COVER SHEET

Xylanibacter oryzae gen. nov., sp. nov., a novel strictly-anaerobic, Gram-negative, xylanolytic bacterium isolated from rice plant residue in flooded rice-field soil in Japan

Atsuko Ueki[1], Hiroshi Akasaka[1,2], Daisuke Suzuki[1], Satoshi Hattori[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

Author for correspondence: Atsuko Ueki. Tel: +81 235 28 2866. Fax: +81 235 28 2846.

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

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

Key words: *Prevotella*, anaerobic Gram-negative rods, hemin, propionate production, rice field soil

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain KB3^T is AB078826.

ABSTRACT

A strictly anaerobic, xylanolytic bacterium, strain KB3^T, isolated from rice plant residue in flooded anoxic rice-field soil in Japan was characterized phenotypically and phylogenetically. Cells were Gram-negative, non-motile, non-spore-forming, short to filamentous rods. Growth of the strain was remarkably stimulated with the addition of hemin to the medium. The strain utilized various sugars including xylan, xylose, pectin and carboxymethylcellulose and produced acetate, propionate and succinate with a small amount of malate. Propionate production was stimulated with the addition of B-vitamin mixture or cobalamin to the medium. The strain was slightly acidophilic with the optimum pH 5.7-6.2 and the optimum growth temperature was

30ºC. Oxidase, catalase and nitrate-reducing activities were negative. Aesculin was hydrolyzed. The major cellular fatty acids were anteiso-$C_{15:0}$ and iso-3-OH $C_{17:0}$. Menaquinones MK-12(H_2) and MK-13(H_2) were the major respiratory quinones. The genomic DNA G + C content was 43.6%. Phylogenetic analysis based on 16S rRNA gene sequence placed the strain in the phylum "*Bacteroidetes*". The closest related species was *Prevotella bivia* with 16S rRNA gene sequence similarity of 89.5%. *Prevotella albensis* and *Prevotella oulorum* were the next closest known species with sequence similarity of 89.1%. Based on the comprehensive consideration for the differences in the phylogenetic, ecological, physiological and chemotaxonomic characteristics of strain KB3^T from those of the related species, *Xylanibacter oryzae* gen. nov., sp. nov. is proposed to accommodate the strain. The type strain of the novel species is KB3^T (= JCM 13648^T = DSM 17970^T).

MAIN TEXT

Rice is widely cultivated as the principal food using irrigated fields in Japan. In anoxic rice-field soil during the flooding period, 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, thus producing methanogenic substrates such as acetate, formate and H_2. Methane produced depending on these substrates is emitted to the atmosphere as one of the major greenhouse gases (Takai, 1970; Seiler *et al*., 1984; Boon, 2000; Khalil, 2000; Wassmann *et al*., 2000a, b).

We have isolated various fermentative anaerobes from samples of rice plant residue as well as living rice roots in irrigated rice field soil in the course of investigation on microbes in anoxic rice field soil (Satoh *et al*. 2002; Akasaka *et al*., 2003a; Akasaka *et al*., 2004). We have previously described two novel anaerobic,

propionate-producing species for the strains isolated from rice straw samples (Akasaka *et al*., 2003b; Ueki *et al*., 2006). In this study, we have characterized one of the isolates (strain KB3^T) from stubble and roots, which were pylogenetically distant from any related recognized species. The isolate was a strictly anaerobic, xylanolytic and propionate-producing bacterium consisting of Gram-negative, non-motile rod-like cells. Based on the phylogenetic, ecological, physiological and chemotaxonomic characteristics, we propose a novel genus and species in the phylum "*Bacteroidetes*" to accommodate the strain.

Strain KB3^T (= JCM 13648^T = DSM 17970^T) was isolated from a rice plant residue (rice stubble and roots) sample 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. Cultivation practices for rice plants and other conditions of the fields were described previously (Ueki *et al*., 2000). The strain was isolated by the anaerobic roll tube method for enumeration of anaerobic fermentative bacteria by the colony-counting method (Hungate, 1966; Holdeman *et al*., 1977). Plant residue samples (rice stubble and roots) homogenized by a Waring blender (10000 rpm, 10 min.) under N_2 gas were used as sources for isolation (Akasaka *et al*., 2003a, 2004).

The strain was cultivated anaerobically at 30ºC unless otherwise stated by using peptone/yeast extract (PY) medium as basal medium with oxygen-free, 95% N_2/ 5% CO_2 mixed gas as the headspace as described by Akasaka *et al*. (2003a). 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 strain in agar slants. PY liquid medium supplemented with hemin (at a final concentration of 5 mg l^{-1}) (Holdeman

et al., 1977). and the B-vitamin mixture (10 ml l^{-1}) (PYHV medium) as well as 10 g glucose l^{-1} (PYHVG medium) was used for the cultivation of the strain for various physiological tests and chemotaxonomic analyses of the cells unless otherwise stated. Since strain KB3^T was slightly acidophilic as described below, pH of liquid media was usually adjusted to about pH 6.0. 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 strain 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 80^{o}C 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 characterization was performed according to the methods as described by Holdeman *et al*. (1977) and Ueki *et al*. (2006).

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 NCIMB Japan based on the MIDI microbial identification system (Microbial ID) of MOORE (Moore *et al*., 1994). Microbial identification system of the TSBA40 was also used to confirm the identification. Isoprenoid quinones were extracted as described by Komagata & Suzuki (1987) and analyzed by using a mass spectrometer (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 µBondpack C18 column (3.9 × 300 mm; Waters).

DNA extraction and PCR amplification were performed according to the method described by Akasaka *et al*. (2003a). 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). Multiple alignments of the sequence with reference 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.

Cells of strain KB3^T were Gram-negative rods. The strain grew very slowly in PYG liquid medium without hemin. The addition of hemin to the medium greatly enhanced the growth as described below and morphology of the cells was significantly changed depending on the growth medium. Most of the cells

grown in the presence of hemin were short rods (0.6-0.7 × 2.2-2.6 μm) with some longer rods (4-10 μm) (Fig. 1A), while cells grown on PY4S agar slants (without hemin) often occurred in filamentous rods (20-50 μm long) with some chains of cells (Fig. 1B). Cells were non-motile as observed phase-contrast microscopy. Colonies on PY4S agar were translucent and thin with smooth surface and were 0.5-0.8 mm diameter after 3-4 days of incubation. The strain could not 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.

The specific growth rate (μ) of strain KB3^T in PYG liquid medium (without hemin) was very low at 0.015 h^{-1} at 30ºC. In the presence of hemin (5 mg l^{-1}) (PYHG medium), the strain grew very rapidly at a growth rate of 0.29-0.33 h^{-1}. Hemin at a lower concentration (0.5 mg l^{-1}) also supported the rapid growth Further addition of the B-vitamin mixture to the medium (PYHVG medium) did not apparently affect the growth rate, while the addition changed the fermentation products of the strain as described below. Vitamin K did not affect the growth and fermentation of the strain.

When cells were cultivated to stationary growth phase in the presence of hemin and were used as an inoculum for growth experiments, the growth of the strain was often found to be considerably delayed irrespective of the hemin concentrations in the medium. Thus, viability or culturability of the cells grown in the presence of hemin seemed to be significantly reduced soon in the stationary phase. Thus, for stable and reproducible growth experiments, it was essential to use cells in the late-exponential or early-stationary growth phase. Cells cultivated without hemin did not show any significant decline of viability even at the stationary phase. Thus, PY4S agar, as a medium without hemin, was usually used for maintenance of the strain as slant cultures.

Both catalase and oxidase were negative and the strain did not reduce nitrate. The strain utilized arabinose, ribose, xylose, fructose, galactose, glucose, mannose, rhamnose, cellobiose, lactose, maltose, sucrose, trehalose, carboxymethylcellulose (CMC), soluble starch, xylan, pectin, salicin and pyruvate as growth substrates. Acids were produced from all substrates used, while gas was not. The strain did not use sorbose, melezitose, cellulose powder, filter paper, inulin, glycerol, inositol, mannitol, fumarate, lactate, malate and succinate.

The major products of the strain in PYHG medium were acetate and succinate, while propionate production was significantly stimulated in the presence of the B-vitamin mixture (Table 1). The strain also produced substantial amounts of acetate, propionate and succinate from xylan, and with this substrate propionate production was also significantly stimulated by the addition of the B-vitamin mixture. The vitamin mixture could be completely replaced by cyanocobalamin of the same concentration in the vitamin mixture (at a final concentration of 10 μg l^{-1}). Propionate was also a major product from pectin, CMC and pyruvate in the presence of vitamin, while succinate was a minor product. A small amount of malate was usually detected. Formate was not detected from any of the substrates examined.

Aesculin was hydrolyzed, but gelatin was not. Production of urease, hydrogen sulfide and indole were negative. The strain could not grow in the presence of 0.1% (w/v) of bile salts, irrespective of the presence or absence of hemin, demonstrating that the strain was sensitive to bile salts.

Strain KB3^T was slightly acidophilic with pH optimum at 5.7-6.2 and pH range of 4.7-7.3 for growth. The growth was significantly delayed even at the initial pH 7.3. When grown in PYHVG medium with the initial

pH 6.1, the final pH was 4.1. Growth temperature range for growth was 10-37°C with optimum temperature of 30°C. The growth rate at 37°C (μ = 0.194 h^{-1}) was significantly lower than that at 30°C (0.29-0.33 h^{-1}) and the strain could not grow at 37°C in the absence of hemin. NaCl concentration range for growth was 0-0.5% (w/v).

The major CFAs of strain KB3^T were anteiso-$C_{15:0}$ (42.2%) and iso-3-OH $C_{17:0}$ (21.8%) with lower amounts of 3-OH $C_{17:0}$ (5.2%), $C_{15:0}$ (4.7%), anteiso-3-OH $C_{17:0}$ (3.7%), iso-$C_{15:0}$ (3.7%), iso-$C_{17:0}$ (3.3%) and $C_{16:0}$ (3.1%). Unsaturated fatty acids were not detected. The predominant respiratory quinones of the strain were menaquinones MK-12(H_2) and MK-13(H_2). G + C content of genomic DNA was 43.6%.

Strain KB3^T was assigned to the phylum "*Bacteroidetes*" (Garrity & Holt, 2001) based on 16S rRNA gene sequence (Fig. 2). The closest recognized species of strain KB3^T were *Prevotella bivia* ATCC 29303^T (originally described as *Bacteroides bivius*) (Holdeman & Johnson, 1977; Holdeman *et al*., 1984) with sequence similarity of 89.5%, and *Prevotella albensis* DSM 11370^T (formerly *Prevotella ruminicola* subsp. *ruminicola* biovar. 7) (Holdeman *et al*., 1984; Avgustin *et al*., 1997) and *Prevotella oulorum* ATCC 43324^T (Shah *et al*., 1985) were the next closely related species (both with sequence similarity of 89.1%). The fourth and fifth closely related species were also the species in the genus *Prevotella* (*P. corporis* and *P. loescheii*, with 88.8% and 88.7% similarity, respectively) (Holdeman *et al*., 1984). These closely related species of strain KB3^T are all isolates from mammalian species, including the urogenital region of humans (*P. bivia*), rumen (*P. albensis*), oral cavities of humans (*P. oulorum* and *P. loescheii*) and other human clinical specimens (*P. corporis*) (Holdeman *et al*., 1984; Shah *et al*., 1985).

Some characteristics of strain KB3^T and the three most closely related species are compared in Table 2. Phylogenetic analysis showing similarity values less than 90% of 16S rRNA gene sequence with the closest relative indicated that strain KB3^T only distantly related to the recognized species. The genus *Prevotella* mainly consists of species from human oral or urogenital sources with important exceptions isolated from the rumen such as *Prevotella ruminicola*, *P. albensis*, *Prevotella brevis* and *Prevotella bryantii* (Shah & Collins, 1989, 1990; Paster *et al*., 1994; Avgustin *et al*., 1997). Strain KB3^T was isolated from an extremely different environment, rice plant residue in anoxic rice field soil, from the known habitats of the *Prevotella* species. The optimum growth temperature of strain KB3^T at 30^{o}C may reflect the ecological difference of the strain from those of the related species. In addition to the low similarity level of 16S rRNA gene sequence, the obvious ecological difference strongly indicates that strain KB3^T should represent a new taxon in the phylum "*Bacteroidetes*", living in anaerobic environments other than mammals.

Many species in the genus *Prevotella* have a requirement for hemin for growth, and the closest relatives of strain KB3^T, *P. bivia, P. albensis and P. oulorum*, also require hemin for growth or are usually cultivated in the presence of hemin in the medium (Holdeman *et al*., 1984; Shah *et al*., 1985). Growth of strain KB3^T was also strongly stimulated by the addition of hemin to the medium. Since the hemin requirement of *Prevotella* species has often been considered in relation to heme or hemoglobin derived from host animals (Leung & Folk, 2002), it is of interest that hemin also stimulated the growth of a bacterium isolated from a plant residue sample in flooded soil. Although cells of *Prevotella* or *Bacteroides* species are known to be often pleomorphic depending on the culture conditions (Holdeman *et al*., 1984), the morphology of long

filamentous rods cultivated in the absence of hemin seems to give a unique feature to strain KB3^T together with the slightly acidophilic property.

Ranges of substrate utilization of the two related species (*P. bivia* and *P. oulorum*) are relatively restricted and these species are not able to utilize carbohydrates such as arabinose, xylose and cellobiose. Furthermore, utilization of polymers such as xylan, pectin and CMC is never reported. Strain KB3^T is able to use various carbohydrates including polymers (xylan, pectin, CMC and starch) and disaccharides (e.g., cellobiose, trehalose and sucrose) as well as hexoses and pentoses including arabinose and xylose. *P. albensis* isolated from rumen utilizes a relatively wide range of carbohydrate including xylose and xylan, which were key characteristics to divide *Prevotella ruminicola* into subspecies (*P. ruminicola* subsp. *ruminicola* and *P. ruminicola* subsp. *brevis*) (Holdeman *et al*., 1984). *P. albensis*, which was established from the former subspecies, is reported to utilize pectin strongly as well as arabinose and cellobiose. Thus, strain KB3^T has common characteristics with *Prevotella* species from rumen as far as the utilization of polymers, however, the range of carbohydrate utilization of strain KB3^T is different from that of *P. albensis* (e.g., mannose, sucrose, trehalose and CMC) (Table 1).

P. bivia and *P. oulorum* produce acetate and succinate as major acids from glucose and production of propionate has never been reported. As for *P. albensis*, major acids from glucose are succinate, acetate and formate. Propionate is not recognized as a major acid, if not produced (Holdeman *et al*., 1984; Shah *et al*., 1985). Strain KB3^T produced a substantial amount of propionate from xylan irrespective of the presence of hemin. In the presence of the B-vitamin mixture, propionate was usually the major end-product from

substrates examined. Since the vitamin mixture could be completely replaced by cyanocobalamin, strain KB3^T was considered as a propionate-producing bacterium depending on the supply of exogenous cobalamin. A propionate-producing bacterium, *Propionicimonas paludicola*, which requires cobalamin for growth and propionate production has been also isolated from the rice plant residue samples in the same rice field (Akasaka *et al*., 2003b). Cobalamin requirement to produce propionate seems to be rather common physiological characteristics in anaerobic bacteria living in anoxic environments like flooded soil.

G + C content of genomic DNA of strain KB3^T (43.6%) is a similar level to those of the related species (Table 2) (Holdeman *et al*., 1984; Shah *et al*., 1985). It is reported that the major CFAs of species in the two genera of *Bacteroides* and *Prevotella* 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 iso-$C_{15:0}$ and $C_{16:0}$ were only minor components of CFAs of strain KB3^T, the overall pattern of the CFAs composition with anteiso-$C_{15:0}$ and iso-3-OH $C_{17:0}$ as major components seems to be in the variation of those of *Bacteroides* and *Prevotella* species reported. However, since the closest relative of strain KB3^T, *P. bivia*, has unsaturated fatty acid ($C_{18:1}$) as the most dominant CFAs (18.9%) together with anteiso-$C_{15:0}$ (16.9%) and iso-3-OH $C_{17:0}$ (17.9%) as major CFAs (Sakamoto *et al*., 2004), the CFAs composition of strain KB3^T is rather different from that of the closest relative.

P. bivia possesses menaquinones MK-9 (5%), MK-10 (70%) and MK-11 (23%) (Sakamoto *et al*., 2005) and *P. oulorum* has MK-10 (Shah *et al*., 1985). *Prevotella* species from rumen usually has menaquinone MK-11 as one of predominant menaquinones (Shah & Collins, 1980). Since strain KB3^T possessed menaquinones MK-12(H_2) and MK-13(H_2), the menaquinone composition is rather different from those of the related *Prevotella* species.

Strain KB3^T were isolated from rice plant residue (plant stubble and roots) together with very closely related strains (strains KB10, KB11 and KB13; accession number for the 16S rRNA gene sequences AB07880, AB078831 and AB078833, respectively; strain KB10 = JCM 13649 = DSM 17971), (Akasaka *et al*., 2003a) (Fig. 2). The population density of the bacterial group of strain KB3^T determined by the dilution-colony-counting method was enumerated at the order of 10^9 CFU/g of dry weight. Thus, the bacterial group seems to be one of dominant groups living in plant residue in rice field soil, which has functions in decomposing plant biomass such as hemicellulose and pectin as well as substrates derived from the decomposition of these components including solubilized cellulose.

Strain KB3^T is phylogenetically distant from the closest related species all derived from mammals and has distinct characteristics from those of the related species. Analyses using various environmental clones from various samples other than animals have shown that bacterial groups belonging to the phylum "*Bacteroidetes*" are frequently detected as one of predominant groups and most of them have never been cultivated (Lydell *et al*., 2004; Chouari *et al*., 2005). Strain KB3^T may represent such uncultured bacterial groups mainly taking roles in anaerobic decomposition of plant biomass.

On the basis of above-mentioned comprehensive analyses of the phenotypic, chemotaxonomic and phylogenetic characteristics as well as the ecological property, we propose here a novel genus, *Xylanibacter* gen. nov., with *Xylanibacter oryzae* sp. nov. as the type species to accommodate strain KB3^T.

Description of *Xylanibacter* gen. nov.

Xylanibacter (Xy.la.ni.bac'ter. N.L. n. *xylanum* xylan; N.L. masc, n. *bacter* a rod; N.L. masc, n. *Xylanibacter* rod decomposing xylan).

Cells are Gram-negative, non-spore-forming, non-motile, short to filamentous rods. Strictly anaerobic. Chemoorganotroph. Optimum growth temperature is 30°C. Oxidase, catalase and nitrate-reducing activities are negative. Utilize various sugars including xylan and produce acetate, propionate and succinate as major fermentation end products. Major cellular fatty acids are anteiso-$C_{15:0}$ and iso-3-OH $C_{17:0}$. Major respiratory quinones are MK-12(H_2) and MK-13(H_2). The type species is *Xylanibacter oryzae.*

Description of *Xylanibacter oryzae* sp. nov.

Xylanibacter oryzae (o'ry.zae. L. fem. n. *oryza* rice and the genus name of rice; L. gen. n. *oryzae* from/of rice or rice plants, referring to rice-plant residue from which the strain was isolated).

Has the following properties in addition to those given for the genus. Hemin significantly stimulates growth. In the presence of B-vitamin mixture as well as hemin, acetate, propionate and succinate are produced as major fermentation products. Slightly acidophilic and grows with pH optimum at 5.7-6.2 (pH range 4.7-7.3). Growth temperature range is 15-35°C with optimum at 30°C. Growth at 37°C is significantly delayed. NaCl concentration range for growth is 0-0.5% (w/v) in PYG medium containing hemin and B-vitamin mixture. Utilizes arabinose, ribose, xylose, fructose, galactose, glucose, mannose, rhamnose, cellobiose, lactose, maltose, sucrose, trehalose, CMC, soluble starch, xylan, pectin, salicin and pyruvate as growth substrates. Acids are produced from these substrates, while gas is not. Does not use sorbose, melezitose, cellulose powder, filter paper, inulin, glycerol, inositol, mannitol, fumarate, lactate, malate and succinate. Aesculin is hydrolyzed, but gelatin is not. Urease is negative. Hydrogen sulfide and indole are not produced. Does not

grow in the presence of bile salts. The genomic DNA G + C content is 43.6%. The type strain, KB3^T (= JCM 13648^T = DSM 17970^T), was isolated from stubble and roots of rice plant residue in anoxic rice-field soil in Japan.

ACKNOWLEDGMENTS

We are grateful to Yoshimi Ohtaki for her technical assistance on physiological examinations of the strain. This work was partly supported by a Grant-in-Aid for Scientific Research (no. 16580271) from the Japan Society for Promotion of Science and also by the Project for Development of Technology for Analyzing and Controlling the Mechanism of Biodegrading and Processing supported by the New Energy and Industrial Technology Development of Organization (NEDO).

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Figure legends

Fig. 1. Phase-contrast photomicrographs of cells of strain KB3^T grown anaerobically in PYG liquid medium with both hemin and the B-vitamin mixture (A) or on an agar slant of PY4S medium without either hemin or the B-vitamin mixture (B). Bar, 10 µm.

Fig. 2. Neighbor-joining tree showing the phylogenetic relationship of strain KB3^T and related species in the phylum *Bacteroidetes* based on 16S rRNA gene sequences. 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.

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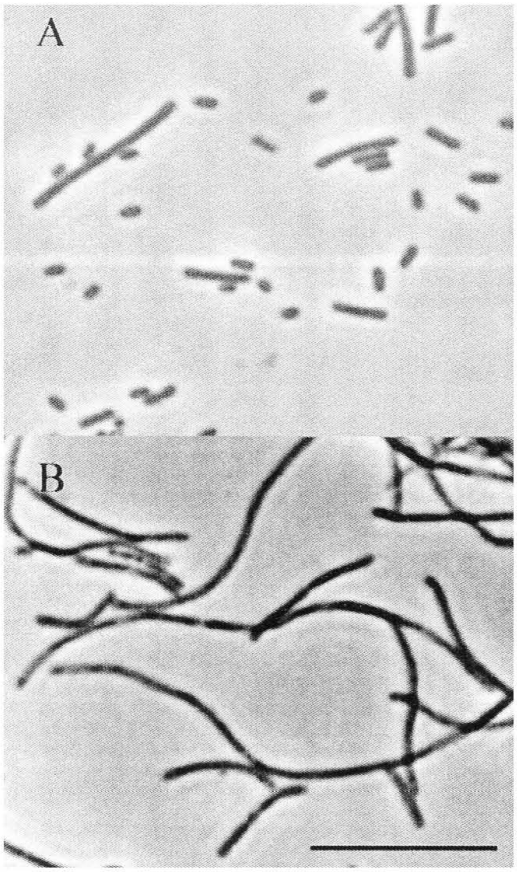

Fig. 2

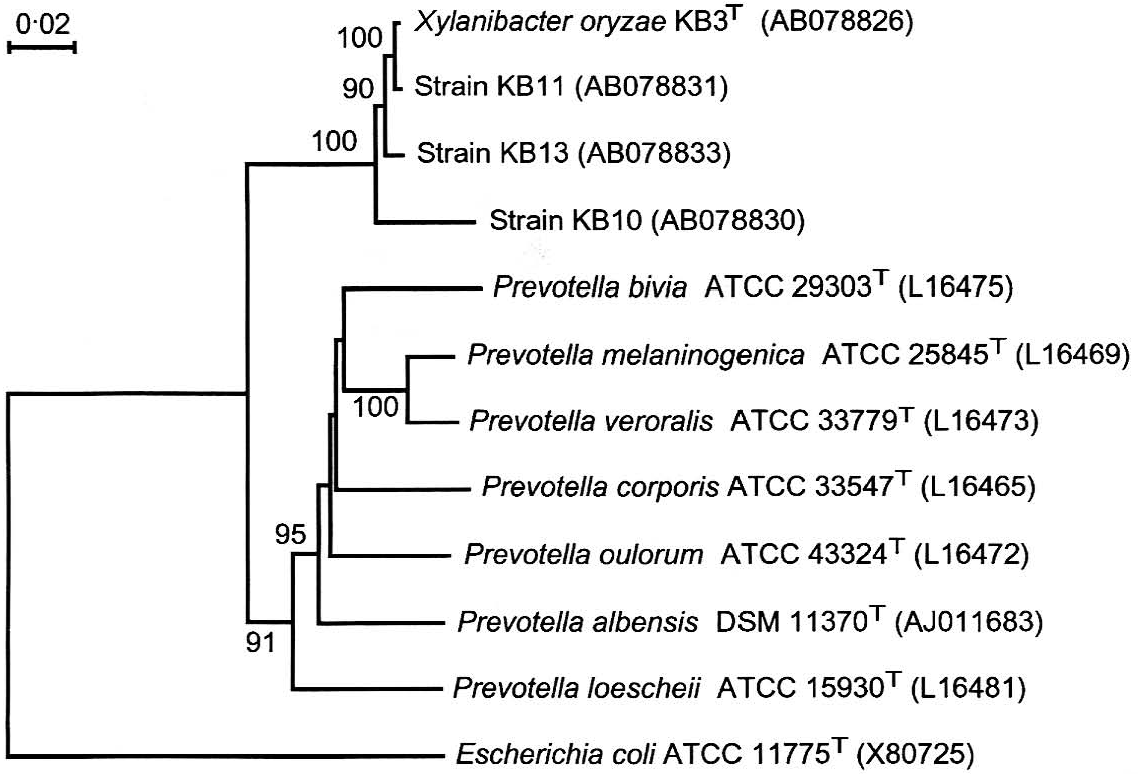
0·02
100
90
100
100
95
91
Xylanibacter oryzae KB3T (AB078826)
Strain KB11 (AB078831)
Strain KB13 (AB078833)
Strain KB10 (AB078830)
Prevotella bivia ATCC 29303T (L16475)
Prevotella melaninogenica ATCC 25845T (L16469)
Prevotella veroralis ATCC 33779T (L16473)
Prevotella corporis ATCC 33547T (L16465)
Prevotella oulorum ATCC 43324T (L16472)
Prevotella albensis DSM 11370T (AJ011683)
Prevotella loescheii ATCC 15930T (L16481)
Escherichia coli ATCC 11775T (X80725)

Table 1. Fermentation products of strain KB3^T from different substrates with or without vitamin in PY medium supplemented with hemin. Concentration of substrates: pyruvate, 30 mM, others, 1% (w/v). +, with B-vitamin mixture, -, without B-vitamin mixture, +*, cyanocobalamin (10 µg l^{-1}) in place of B-vitamin mixture. tr, trace, ND, not detected.

Substrate	Vitamin	Fatty acid (mmol l^{-1})			
		Acetate	Propionate	Succinate	Malate
Glucose	–	6.1	0.3	14.1	0.2
	+	9.4	6.1	15.7	0.3
Xylan	–	7.4	3.4	8.3	tr
	+	9.2	12.4	4.4	0.1
	+*	7.2	10.9	3.8	0.1
Pectin	+	3.3	8.6	0.1	ND
CMC	+	2.4	11.4	0.6	ND
Pyruvate	+	15.1	11.8	0.5	0.1

Table 2. Some differential characteristics of the species related to strain KB3^T. Strains: 1. Strain KB3^T 2. *Prevotellla bivia* ATCC 29303^T (data from Holdeman *et al* ., 1984); 3. *Prevotella albensis* DSM 11370^T (data from Holdeman *et al* ., 1984; Avgustin *et al* ., 1997; Data of major cellular fatty acids and predominant quinones are those of *Bacteroides ruminicola* subsp. *ruminicola* from Shah & Collins,1980). 4. *Prevotella oulorum* ATCC 43324^T (data from Shah *et al* ., 1985). +, Positive; -, negative; nd, no data available. F, Formate; A, Acetate; P/p, Propionate; S, Succinate; m, Malate; ib, iso-Butyrate; iv, iso-Valerate. Lower-case letters indicate minor products. Products in parentheses may or may not be detected.

Characteristics	1	2	3	4
Habitat	Plant residue in rice field soil	Urogential clinical specimen	Rumen	Oral cavity
Optimum temp. (ºC)	30	37	37	37
Optimum pH	5.7-6.2	6.7-6.9	6.7-6.9	6.8
G+C (%)	43.6	40.0	39-43	45-46
Major celluar fatty acids	anteiso-$C_{15:0,}$ iso-3-OH $C_{17:0}$	anteiso-$C_{15:0,}$ iso-3-OH $C_{17:0,}$ $C_{18:1}$	anteiso-$C_{15:0,}$ $C_{15:0}$	anteiso-$C_{15:0}$, iso-$C_{15:0,}$ iso-$C_{17:0}$
Predominant quinones	MK-12(H_2) MK-13(H_2)	MK-9, MK-10, MK-11	MK-11, MK-12	MK-10
Gelatin hydrolysis	-	+	-	-
Aesculin hydrolysis	+	-	+	+
Products from glucose	A, P, S (m)	A, S (ib, iv)	A, S, F (p, ib, iv)	A, S
Acids production from:				
Arabinose	+	-	+	-
Xylose	+	-	+	-
Mannose	+	+	-	+
Cellobiose	+	-	+	-
Sucrose	+	-	-	+
Trehalose	+	-	-	-
CMC	+	nd	-	nd
Inulin	-	-	-	+
Pectin	+	nd	+	nd
Xylan	+	nd	+	nd
Salicin	+	-	+	-