaka, H., Suzuki, D. & Ueki, K. (2006a). *Paludibacter propionicigenes* gen. nov., sp. nov., a novel strictly anaerobic, Gram-negative, propionate-producing bacterium isolated from plant residue in irrigated rice-field soil in Japan. *Int J Syst Environ Microbiol* **56**, 39-44.

Ueki, A., Akasaka, H., Suzuki, D., Hattori, S. & Ueki, K. (2006b). *Xylanibacter oryzae* gen. nov., sp. nov., a novel strictly anaerobic, Gram-negative xylanolytic bacterium isolated from rice-plant residue in flooded

rice-filed soil in Japan. *Int J Syst Environ Microbiol* **56**, 2215-2221.

Wassmann, R., Neue, H. U., Lantin, R. S., Makarim, K., Chareonsilp, N., Buendia, L. V. & Rennenberg, H. (2000). Characterization of methane emissions from rice fields in Asia. II. Differences among irrigated, rainfed, and deepwater rice. *Nutr Cycl Agroecosys* **58**, 13-22.

Watabe, J., Benno, Y. & Mitsuoka, T. (1983). Taxonomic study of *Bacteroides oralis* and related organisms and proposal of *Bacteroides veroralis* sp. nov. *Int J Syst Bacteriol* **33**, 57-64.

Figure legends

Fig. 1. Phase-contrast photomicrograph of cells of strain KB7^T grown anaerobically on agar slants of PY4S. Bar, 10 µm.

Fig. 2. Neighbor-joining tree showing the phylogenetic relationship of strains KB7^T and A42, all species in the genus *Prevotella* and the type species of related genera (*Bacteroides*, *Porphyromonas*, *Rikenella* and *Xylanibacter*) based on 16S rRNA gene sequences. The phylogenetic tree was constructed by using 1111 nt of the 16S rRNA gene sequence. Bootstrap values (expressed as percentages of 1000 replications) above 50% are shown at branch nodes. Bar, 2% estimated difference in nucleotide sequence position. The sequence of *Escherichia coli* ATCC 11775^T, which belongs to the *Gammaproteobacteria* (Garrity & Holt, 2001), was used as the outgroup.

Fig. 1

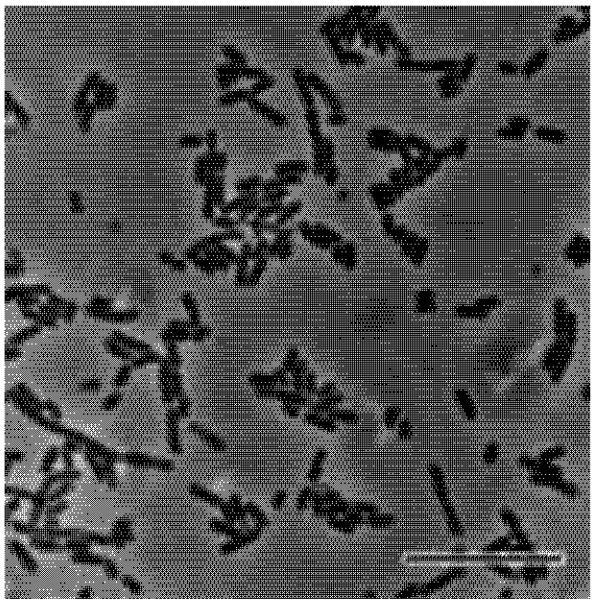

Fig. 2

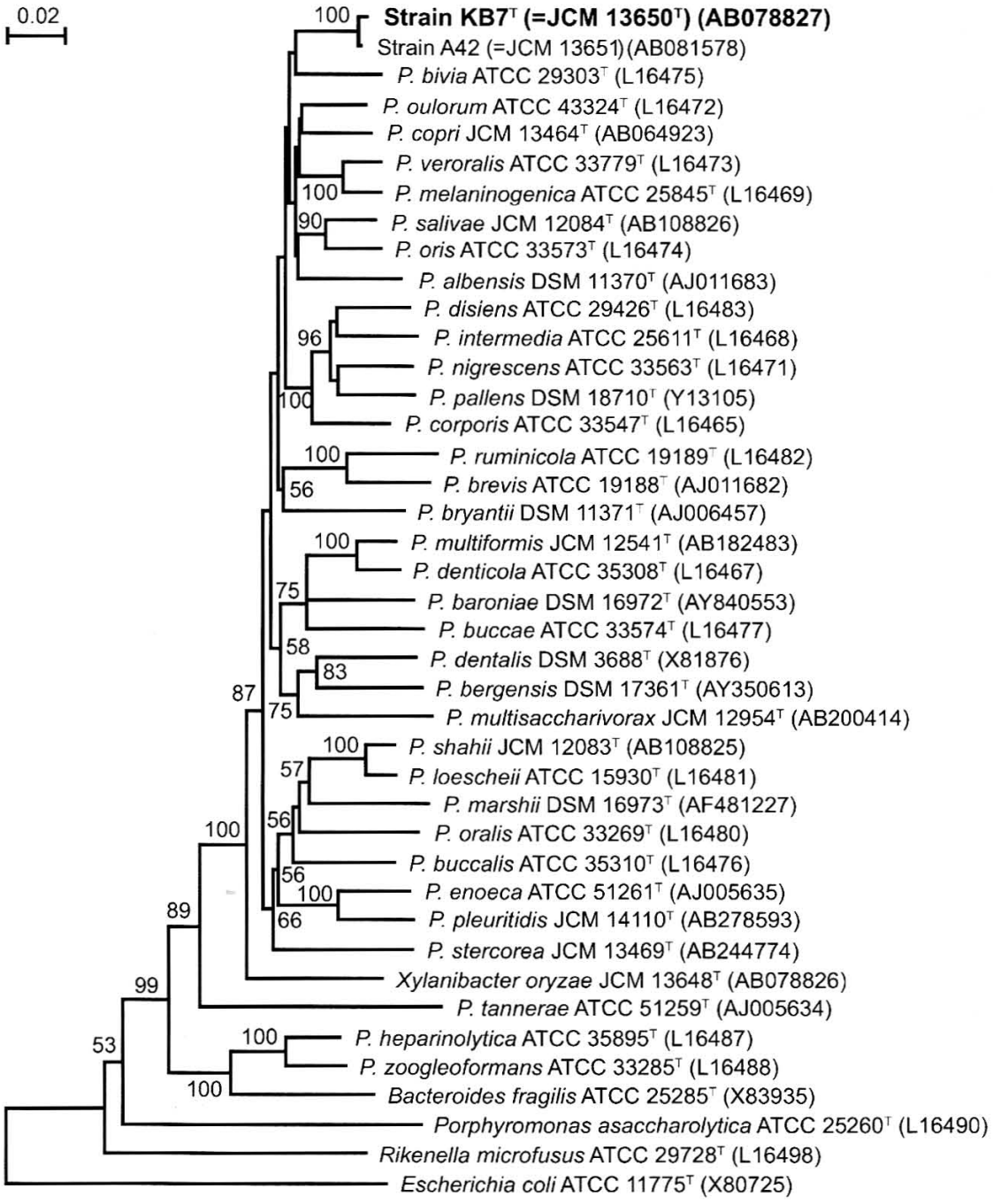
0.02
100
Strain KB7^{T} (=JCM 13650^{T}) (AB078827)
Strain A42 (=JCM 13651)(AB081578)
P. bivia ATCC 29303^{T} (L16475)
P. oulorum ATCC 43324^{T} (L16472)
P. copri JCM 13464^{T} (AB064923)
P. veroralis ATCC 33779^{T} (L16473)
100
P. melaninogenica ATCC 25845^{T} (L16469)
90
P. salivae JCM 12084^{T} (AB108826)
P. oris ATCC 33573^{T} (L16474)
P. albensis DSM 11370^{T} (AJ011683)
P. disiens ATCC 29426^{T} (L16483)
96
P. intermedia ATCC 25611^{T} (L16468)
P. nigrescens ATCC 33563^{T} (L16471)
100
P. pallens DSM 18710^{T} (Y13105)
P. corporis ATCC 33547^{T} (L16465)
100
P. ruminicola ATCC 19189^{T} (L16482)
56
P. brevis ATCC 19188^{T} (AJ011682)
P. bryantii DSM 11371^{T} (AJ006457)
100
P. multiformis JCM 12541^{T} (AB182483)
P. denticola ATCC 35308^{T} (L16467)
75
P. baroniae DSM 16972^{T} (AY840553)
P. buccae ATCC 33574^{T} (L16477)
58
P. dentalis DSM 3688^{T} (X81876)
83
P. bergensis DSM 17361^{T} (AY350613)
87
75
P. multisaccharivorax JCM 12954^{T} (AB200414)
100
P. shahii JCM 12083^{T} (AB108825)
57
P. loescheii ATCC 15930^{T} (L16481)
P. marshii DSM 16973^{T} (AF481227)
100
56
P. oralis ATCC 33269^{T} (L16480)
P. buccalis ATCC 35310^{T} (L16476)
56
100
P. enoeca ATCC 51261^{T} (AJ005635)
89
66
P. pleuritidis JCM 14110^{T} (AB278593)
P. stercorea JCM 13469^{T} (AB244774)
Xylanibacter oryzae JCM 13648^{T} (AB078826)
99
P. tannerae ATCC 51259^{T} (AJ005634)
100
53
P. heparinolytica ATCC 35895^{T} (L16487)
P. zoogleoformans ATCC 33285^{T} (L16488)
100
Bacteroides fragilis ATCC 25285^{T} (X83935)
Porphyromonas asaccharolytica ATCC 25260^{T} (L16490)
Rikenella microfusus ATCC 29728^{T} (L16498)
Escherichia coli ATCC 11775^{T} (X80725)

Table 1. Characteristics that differentiate strain KB7^T and other related *Prevotella* species.

Strains: 1, KB7^T; 2, *P. oulorum* NCTC 11871^T (Shah *et al*., 1985); 3, *P. veroralis* ATCC 33779^T (Watabe *et al*., 1983; Shah *et al*., 1985); 4. *P. melaninogenica* ATCC 25845^T (Holdeman *et al*., 1984; Sakamoto *et al*., 2005a).

+, positive; -, negative; n.d., no data available. F, formate (minor product); A, acetate; S, succinate.

Characteristic	1	2	3	4
Habitat	Plant residue in rice-field soil	Gingival crevice	Oral cavity	Gingival crevice, clinical specimens
Optimum growth temperature (°(	30	37	37	37
DNA G+C content (mol%)	39.2	45-46	42.1	36-40
Predominant quinones	MK-11, MK-11(H_2)	MK-10, MK-11, MK-9	MK-10, MK-11, MK-12	MK-10, MK-11
Catlase	-	+	n.d.	-
Vitamin K requirement	-	+*	+*	+
Gelatin hydrolysis	-	-	-	+
Products from glucose	A, S, F	A, S	A, S	A, S, F
Acid production from				
Arabinose	+	-	-	-
Xylose	+	-	-	-
Cellobiose	+	-	+	-
Rhamnose	+	-	-	-
Inulin	-	+	+	+
Xylan	+	-	+	n.d.
Salicin	+	-	-	-

*Vitamin K is usually added to the medium.

Table 2. Cellular fatty acids composition (%) of strain KB7^T and other related *Prevotella* species. Strains: 1, KB7^T; 2, *P. oulorum* NCTC 11871^T (Shah *et al*., 1985); 3, *P. veroralis* JCM 6290^T (Sakamoto *et al*., 2004); 4. *P. melaninogenica* JCM 6325^T (Sakamoto *et al*., 2004).
Tr, Trace; -, not detected.

Fatty acid	1	2	3	4
Saturated straight-chain:				
$C_{14:0}$	3.9	0.9	Tr	1.2
$C_{15:0}$	10.1	0.8	-	Tr
$C_{16:0}$	3.3	6.3	5.5	9.1
$C_{17:0}$	-	0.2	-	-
$C_{18:0}$	0.3	4.5	0.9	1.2
Unsaturated straight-chain:				
$C_{16:1}$	-	Tr	1.2	2.0
$C_{18:2}$	-	-	1.4	2.3
$C_{18:1}$	-	0.8	17.2	17.9
Hydroxy acids:				
iso-$C_{15:0}$ 3-OH	-	0.2	0.6	-
$C_{15:0}$ 3-OH	-	0.1	-	-
iso-$C_{16:0}$ 3-OH	3.9	0.2	1.3	1.3
$C_{16:0}$ 3-OH	6.8	2.5	2.8	4.8
iso-$C_{17:0}$ 3-OH	9.1	6.8	13.5	9.6
anteiso-$C_{17:0}$ 3-OH	2.1	-	1.5	1.7
$C_{17:0}$ 3-OH	0.6	0.2	Tr	-
Saturated branched-chain:				
iso-$C_{13:0}$	4.4	-	Tr	-
anteiso-$C_{13:0}$	1.9	0.8	-	-
iso-$C_{14:0}$	7.9	1.4	3.6	2.8
iso-$C_{15:0}$	11.9	15.5	6.7	8.2
anteiso-$C_{15:0}$	27.3	37.0	23.5	25.2
iso-$C_{16:0}$	-	3.4	3.3	1.9
iso-$C_{17:0}$	0.1	11.8	2.1	1.2
anteiso-$C_{17:0}$	0.2	6.3	3.4	2.1