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

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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 by the addition of haemin to the medium. The novel 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 by the addition of a B-vitamin mixture or cobalamin to the medium. The novel strain was slightly acidophilic with an optimum pH 5.7–6.2 and the optimum growth temperature was 30 °C. Oxidase, catalase and nitrate-reducing activities were negative. Aesculin was hydrolysed. The major cellular fatty acids were anteiso-C_{15:0} and iso-3-OH C_{17:0}. The major respiratory quinones were menaquinones MK-12(H₂) and MK-13(H₂). The genomic DNA G + C content was 43.6 mol%. Phylogenetic analysis based on 16S rRNA gene sequence placed the strain in the phylum *Bacteroidetes*. The closest related species was *Prevotella bivia* with a 16S rRNA gene sequence similarity of 89.5%. *Prevotella albensis* and *Prevotella oulorum* were the next closest recognized species with sequence similarities of 89.1%. Based on a comprehensive examination of the differences in phylogenetic, ecological, physiological and chemotaxonomic characteristics of strain KB3^T and those of related species, a novel genus and species, *Xylanibacter oryzae* gen. nov., sp. nov., is proposed to accommodate strain KB3^T. The type strain of the novel species is KB3^T (=JCM 13648^T = DSM 17970^T).

As the principal food crop, rice is widely cultivated in Japan using irrigated fields. Diverse fermentative bacterial groups play a key role in the decomposition of organic matter in anoxic rice-field soil during the flooding period. Plant residues such as rice straw, stubble and roots ploughed into the soil are utilized by bacteria and methanogenic substrates such as acetate, formate and H₂ are produced. The methane produced from these substrates is released into the atmosphere as one of the major greenhouse gases (Takai, 1970; Seiler *et al.*, 1984; Boone, 2000; Khalil, 2000; Wassmann *et al.*, 2000a, b).

During the course of an investigation into microbes in anoxic rice-field soil, we have isolated various fermentative anaerobes from samples of rice-plant residue, as well as living rice roots, in irrigated rice-field soil (Satoh *et al.*, 2002; Akasaka *et al.*, 2003a, 2004). We have previously described two novel anaerobic, propionate-producing species that were isolated from rice straw samples (Akasaka *et al.*, 2003b; Ueki *et al.*, 2006). In this study, a novel strain, KB3^T, one of a group of isolates from stubble and roots that were phylogenetically distant from any related recognized species, was characterized. The novel isolate was a strictly anaerobic, xylanolytic and propionate-producing bacterium consisting of Gram-negative, non-motile rod-like cells. Based on phylogenetic, ecological, physiological and chemotaxonomic characteristics, we propose a novel genus and species in the phylum *Bacteroidetes* to accommodate strain KB3^T.

Strain KB3^T was isolated from a sample of rice-plant residue (rice stubble and roots) collected from irrigated rice-field soil at the Shonai Branch of the Yamagata Agricultural

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

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

Experimental Station (Tsuruoka, Yamagata, Japan) during the flooding period of the field. Cultivation practices for rice plants and other conditions of the fields have been described previously (Ueki *et al.*, 2000). The strain was isolated by the anaerobic roll tube method for the enumeration of anaerobic fermentative bacteria by the colony-counting method (Hungate, 1966; Holdeman *et al.*, 1977). Plant residue samples (rice stubble and roots) homogenized using a Waring blender (10 000 r.p.m., 10 min) under N₂ gas were used as sources for the isolation of novel strains (Akasaka *et al.*, 2003a, 2004).

The novel strain was cultivated anaerobically at 30 °C unless otherwise stated. Peptone-yeast extract (PY) medium was used as the basal medium with oxygen-free, 95 % N₂/5 % CO₂ mixed gas as the headspace as described by Akasaka *et al.* (2003a). PY medium supplemented with (l⁻¹) 0.25 g glucose, cellobiose, maltose and soluble starch and 15 g agar (Difco) was designated PY4S agar and used for the maintenance of the novel strain in agar slants. For the cultivation of the strain for various physiological tests and chemotaxonomic analyses, PYHV medium [PY liquid medium supplemented with haemin (at a final concentration of 5 mg l⁻¹; Holdeman *et al.*, 1977) and B-vitamin mixture (10 ml l⁻¹)] and PYHVG medium (PYHV medium supplemented with 10 g glucose l⁻¹) were used, unless otherwise stated. Since strain KB3^T was slightly acidophilic, as described below, the 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 B₁₂), 0.3 mg *p*-amino-benzoic 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 OD₆₆₀.

Growth of the novel strain under aerobic conditions was examined by plate culture on nutrient agar (Nissui Pharmacy) and PY4S agar modified to exclude Na₂CO₃, cysteine-HCl-H₂O and sodium resazurine in PY basal medium. Spore formation was assessed by observation of cells after Gram-staining and by growth of cells exposed to 80 °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 a concentration of 10 g l⁻¹ (for sugars and sugar alcohols) or 30 mM (organic acids). Bile sensitivity was determined by the addition of bile salts (Oxoid; 0.1–0.5 %, w/v) to PYHVG and PYG media (Lawson *et al.*, 2002). Fermentation products were analysed by GC or HPLC as described previously (Ueki *et al.*, 1986; Akasaka *et al.*, 2003a). Other characterizations were performed according to the methods 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 analysed by GC (HP6890; Hewlett

Packard or G-3000; Hitachi) equipped with a HP Ultra2 column. CFAs were identified by equivalent chain-length (ECL) (Miyagawa *et al.*, 1979; Ueki & Suto, 1979) according to Moore's protocol of NCIMB Japan based on the MIDI microbial identification system (Microbial ID) (Moore *et al.*, 1994). The microbial identification system of the TSBA40 was also used for confirmation of identification. Isoprenoid quinones were extracted as described by Komagata & Suzuki (1987) and analysed by using a mass spectrometer (JMS-SX102A; JEOL). Genomic DNA was extracted according to the method of Kamagata & Mikami (1991). Extracted DNA was digested with P1 nuclease by using a Yamasa GC kit (Yamasa shoyu) and the DNA G + C content was measured by HPLC (L-7400; Hitachi) 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). 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). 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 neighbour-joining method (Saitou & Nei, 1987) by using CLUSTAL W (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 haemin. The addition of haemin to the medium greatly enhanced growth as described below and the morphology of the cells was significantly altered depending on the growth medium. Most of the cells grown in the presence of haemin 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 haemin) often occurred as filamentous rods (20–50 µm long) with some chains of cells (Fig. 1b). Cells were non-motile as observed by phase-contrast microscopy. Colonies on PY4S agar were translucent and thin with a smooth surface and were 0.5–0.8 mm diameter after 3–4 days of incubation. The novel strain could not grow in air either on PY4S or nutrient agar. Spore formation was not observed and cells treated at 80 °C for 10 min did not grow.

The specific growth rate (µ) of strain KB3^T in PYG liquid medium (without haemin) was very low at 0.015 h⁻¹ at 30 °C. In the presence of haemin (5 mg l⁻¹; PYHG medium), the strain grew very rapidly with a growth rate of 0.29–0.33 h⁻¹. Haemin at a lower concentration (0.5 mg l⁻¹) also supported rapid growth. Addition of the B-vitamin mixture (PYHVG medium) did not apparently affect the growth rate, but the fermentation products of the strain were changed, as described below. Vitamin K did not affect the growth or fermentation of the novel strain.

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

When cells were cultivated to the stationary growth phase in the presence of haemin and then used as an inoculum for growth experiments, the growth of the strain was often found to be considerably delayed, irrespective of the haemin concentration of the medium. Thus, the viability or culturability of the cells grown in the presence of haemin appeared to be significantly reduced early 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 haemin did not show any significant decline of viability, even at the stationary phase. Thus, PY4S agar, a medium without haemin, was usually used for the maintenance of the novel strain as slant cultures.

Both catalase and oxidase activities were negative and the novel strain did not reduce nitrate. Strain KB3^T 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, but gas was not. Strain KB3^T did not use sorbose, melezitose, cellulose

powder, filter paper, inulin, glycerol, inositol, mannitol, fumarate, lactate, malate or succinate.

The major products of the novel strain in PYHG medium were acetate and succinate. Propionate production was significantly stimulated in the presence of the B-vitamin mixture (Table 1). Strain KB3^T 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 B-vitamin mixture could be completely replaced by cyanocobalamin at the same concentration as in the vitamin mixture (final concentration of 10 μ g l⁻¹). Propionate was also a major product from pectin, CMC and pyruvate in the presence of the vitamin mixture, 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 hydrolysed, but gelatin was not. Production of urease, hydrogen sulfide and indole were negative. The novel strain could not grow in the presence of 0.1 % (w/v) bile salts, irrespective of the presence or absence of haemin, demonstrating that the strain was sensitive to bile salts.

Strain KB3^T was slightly acidophilic with the optimum pH at 5.7–6.2 and a pH range of 4.7–7.3 for growth. Even at an initial pH of 7.3, growth was significantly delayed. When the novel strain was grown in PYHVG medium at an initial pH of 6.1, the final pH was 4.1. The temperature range for growth was 10–37 °C, with an optimum temperature of 30 °C. The growth rate at 37 °C ($\mu = 0.194$ 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 haemin. The NaCl concentration range for growth was 0–0.5 % (w/v).

Table 1. Fermentation products of strain KB3^T from different substrates with or without vitamin in PY medium supplemented with haemin

Concentration of substrates: pyruvate, 30 mM, others, 1 % (w/v). +, With B-vitamin mixture; –, without 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

*Cyanocobalamin (10 μ g l⁻¹) in place of B-vitamin mixture.

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(H₂) and MK-13(H₂). The G+C content of genomic DNA was 43.6 mol%.

Strain KB3^T was assigned to the phylum *Bacteroidetes* (Garrity & Holt, 2001) based on the 16S rRNA gene sequence (Fig. 2). The closest recognized species to strain KB3^T was *Prevotella bivia* ATCC 29303^T (originally described as *Bacteroides bivius*) (Holdeman & Johnson, 1977; Holdeman *et al.*, 1984) with a gene sequence similarity of 89.5 %. *Prevotella albensis* DSM 11370^T (formerly *Bacteroides 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 most closely related species (both with gene sequence similarity of 89.1 %). The fourth and fifth most closely related species were also species of the genus *Prevotella* (*Prevotella corporis* and *Prevotella loescheii*, with 88.8 and 88.7 % gene sequence similarity, respectively) (Holdeman *et al.*, 1984). The species found to be most closely related to 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 revealed that strain KB3^T showed 16S rRNA gene sequence similarity values of less than 90 % with the closest recognized species. The genus *Prevotella* mainly consists of species from human oral or urogenital sources with important exceptions isolated from rumen, such as *P. 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 a

very different environment, rice-plant residue from anoxic rice-field soil, from the known habitats of the recognized *Prevotella* species. The optimum growth temperature of strain KB3^T at 30 °C may reflect the different environmental source of this strain compared with other related species. In addition to the low 16S rRNA gene sequence similarity values, the obvious ecological difference strongly indicates that strain KB3^T represents a novel taxon in the phylum *Bacteroidetes* that inhabits anaerobic environments other than mammals.

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

The ranges of substrate utilization of the 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 carbohydrates, including xylose and xylan, which were

Fig. 2. Neighbour-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. The sequence of *Escherichia coli* ATCC 11775^T, which belongs to the *Gammaproteobacteria* (Garrity & Holt, 2001), was used as the outgroup. Bar, 2 % estimated difference in nucleotide sequence position.

Table 2. Characteristics that differentiate strain KB3^T from related species

Strains: 1, strain KB3^T; 2, *Prevotella 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 for 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.

Characteristic	1	2	3	4
Habitat	Plant residue in rice-field soil	Urogenital clinical specimen	Rumen	Oral cavity
Optimum temperature (°C)	30	37	37	37
Optimum pH	5.7–6.2	6.7–6.9	6.7–6.9	6.8
DNA G + C content (mol%)	43.6	40.0	39–43	45–46
Major cellular 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
Acid production from:				
Arabinose	+	–	+	–
Cellobiose	+	–	+	–
CMC	+	ND	–	ND
Inulin	–	–	–	+
Mannose	+	+	–	+
Pectin	+	ND	+	ND
Salicin	+	–	+	–
Sucrose	+	–	–	+
Trehalose	+	–	–	–
Xylan	+	ND	+	ND
Xylose	+	–	+	–

key characteristics used to divide *Bacteroides ruminicola* into subspecies (*B. ruminicola* subsp. *ruminicola* and *B. ruminicola* subsp. *brevis*) (Holdeman *et al.*, 1984). *P. albensis*, established from the former subspecies, is reported to utilize pectin strongly as well as arabinose and cellobiose. Thus, strain KB3^T has characteristics common to *Prevotella* species isolated from rumen as regards 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. For *P. albensis*, the major acids from glucose are succinate, acetate and formate. Propionate is not recognized as a major acid (Holdeman *et al.*, 1984; Shah *et al.*, 1985). Strain KB3^T produced a substantial amount of propionate from xylan, irrespective of the presence of haemin. In the presence of the B-vitamin mixture, propionate was usually the major end product from the substrates tested. Since the vitamin mixture could be completely replaced by cyanocobalamin, strain KB3^T is considered to be 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 also has been isolated from rice-plant residue samples from the same rice-field (Akasaka *et al.*, 2003b). The requirement for cobalamin for the production of propionate seems to be a rather common physiological characteristic of anaerobic bacteria living in anoxic environments such as flooded soil.

The G + C content of the genomic DNA of strain KB3^T (43.6 mol%) is similar to those of related species (Table 2) (Holdeman *et al.*, 1984; Shah *et al.*, 1985). It has been reported that the major CFAs of species in the genera *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 the CFAs for strain KB3^T, the overall pattern of the CFA content with anteiso-C_{15:0} and iso-3-OH C_{17:0} as major components seems to be within the variation of those reported for species of the genera *Bacteroides* and *Prevotella*. However, since the closest relative of strain KB3^T, *P. bivia*, has unsaturated fatty acid (C_{18:1}) as the dominant CFA (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 CFA composition of strain KB3^T is rather different from that of its 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 have menaquinone MK-11 as one of predominant menaquinones (Shah & Collins, 1980). Since strain KB3^T possesses menaquinones MK-12(H₂) and MK-13(H₂), the menaquinone composition is rather different from those of related *Prevotella* species.

Strain KB3^T was isolated from rice-plant residue (plant stubble and roots) together with some very closely related strains [strains KB10 (=JCM 13649=DSM 17971), KB11 and KB13; GenBank 16S rRNA gene sequence accession numbers AB07880, AB078831 and AB078833, respectively] (Akasaka *et al.*, 2003a) (Fig. 2). The population density of this bacterial group, determined by the dilution-colony-counting method, was enumerated at the order of 10⁹ c.f.u. g⁻¹ dry weight. Thus, this bacterial group seems to be one of the dominant groups living in plant residues in rice-field soil and 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 that are all derived from mammals and has characteristics that are distinct from these related species. Analyses using various environmental clones from samples isolated from sources other than animals have shown that bacterial groups belonging to the phylum *Bacteroidetes* are frequently detected as one of the predominant groups and most of them have never been cultivated (Lydell *et al.*, 2004; Chouari *et al.*, 2005). Strain KB3^T may represent such an uncultured bacterial group taking a role in anaerobic decomposition of plant biomass.

On the basis of the comprehensive analyses of the phenotypic, chemotaxonomic and phylogenetic characteristics described above and its ecological origin, we propose a novel genus, *Xylanibacter* gen. nov., with strain KB3^T representing the type strain of the type species, *Xylanibacter oryzae* sp. nov.

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. Chemo-organotroph. 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(H₂) and MK-13(H₂). 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. Haemin significantly stimulates growth. In the presence of B-vitamin mixture as well as haemin, acetate, propionate and succinate are produced as major fermentation products. Slightly acidophilic and grows with an optimum pH of 5.7–6.2 (pH range, 4.7–7.3). Growth temperature range is 15–35 °C with the 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 haemin 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, but gas is not. Does not use sorbose, melezitose, cellulose powder, filter paper, inulin, glycerol, inositol, mannitol, fumarate, lactate, malate or succinate. Aesculin is hydrolysed, 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 mol%.

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.

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