Naxibacter alkalitolerans gen. nov., sp. nov., a novel member of the family '*Oxalobacteraceae*' isolated from China

Ping Xu,[1,2]† Wen-Jun Li,[1]† Shu-Kun Tang,[1] Yu-Qin Zhang,[1] Guo-Zhong Chen,[1] Hua-Hong Chen,[1] Li-Hua Xu[1] and Cheng-Lin Jiang[1]

[1]The Key Laboratory for Microbial Resources of the Ministry of Education, Yunnan Institute of Microbiology, Yunnan University, Kunming, Yunnan, 650091, People's Republic of China

[2]New Drug R & D, North China Pharmaceutic Corp., Shijiazhuang, 050015, People's Republic of China

Correspondence
Cheng-Lin Jiang
lihxu@ynu.edu.cn *or*
wjli@ynu.edu.cn

A taxonomic study was performed on strain YIM 31775^T, which was isolated from a soil sample collected from Yunnan Province, China. The isolate was chemo-organotrophic, aerobic and Gram-negative. Cells were short rods and motile, with one or more polar flagella. Growth temperature and pH ranged from 4 to 55 °C and 6·5 to 12·0, respectively; the optimum growth temperature and pH were 28–37 °C and 7·0–9·0, respectively. Q-8 was the predominant respiratory lipoquinone. The major fatty acids were $C_{16:1}\omega7c$ (42·4 %) and $C_{16:0}$ (28·1 %). The DNA G+C content was 62·4 ± 0·3 mol%. Phylogenetic analysis based on the 16S rRNA gene sequence indicated that strain YIM 31775^T should be placed within the family 'O*xalobacteraceae*', in which it formed a distinct lineage. Based on the high 16S rRNA gene sequence divergence and phenotypic characteristics, it is proposed that strain YIM 31775^T should be classified as representing a novel member of the family 'O*xalobacteraceae*', for which the name *Naxibacter alkalitolerans* gen. nov., sp. nov. is proposed. The type strain is YIM 31775^T (=CCTCC AA 204003^T=KCTC 12194^T).

In the course of a screening programme for new antibiotics, a Gram-negative strain, YIM 31775^T, which contained genes encoding both type I and type II polyketide biosynthesis pathways, was isolated from a soil sample collected from Yunnan Province, China (Xu *et al.*, 2003b). 16S rRNA gene sequence analysis showed that the isolate belonged to the class '*Betaproteobacteria*'. The morphological, physiological and chemical characteristics, and phylogenetic analyses, showed that the strain should be classified as representing a novel member of the family '*Oxalobacteraceae*', order '*Burkholderiales*', class '*Betaproteobacteria*' (Garrity & Holt, 2001). In this report we propose the name *Naxibacter alkalitolerans* gen. nov., sp. nov.

Strain YIM 31775^T was isolated from a soil sample after incubation for 2 weeks at 28 °C on Water Proline agar (1 % proline/tap water). Biomass for molecular systematic and chemotaxonomic studies was obtained after incubation at 37 °C for 3 days in shake flasks containing tryptone soy broth (TSB; Oxoid). Colony morphology was determined after 3 days incubation at 28 °C on TSB agar. Colour determination was done with colour chips from the ISCC–NBS Colour Charts Standard Samples no. 2106 (Kelly, 1964). Gram (Lányí, 1987) and Ziehl-Neelsen (Gordon, 1967) preparations were observed by light microscopy (Olympus microscope BH-2). Cell motility was studied on Luria–Bertani swarming agar (0·3 %, w/v). Cellular morphology was studied by using a Hitachi H-800 transmission electron microscope.

The physiological and biochemical tests were all performed at 37 °C. Unless indicated otherwise, standard methods for phenotypic characterization of the strain were employed, as described by O'Brien & Colwell (1987). Catalase activity was determined by production of bubbles after the addition of a drop of 3 % H_2O_2. Some physiological properties were tested by using API ID32 E and API 20NE test kits (bioMérieux). The temperature range and the optimum temperature for growth were tested at 4–65 °C on TSB agar. The pH range and the optimum pH for growth were examined at pH 4·0–13·0, by using the following buffer system: pH 4·0–5·0: 0·1 M citric acid/0·1 M sodium citrate; pH 6·0–8·0: 0·1 M KH_2PO_4/0·1 M NaOH; pH 9·0–10·0: 0·1 M $NaHCO_3$/0·1 M Na_2CO_3; pH 11·0: 0·05 M

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†These authors contributed equally to this work.

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Na_2HPO_4/0·1 M NaOH; pH 12·0–13·0: 0·2 M KCl/0·2 M NaOH. Negative controls for each buffer were used and the final pH was determined by using an indicator of acidity (ORION). The liquid cultures were cultivated in tubes at 28 °C for 2–3 weeks. Tolerance to sodium chloride (1, 3, 7, 10 and 13 %) and phenol (0·1, 0·2, 0·5 and 1·0 %) was tested using TSB agar. Susceptibility to antibiotics was determined by using the disc-diffusion plate method. The following antibiotics were tested (concentration per millilitre in parentheses) with TSB agar: amikacin (30 μg), aureomycin (30 μg), ciprofloxacin (10 μg), chloramphenicol (30 μg), erythromycin (15 and 30 μg), gentamicin sulfate (10 μg), kanamycin (15 μg), netilmicin (10 μg), novobiocin (5 and 30 μg), oleandomycin (10 μg), penicillin G (10 U), polymyxin B (10 and 300 U), streptomycin sulfate (10 and 25 μg), terramycin (2·5 and 30 μg), tetracycline (10 and 30 μg), tobramycin sulfate (10 μg) and vancomycin (10 μg).

Biomass for quantitative fatty acid analysis was obtained from TSB agar that had been incubated for 3 days at 28 °C. Analysis of the whole-cell fatty acid pattern followed the MIDI system (Microbial ID) (Kroppenstedt, 1985; Meier *et al.*, 1993). Polar lipids were determined by using the method of Minnikin *et al.* (1979). Isoprenoid quinones were isolated from lyophilized cells by using the method of Collins *et al.* (1977), and analysed by HPLC (Groth *et al.*, 1997).

Bacterial genomic DNA preparation, PCR and 16S rRNA gene sequencing were carried out as described previously (Xu *et al.*, 2003a; Cui *et al.*, 2001). The nearly complete 16S rRNA gene sequence of strain YIM 31775^T (1526 nucleotides) was aligned with corresponding almost complete sequences of members of the class '*Betaproteobacteria*', retrieved from the DDBJ, EMBL and GenBank databases by using BLAST (Altschul *et al.*, 1997) and BLAST 2 sequences (Tatusova & Madden, 1999). Following determination of the phylogenetic position within the class '*Betaproteobacteria*', the dendrogram was restricted to the nearest neighbours. CLUSTAL W (Thompson *et al.*, 1994) was used to estimate the evolutionary distances (K_{nuc} value; Kimura, 1980) and similarity values were used to reconstruct a phylogenetic tree by the neighbour-joining method (Saitou & Nei, 1987). The topology of the tree was evaluated by performing a bootstrap analysis (Felsenstein, 1985) using 1000 resamplings. *Ralstonia solanacearum* ATCC 11696^T (GenBank/EMBL/DDBJ accession no. X67036) was used as an outgroup. The chromosomal DNA for genomic DNA G+C content analysis was prepared by following the method of Marmur (1961). The DNA G+C content of strain YIM 31775^T was determined by using the thermal denaturation method (Mandel & Marmur, 1968).

Colonies of strain YIM 31775^T were 1·1–1·4 mm in diameter, circular, entire, convex, glistening, butyraceous and opaque, and had a pale white–yellow to straw-colour on nutrient agar plates. Cells were rod-shaped, 0·5–0·8 × 1·35–2·0 μm in size, Gram-negative, motile with one or more polar flagella, and chemo-organotrophic with respiratory-type metabolism. Spores and polyhydroxyalkanoates were not formed. The strain could not grow in the presence of sodium chloride at 3 %. The temperature range for growth was 4–55 °C, with optimum growth occurring at 28–37 °C. The pH range for growth was 6·5–12·0, with optimum growth occurring between pH 7·0 and 9·0. Tests for gelatin, melanin production and H_2S production were positive; however, those for indole production, resistance to KCN, milk coagulation and peptonization were negative. Nitrate was not reduced to nitrite. Other physiological and biochemical results are listed in Table 1 and in the species description.

Phylogenetic analysis of the 16S rRNA gene sequence of strain YIM 31775^T showed that the isolate was a member of the class '*Betaproteobacteria*' (Garrity & Holt, 2001). In the phylogenetic tree, strain YIM 31775^T formed a distinct lineage within the family '*Oxalobacteraceae*' (Garrity & Holt, 2001), and belonged to a cluster of species including *Telluria mixta*, *Telluria chitinolytica*, *Massilia timonae*, *Duganella zoogloeoides*, *Janthinobacterium agaricidamnosum* and *Janthinobacterium lividum* (see Fig. 1). The 16S rRNA gene sequence similarity values between strain YIM 31775^T and its closest relatives, *J. agaricidamnosum* DSM 9628^T, *J. lividum* DSM 1522^T, *D. zoogloeoides* IAM 12670^T and *M. timonae* CIP 105350^T, were 95·5, 95·2, 95·0 and 94·9 %, respectively. The next closest relatives, *Herbaspirillum*, *Telluria* and *Collimonas* species, showed much lower similarity values (<93·6 %) (see Fig. 1). The 16S rRNA gene sequence similarity between strain YIM 31775^T and the other '*Betaproteobacteria*' reference strains was less than 91·6 %. The low level of sequence similarity between the novel strain and other bacteria belonging to the family 'O*xalobacteraceae*' and related genera clearly demonstrates that YIM 31775^T may be a representative of a new genus.

The cellular fatty acid profiles of YIM 31775^T are given in detail in the species description. The major fatty acids were $C_{16:0}$ and $C_{16:1}\omega7c$, whereas those for the two type species of the genus *Janthinobacterium* (Lincoln *et al.*, 1999) were $C_{16:0}$, $C_{16:1}\omega7c$ and $C_{17:0}$ cyclo. For the type strains of the genera *Duganella* (De Boer *et al.*, 2004) and *Massilia* (Lindquist *et al.*, 2003), the major fatty acid components were $C_{16:0}$, $C_{16:1}\omega7c$ and $C_{18:1}\omega7c$. Additionally, the presence of $C_{18:1}\omega9c$ in strain YIM 31775^T was a characteristic that clearly distinguished the isolate from species of the genera *Janthinobacterium*, *Duganella* and *Massilia* (De Boer *et al.*, 2004; Lincoln *et al.*, 1999; Lindquist *et al.*, 2003). The major hydroxyl fatty acids of the genus *Duganella* were $C_{10:0}$ 3-OH, $C_{12:0}$ 2-OH and $C_{14:0}$ 2-OH, whereas the novel isolate YIM 31775^T had only $C_{10:0}$ 3-OH and $C_{12:0}$ 2-OH; this was an additional distinguishing characteristic.

The major isoprenoid quinone detected in strain YIM 31775^T was Q-8 (93·4 %), and a minor amount of Q-7 (6·6 %) was also present. Polar lipids of strain YIM 31775^T included phosphatidylglycerol, phosphatidylethanolamine

Table 1. Phenotypic properties separating strain YIM 31775^T from related taxa

Taxa: 1, *Massilia*; 2, *Duganella zoogloeoides*; 3, *Janthinobacterium*; 4, *Telluria*; 5, YIM 31775^T (data from this study and Lincoln *et al.*, 1999; Bowman *et al.*, 1993; La Scola *et al.*, 1998; Lindquist *et al.*, 2003; Hiraishi *et al.*, 1997). +, Positive; (+), weakly positive; −, negative; V, variable among species or strains; polar (m), polar monotrichous; ND, no data.

Characteristic	1	2	3	4	5
Cell diameter >1 μm	+	−	+	−	−
Flagellation	Polar (m)	Polar (m)	ND	Polar (m)	Polar (one to more)
Production of zoogloeae	−	+	−	−	−
Growth on nutrient agar	ND	+	+	−	+
Pigment production	−	Non-diffusible yellow pigment	Purple pigment	Non-diffusible yellow pigment	Melanin
Hydrolysis of gelatin	+	+	−	+	+
Hydrolysis of starch	+	+	ND	+	−
Urease production	−	+	−	V	+
Oxidase production	+	+	+	(+)	−
Utilization as sole carbon source:					
Glucose	−	+	+	+	+
Sucrose	−	+	+	+	−
Fructose	−	+	+	+	+
Galactose	−	+	V	+	+
Lactose	+	+	−	+	+
Rhamnose	+	+	V	+	−
D-Trehalose	−	−	V	+	−
Citrate	−	+	−	+	−
Malonate	+	+	ND	+	−
L-Tartrate	−	+	ND	+	−
Acetate	ND	−	V	−	−
Acid formed from:					
Glucose	−	+	+	−	+
Sucrose	−	+	+	−	−
Maltose	−	+	ND	−	+
Mannose	−	+	ND	−	+
G+C content (mol%)	62–67	63–64	61–67	67–72	62–63
Isolation	Blood	Sewage and polluted water	Mushroom pathogen	Soil	Soil

and phosphatidylinositol mannosides. The predominant phosphatidylglycerol and phosphatidylethanolamine and the presence of ubiquinone 8 conform with the inclusion of strain YIM 31775^T within the class '*Betaproteobacteria*' (Wilkinson, 1988; Suzuki *et al.*, 1993). The G+C content of genomic DNA of strain YIM 31775^T was 62–63 mol%, which is similar to the G+C content of recognized species of the genera *Janthinobacterium*, *Duganella* and *Massilia*, but is notably lower than that of the genus *Telluria* (Table 1).

From Table 1, it can also be seen that YIM 31775^T shares some metabolic properties with strains of the genera *Janthinobacterium*, *Duganella*, *Massilia* and *Telluria*. However, the inability of strain YIM 31775^T to metabolize rhamnose distinguishes it from the strains of these genera. The characteristics of starch hydrolysis, oxidase production and pigment production of strain YIM 31775^T also differentiate it from the genera *Janthinobacterium*, *Duganella*, *Massilia* and *Telluria* (Table 1). Furthermore, unlike recognized species of the genera *Janthinobacterium* and *Massilia*, YIM 31775^T is a soil bacterium with no apparent pathogenic or symbiotic relationship with mushrooms (Lincoln *et al.*, 1999; La Scola *et al.*, 1998). Therefore, the novel strain should not be classified within known genera of the family '*Oxalobacteraceae*', order '*Burkholderiales*', class '*Betaproteobacteria*'. On the basis of both the phylogenetic and phenotypic distinctions, we propose that strain YIM 31775^T should be classified as representing a novel species within a new genus, for which the name *Naxibacter alkalitolerans* gen. nov., sp. nov. is proposed.

Description of *Naxibacter* gen. nov.

Naxibacter (Na.xi.bac′ter. N.L. n. *Naxi* referring to the Naxi nationality, who lived in Lijiang, Yunnan Province, China, from where the organism was isolated; n. *bacter* from

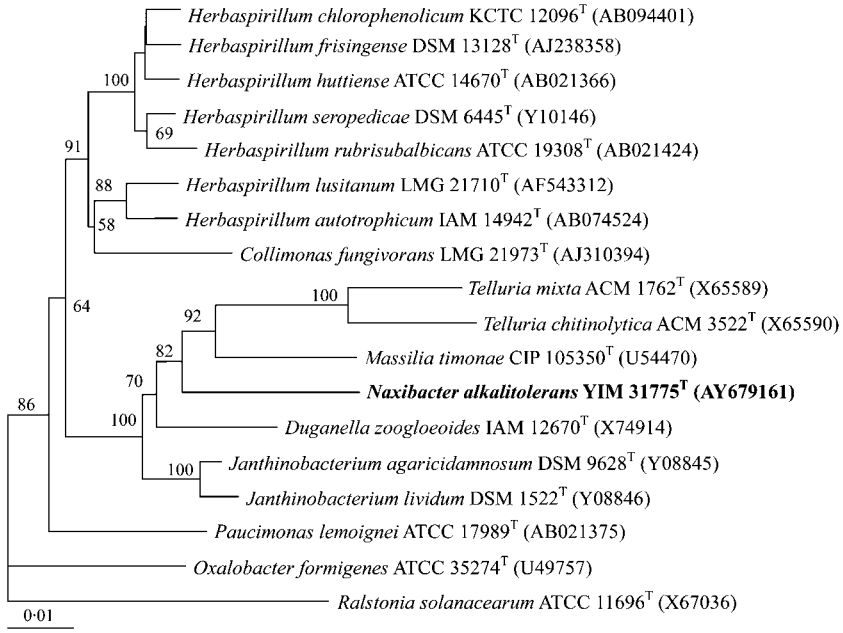

Fig. 1. Phylogenetic dendrogram obtained by neighbour-joining analysis based on 1398 bp of 16S rRNA gene sequences, showing the position of strain YIM 31775^T among its phylogenetic neighbours. Numbers on branch nodes are bootstrap values expressed as a percentage (1000 resamplings). Sequence accession numbers are given in parentheses. The sequence of *Ralstonia solanacearum* ATCC 11696^T (X67036) was used as the root. Bar, 0·01 substitution per nucleotide position.

Gr. n. *baktron* rod; N.L. masc. n. *Naxibacter* rod-shaped microbe from the place in which the Naxi nationality lived).

Gram-negative, oxidase-negative and catalase-positive. Aerobic and chemo-organotrophic. Cells are non-spore-forming rods with rounded ends, and motile with one or more polar flagella. The major cellular fatty acids are $C_{16:1}\omega7c$ and $C_{16:0}$; the hydroxylated fatty acids are $C_{10:0}$ 3-OH and $C_{12:0}$ 2-OH. The main polar lipids are phosphatidylglycerol, phosphatidylethanolamine and phosphatidylinositol mannosides. Q-8 is the predominant respiratory quinone. The G+C content of genomic DNA is about 62–63 mol%. The genus is a member of the class '*Betaproteobacteria*'.

The type species is *Naxibacter alkalitolerans*.

Description of *Naxibacter alkalitolerans* sp. nov.

Naxibacter alkalitolerans (al.ka.li.to′le.rans. Arabic article *al* the; Arabic n. *qaliy* ashes of saltwort; French n. *alcali* alkali; N.L. n. *alkali* alkali; L. part. adj. *tolerans* tolerating; N.L. part. adj. *alkalitolerans* alkali-tolerating).

In addition to the characteristics that define the genus, the species has the characteristics described below. Colonies are 1·1–1·4 mm in diameter, circular, entire, convex, glistening, butyraceous, opaque and with pale white–yellow to straw-colour on nutrient agar plates. Cells are 0·45–0·8 μm wide and 1·35–2 μm long. Endospores are not observed. Polyhydroxyalkanoates are not formed. The isolate cannot grow in the presence of sodium chloride at 3 %. Temperature range for growth is 4–55 °C, with optimum growth occurring at 28–37 °C. pH range for growth is 6·5–12·0, with optimum growth occurring between pH 7·0 and 9·0. Dextrin, dulcitol, glycerol, inositol, mannose, mannitol, melibiose, melezitose, raffinose, ribose, salicin and xylitol are utilized as sole carbon and energy sources, but not adonitol, arabitol, galacturonate, inositol, oxalate, sorbitol or 5-ketogluconate. Nitrate, L-threonine, L-valine, L-hydroxyproline, L-lysine, L-tryptophan, L-proline, L-tyrosine, phenylalanine, L-histidine and L-asparagine (weak) are used as sole nitrogen sources, but not L-methionine, L-glutamic acid, glycine, L-arginine or L-cysteine. Urea, acetamide, xanthine, hypoxanthine, aesculin, keratin, chitin and DNA are hydrolysed. Tweens 20 and 80 are degraded, but not cellulose, starch, allantoin, glucosamine, amygdalin or adenine. Tests for gelatin, melanin production and H_2S production are positive; however, those for nitrate reduction, indole production, resistance to KCN, milk coagulation and peptonization are negative. Tests for lipase, ornithine decarboxylase, β-glucosidase, β-galactosidase, α-glucosidase and α-galactosidase are positive. Tests for arginine dihydrolase, lysine decarboxylase and *N*-acetyl-β-glucosaminidase are negative. Resistant to lysozyme, penicillin G, vancomycin, polymyxin B, tobramycin sulfate, gentamicin sulfate, netilmicin, oleandomycin and ciprofloxacin (weak), but sensitive to erythromycin, terramycin, aureomycin, tetracycline, streptomycin sulfate, amikacin, novobiocin, kanamycin, nalidixic acid and chloramphenicol. The cellular fatty acid profiles are $C_{16:1}\omega7c$ (42·4 %), $C_{16:0}$ (28·1 %), $C_{17:0}$ cyclo (6·8 %), $C_{12:0}$ (6·1 %), $C_{18:1}\omega7c$ (4·3 %), $C_{10:0}$ 3-OH (4·2 %), $C_{14:0}$ (3·4 %), $C_{18:1}\omega9c$ (1·2 %), $C_{10:0}$ (0·5 %), $C_{18:0}$ (0·4 %), $C_{12:0}$ 2-OH (0·3 %) and $C_{15:0}$ (0·2 %). The isoprenoid quinones are Q-8 (93·4 %) and Q-7 (6·6 %). The phospholipids are phosphatidylglycerol, phosphatidylethanolamine and phosphatidylinositol mannosides. The G+C content of genomic DNA is 62·4±0·3 mol%.

The type strain is YIM 31775^T (=CCTCC AA 204003^T=KCTC 12194^T), which was isolated from soil in Lijiang, Yunnan Province, China.

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