Reclassification of *Xylanibacter oryzae* Ueki *et al.* 2006 as *Prevotella oryzae* comb. nov., with an emended description of the genus *Prevotella*

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16S rRNA gene sequence information has indicated that *Xylanibacter oryzae* has a close relationship with the genus *Prevotella*. To clarify the taxonomic position of *X. oryzae*, we determined the sequence of *hsp60* as it represents an alternative phylogenetic marker for identification and classification of Gram-negative anaerobic rods. On the basis of *hsp60* sequences, *X. oryzae* was located within the genus *Prevotella*, indicating that the species does not represent a distinct taxon at the genus level. Statistical tests (the Shimodaira–Hasegawa test and the approximately unbiased test) supported the finding that *X. oryzae* is monophyletic with members of the genus *Prevotella* and thus belongs to the genus. On the basis of the phylogenetic findings, we propose that *X. oryzae* should be reclassified as *Prevotella oryzae* comb. nov.; the type strain is KB3^T (=JCM 13648^T =DSM 17970^T). An emended description of the genus *Prevotella* is also provided.

A strictly anaerobic, xylanolytic bacterium, strain KB3^T, was isolated from rice-plant residue in flooded anoxic rice-field soil in Japan (Ueki *et al.*, 2006). This bacterium was phylogenetically related to members of the genus *Prevotella* (about 89% 16S rRNA gene sequence similarity). The genus *Prevotella* in the phylum *Bacteroidetes* comprises a variety of species and 16S rRNA gene sequence similarities among its members range from 85.6 to 97.7% (mean $\pm$ SEM 89.6 $\pm$ 0.1%) (Sakamoto & Ohkuma, 2010). Although strain KB3^T could have been assigned to the genus *Prevotella* from the viewpoint of sequence similarity, Ueki *et al.* (2006) proposed a novel species in a new genus, *Xylanibacter oryzae*, on the basis of phylogenetic, ecological, physiological and chemotaxonomic characteristics. The creation of the genus *Xylanibacter* particularly depended on ecological differences: members of the genus *Prevotella* have been isolated mainly from the oral cavity (some are associated with periodontitis) and also from, for example, the genito-urinary tract, vagina and cervix, which are environments very different from that in which *X. oryzae* was found. Of the members of the genus *Prevotella*, *Prevotella paludivivens* is the only species derived from a natural habitat, i.e. plant residue and rice roots in irrigated rice-field soil, similarly to *X. oryzae* (Ueki *et al.*, 2007).

Recently, *hsp60* has been found to be an alternative phylogenetic marker for the identification and

classification of a broad range of Gram-negative anaerobic rods, including the genera *Bacteroides*, *Barnesiella*, *Butyrivibrio*, *Odoribacter*, *Parabacteroides*, *Paraprevotella*, *Porphyromonas*, *Prevotella* and *Tannerella* (Kitahara *et al.*, 2011, 2012; Sakamoto & Ohkuma, 2010, 2012; Sakamoto *et al.*, 2010a, b). In this study, the taxonomic position of *X. oryzae* was determined using *hsp60* and 16S rRNA gene sequences.

A total of 109 strains of 108 species in 10 genera were obtained from the Japan Collection of Microorganisms (JCM), RIKEN BioResource Center, Wako, Japan. These belonged to the genera *Bacteroides* (38 strains from 38 species), *Barnesiella* (two strains from two species), *Butyrivibrio* (two strains from two species), *Odoribacter* (two strains from two species), *Parabacteroides* (five strains from five species), *Paraprevotella* (two strains from two species), *Porphyromonas* (17 strains from 17 species), *Prevotella* (38 strains from 38 species), *Tannerella* (one strain from one species) and *Xylanibacter* (two strains from one species). The strains were maintained according to the JCM Online Catalogue (<http://www.jcm.riken.jp/JCM/catalogue.shtml>) except for *X. oryzae* JCM 13648^T and JCM 13649 and *P. paludivivens* JCM 13650^T, which grew poorly on the medium specified by the depositor, PY4S agar (Ueki *et al.*, 2006, 2007); therefore, these strains were maintained on Columbia blood agar (Oxoid) supplemented with 5% (v/v) horse blood.

The physiological and biochemical characteristics of *X. oryzae* JCM 13648^T and JCM 13649 were determined in duplicate using the API 20A anaerobe test kit and the Rapid ID 32A anaerobe identification kit, respectively (bioMérieux), according to the manufacturer's

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *Prevotella oryzae* comb. nov. JCM 13648^T and JCM 13649 are AB588018 and AB588019 and for the *hsp60* sequences are AB675483 and AB675484, respectively.

instructions. Fatty acid methyl esters of *X. oryzae* JCM 13648^T and JCM 13649 were obtained from about 40 mg wet cells grown on Columbia blood agar at 30 °C for 72 h by saponification, methylation and extraction, using minor modifications (Kuykendall *et al.*, 1988) of the method of Miller (1982). The cellular fatty acid compositions were determined using the Sherlock Microbial Identification System version 2.99B (MIDI) with the BHIBLA database (version 3.80). Peaks were automatically integrated, fatty acids were identified by equivalent chain-lengths and percentages of the total peak area were calculated. External calibration was done using MIDI calibration mixture 1 (methyl esters of straight-chain saturated fatty acids with length 9–20 carbons and with five hydroxy acids). Iso-prenoid quinones were extracted as described by Komagata & Suzuki (1988) and analysed by HPLC with a Cosmosil 5C₁₈ column (4.6 × 150 mm; NacalaiTesque). The elution solvent was methanol/2-propanol (2 : 1, v/v).

Bacterial genomic DNA was extracted using a High Pure PCR Template Preparation kit (Roche Applied Science). The 16S rRNA sequence was determined as described previously (Sakamoto *et al.*, 2002). The partial *hsp60* sequence (558 bp) was determined as described previously (Sakamoto & Ohkuma, 2010; Sakamoto *et al.*, 2010b). Sequences were aligned using CLUSTAL X version 2.0.12 (Larkin *et al.*, 2007) and corrected by manual inspection. Nucleotide substitution rates (K_{nuc}) were calculated (Kimura, 1980) after gaps and unknown bases were eliminated. The phylogenetic tree was constructed by the neighbour-joining algorithm (Saitou & Nei, 1987). Maximum-likelihood analysis was performed using PhyML version 3.0 (Guindon & Gascuel, 2003), with the optimal model of nucleotide substitution estimated using jModelTest 0.1.1 (Posada, 2008) and the Akaike information criterion. The model selected was GTR+I+G for both genes. Maximum-likelihood analysis was also performed with raxmlGUI 0.95 using the GTRGAMMAI model (Silvestro & Michalak, 2010; Stamatakis, 2006). To estimate the confidence of tree topologies, bootstrap resampling analysis (Felsenstein, 1985) was performed using 1000 replications for both algorithms. The Shimodaira–Hasegawa test (Shimodaira & Hasegawa, 1999) and the approximately unbiased test (Shimodaira, 2002) in CONSEL 0.1i (Shimodaira & Hasegawa, 2001) were used for statistical comparisons of alternative trees of interest. The significance level was <0.05.

Cells of *X. oryzae* JCM 13648^T and JCM 13649 were obligately anaerobic, non-spore-forming, non-motile, Gram-negative, short to filamentous rods. Cells on Columbia blood agar were 0.8–1.0 µm wide and variable in length, mostly 2.0–15.0 µm. Colonies on Columbia blood agar after 72 h at 30 °C were 1–2 mm in diameter, circular, entire, slightly convex, smooth, white-greyish and translucent. The *X. oryzae* strains grew well on Columbia blood agar, similarly to members of the genus *Prevotella*. The phenotypic and biochemical characteristics of the two *X. oryzae* strains were the same and are given in the species description.

Table 1. Cellular fatty acid compositions of *Prevotella oryzae* comb. nov.

Strains: 1, KB3^T (data from Ueki *et al.*, 2006); 2, JCM 13648^T (this study); 3, JCM 13649 (this study). tr, Trace (<1 %); –, not detected.

Fatty acid (%)	1	2	3
Saturated straight-chain			
C _{9:0}	–	tr	–
C _{10:0}	–	tr	–
C _{11:0}	–	tr	–
C _{12:0}	–	tr	–
C _{13:0}	–	tr	–
C _{14:0}	–	tr	tr
C _{15:0}	4.7	1.0	2.2
C _{16:0}	3.1	4.4	6.4
C _{17:0}	–	tr	–
C _{18:0}	–	tr	tr
C _{19:0}	–	tr	–
C _{20:0}	–	tr	–
Unsaturated straight-chain			
C _{13:1} ω1c	–	tr	1.0
C _{16:1} ω7c	–	tr	–
C _{18:2} ω6,9c	–	5.3	7.7
C _{18:1} ω9c	–	2.9	4.0
Hydroxy			
iso-C _{15:0} 3-OH	–	tr	tr
anteiso-C _{15:0} 3-OH	–	tr	–
C _{15:0} 3-OH	–	–	tr
C _{16:0} 3-OH	–	1.7	2.4
iso-C _{17:0} 3-OH	21.8	9.0	6.7
anteiso-C _{17:0} 3-OH	3.7	3.9	3.2
C _{17:0} 3-OH	5.2	1.6	tr
Saturated branched-chain			
iso-C _{13:0}	–	1.1	1.3
iso-C _{14:0}	–	tr	tr
iso-C _{15:0}	3.7	4.2	4.3
iso-C _{16:0}	–	tr	–
iso-C _{17:0}	3.3	tr	tr
anteiso-C _{13:0}	–	tr	tr
anteiso-C _{15:0}	42.2	49.8	50.2
anteiso-C _{17:0}	–	tr	tr
Summed features*			
3	–	5.5	4.0
9	–	1.0	1.0

*Summed features represent two or three fatty acids that cannot be separated by the Microbial Identification System. Summed feature 3 consisted of equivalent chain-length 13.570 and/or iso-C_{15:0} alde. Summed feature 9 consisted of iso-C_{16:0} 3-OH and/or equivalent chain-length 17.157.

The major cellular fatty acids of *X. oryzae* JCM 13648^T and JCM 13649 determined in this study were anteiso-C_{15:0} and iso-C_{17:0} 3-OH, which are as described for *X. oryzae* KB3^T by Ueki *et al.* (2006), although there were differences in the proportions (Table 1). The major menaquinones of strains *X. oryzae* JCM 13648^T and JCM 13649 were MK-12

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationships between 108 type strains of species of Gram-negative anaerobic rods. Bootstrap values (>70%) based on 1000 replicates are shown at branch nodes for neighbour-joining and maximum-likelihood, respectively. Bar, 0.02 substitutions per nucleotide position.

Fig. 2. Neighbour-joining phylogenetic tree based on *hsp60* sequences showing the relationships between 107 type strains of species of Gram-negative anaerobic rods. Bootstrap values (>70%) based on 1000 replicates are shown at branch nodes for neighbour-joining and maximum-likelihood, respectively. Bar, 0.05 substitutions per nucleotide position.

(19%) and MK-13 (77%), which are in agreement with Ueki *et al.* (2006). It should be noted that *X. oryzae* is phenotypically similar to members of the genus *Prevotella* (Shah & Collins, 1990).

Approximately 1500 bp of the 16S rRNA gene sequence was determined for each strain. For the phylogenetic analysis, 1427 bp was used. *X. oryzae* JCM 13648^T and JCM 13649 exhibited 99.7% 16S rRNA gene sequence similarity with each other. *X. oryzae* JCM 13648^T formed a distinct line of descent with high bootstrap support (100%) (Fig. 1). *X. oryzae* JCM 13648^T and all members of the genus *Prevotella* formed a single cluster with high bootstrap support (100%) and were phylogenetically distinct from the two members of the genus *Paraprevotella* (Morotomi *et al.*, 2009). In the *hsp60* sequence analysis, *X. oryzae* JCM 13648^T was located within the genus *Prevotella* although the bootstrap values were low (<70%; Fig. 2). In the *gyrB* sequence analysis (data not shown), *X. oryzae* JCM 13648^T was also located within the genus *Prevotella*. These findings show that *X. oryzae* is not independent of the genus *Prevotella* and our views on the matter were supported by statistical tests with respect to the family *Prevotellaceae*. In the 16S rRNA gene sequence analysis, the log-likelihood differences between the optimal tree and the alternative trees in which *X. oryzae* nested within the genus *Prevotella* were not significant ($P>0.05$). On the other hand, in the *hsp60* sequence analysis, if *X. oryzae* was moved to outside the genus *Prevotella*, the log-likelihood differences were significant (approximately unbiased test $P=0.018$, Shimodaira–Hasegawa test $P=0.015$). These results suggest that *X. oryzae* is monophyletic with the other members of the genus *Prevotella*. In addition, almost all of the 16S rRNA gene signature nucleotides reported for *Prevotella* species (Paster *et al.*, 1994) were present in *X. oryzae*: only the nucleotides T/G at positions 600/638 were different in *X. oryzae*, which had G/T at these positions.

On the basis of the above-mentioned findings, we propose that *X. oryzae* should be reclassified as *Prevotella oryzae* comb. nov. An emended description of the genus *Prevotella* is also provided. The name of the family *Prevotellaceae*, which was proposed in the accepted version of this manuscript, was validly published (Krieg, 2011) after acceptance but before publication of this manuscript.

Emended description of the genus *Prevotella* Shah and Collins 1990

The description is as given by Shah & Collins (1990) and Willems & Collins (1995) with the following modifications. The optimum temperature for growth is 30 or 37 °C. Utilizes various sugars, including xylan, and produces acetate, propionate and succinate as major fermentation end products; occasionally, lower levels of iso-butyrate, iso-valerate, lactate or malate are produced. Respiratory menaquinones, principally MK-10 to MK-13 or MK-11(H₂) to MK-13(H₂), may or may not be present. Habitats are mammals or rice-plant residue (plant stubble and roots). The type species is *Prevotella melaninogenica*.

Description of *Prevotella oryzae* Ueki *et al.* 2006 comb. nov.

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

Basonym: *Xylanibacter oryzae* Ueki *et al.* 2006.

The description is as given by Ueki *et al.* (2006) with the following modifications. Cells on Columbia blood agar are 0.8–1.0 µm wide and variable in length, mostly 2.0–15.0 µm. Colonies on Columbia blood agar after 72 h at 30 °C under anaerobic conditions are 1–2 mm in diameter, circular, entire, slightly convex, smooth, white-greyish and translucent. Acid is produced from raffinose, but not from D-sorbitol and sucrose [acid production from sucrose is given as positive in the description of Ueki *et al.* (2006)]. Catalase is not produced. With Rapid ID 32A, positive for α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, α-arabinosidase, leucyl glycine arylamidase, alanine arylamidase and fermentation of mannose and raffinose, but negative for urease, arginine dihydrolase, 6-phospho-β-galactosidase, β-glucuronidase, β-N-acetylglucosaminidase, glutamic acid decarboxylase, α-fucosidase, nitrate reduction, indole production, alkaline phosphatase, arginine arylamidase, proline arylamidase, phenylalanine arylamidase, leucine arylamidase, pyroglutamic acid arylamidase, tyrosine arylamidase, glycine arylamidase, histidine arylamidase, glutamyl glutamic acid arylamidase and serine arylamidase.

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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