Sphingobium qiguonii sp. nov., a carbaryl-degrading bacterium isolated from a wastewater treatment system

Qiu-Xiang Yan,1 Yong-Xia Wang,2 Shun-Peng Li,1 Wen-Jun Li2 and Qing Hong1

A Gram-staining-negative, catalase-positive, carbaryl-degrading, non-spore-forming, non-motile, rod-shaped bacterium, designated strain X23T, was isolated from a wastewater treatment system. Phylogenetic analysis based on 16S rRNA gene sequence indicated that the strain belongs to the genus Sphingobium. The highest 16S rRNA gene sequence similarity observed for the isolate was 96.6 % with the type strain of Sphingobium amienese. Chemotaxonomic data [major ubiquinone: Q-10; major polar lipids: diphosphatidylglycerol, phosphatidylcholine, phosphatidylglycerol, sphingoglycolipid, phosphatidylethanolamine and unknown aminolipids and phospholipids; major fatty acids: summed feature 7 (C18:1ω7c, C18:1ω9t and/or C18:1ω12t), C16:1ω5c, C14:0 2-OH and C16:0 2-OH] as well as the inability to reduce nitrate and the presence of spermidine as the major polyamine supported the affiliation of the strain to the genus Sphingobium. Based on the phylogenetic analysis, whole-cell fatty acid composition and biochemical characteristics, the strain could be separated from all recognized species of the genus Sphingobium. Strain X23T should be classified as a novel species of the genus Sphingobium, for which the name Sphingobium qiguonii sp. nov. is proposed, with strain X23T (=CCTCC AB 208221T =DSM 21541T) as the type strain.

Carbamate pesticides, such as carbaryl (1-naphthyl N-methylcarbamate), have been used extensively for the control of pests in agriculture. These pesticides can inhibit the activity of acetyl cholinesterase in an irreversible manner and thereby cause insect death (Fahmy et al., 1970). As the acetyl cholinesterases are also present in all vertebrates, the potential for damage by this class of insecticides to non-target organisms could be very high (Kuhr & Dorough, 1976). Soil micro-organisms play a significant role in the bioremediation of pesticide-contaminated soil, and a variety of carbaryl-degrading soil bacteria have been isolated and characterized (Chapalamadugu & Chaudhry, 1991; Hayatsu & Nagata, 1993; Doddamani & Ninnekar, 2001).

The genus Sphingobium was first proposed by Takeuchi et al. (2001) to accommodate a part of the genus Sphingomonas (Yabuuchi et al., 1990; Takeuchi et al., 1993, 2001), due to the latter containing species that were genetically relatively diverse based on 16S rRNA gene sequences. Members of the genus Sphingobium represent environmental isolates that play an important role in the bioremediation and biodegradation of pollutants. At the time of writing, this genus comprised 17 recognized species (www.bacterio.cict.fr/s/sphingobium.html). In this paper, the taxonomic characterization of a carbaryl-degrading bacterium, designated strain X23T, is presented. Based on the results of a polyphasic taxonomic study, this strain was proposed to represent a novel species of the genus Sphingobium.

A conventional enrichment method was employed to isolate the carbaryl-degrading strain (Ou & Sharma, 1989). A carbamate-pesticide-contaminated sludge sample (1.0 g, pH 6.0) collected from the wastewater treatment system of a pesticide manufacturing company in Xuzhou, Jiangsu province, PR China, was inoculated into mineral salts

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain X23T is EU095328.

A transmission electron micrograph of a cell of strain X23T, polar lipid profiles, a neighbour-joining tree based on 16S rRNA gene sequences, and cellular fatty acid profiles are available with the online version of this paper.
medium [MM: 4.8 g K2HPO4, 1.2 g KH2PO4, 1.0 g NH4NO3, 0.2 g MgSO4, 7H2O, 0.4 g Ca(NO3)2·4H2O, 0.5 g NaCl and 0.001 g Fe2(SO4)3 per litre] supplemented with 30 mg carbaryl 1⁻¹ as the sole carbon source and incubated at 28 °C on a rotary shaker at 150 r.p.m. for approximately 1 week. Every 7 days, 5 ml enrichment culture broth was subcultured into 100 ml fresh MM containing 30 mg carbaryl 1⁻¹. After five rounds of transferring, the enrichment culture was serially diluted and spread on modified LB/10 agar (MLB; 1.0 g tryptone 1⁻¹, 0.5 g yeast extract 1⁻¹, 1.0 g NaCl 1⁻¹, pH 7.0) supplemented with 100 mg carbaryl 1⁻¹. Strain X23T was maintained on MLB slants at 4 °C and stored as 20% glycerol suspensions at −80 °C.

Cell morphology and motility were observed by light microscopy (BH-2; Olympus) and transmission electron microscopy (H-7650; Hitachi). Growth at different pH (5–10, in increments of 0.5 pH units) and temperatures (4, 10, 20, 25, 28, 37, 42, 45, 50 and 55 °C) was determined on MLB medium. Catalase activity was determined by assessing bubble production in 3% (v/v) H2O2, and oxidase activity was determined using a 1% (w/v) solution of tetramethyl-p-phenylenediamine (Kovacs, 1956). The effect of oxygen on the growth of strain X23T was carried out according to the method described by Slack (1968). Nitrate reductase, H2S production, indole production, urease and gelatinase tests were performed by using the API 20E kit (bioMérieux). Assimilation and oxidation tests were performed using the API 20NE kit (bioMérieux) and Biolog GN2 system, respectively, following the manufacturers’ recommendations. Some physiological and biochemical tests were also done according to Holt et al. (1994) to confirm the identification. The carbaryl-degrading activity of the strain was analysed by HPLC as described by Yan et al. (2007).

Biomass for quantitative analysis of fatty acids, polar lipids, polyamines and isoprenoid quiones of strain X23T was prepared by scraping cells from MLB plates that had been incubated for 5 days to stationary state at 28 °C. Polar lipids were extracted as described by Minnikin et al. (1984) and identified by two-dimensional TLC and spraying with specific reagents (Collins & Jones, 1980). Phosphatidylcholine and lipids containing free amino groups were identified by spraying with Dragendorff reagent (Sigma) and ninhydrin reagent, respectively. Sugar-containing lipids were detected by using alpha-naphthol reagent (Sigma). The total polar lipid profile was identified by using molybdophosphoric acid reagent. Polyamines were extracted as described by Takeuchi et al. (2001) and analysed by HPLC as described by Hamana et al. (1994). Isoprenoid quiones were extracted by using the method of Collins et al. (1977) and analysed by HPLC as described by Kroppenstedt (1982). Analysis of whole-cell fatty acids followed the methods described by Sasser (1990) using the Microbial Identification System (MIDI). Chromosomal DNA was extracted and purified as described previously (Cui et al., 2001). The G+C content of DNA was determined by melting-point analysis (Marmur & Doty, 1962) using Escherichia coli DH5α DNA as a reference. The nearly complete 16S rRNA gene sequence was obtained by PCR amplification using a set of universal primers, 5’-AGAGTTTGATCCTGGCTCAG-3’ (E. coli bases 8–27) and 5’-TACCTTGTTACGACTT-3’ (E. coli bases 1507–1492), originally presented by Lane (1991). The PCR operating conditions were similar to those described by Suzuki & Yamasato (1994). The PCR product was ligated into the vector pMD18-T (TaKaRa Biotechnology) and then transformed into cells of E. coli DH5α. An automatic sequencer (Applied Biosystems, model 3730) was used to determine the 16S RNA gene sequence. Multiple sequence alignment of 16S rRNA gene sequences was performed by using CLUSTAL X software (Thompson et al., 1997). Phylogenetic analysis was performed by using two tree-making algorithms, namely the neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1981) methods. A maximum-likelihood tree topology was evaluated by means of a bootstrap analysis based on 100 resamplings.

Cells of strain X23T were aerobic, Gram-staining-negative, asporogenous, non-flagellated rods (Supplementary Fig. S1, available in IJSEM Online). The strain required about 3 days to form visible colonies on MLB agar at 28 °C and the colonies were circular, slightly convex with entire margins, opaque, greyish-white and 0.5–0.8 cm in diameter after 5 days of incubation at 28 °C. The strain could grow at pH 5.0–8.0; optimum growth was at pH 6.0–7.0. Growth was not observed at temperatures below 4 or above 42 °C. The optimum growth temperature was around 28 °C. Strain X23T degraded carbaryl. Other phenotypic characteristics of strain X23T are given in Table 1 and in the species description.

Strain X23T possessed the following chemotaxonomic characteristics that supported its assignment to the genus Sphingobium. Cellular fatty acid analysis revealed the presence of summed feature 7, consisting of one or more of C18:1ω7c, C18:1ω9t and C18:1ω12t (63.64 %) as the major non-hydroxylated fatty acids and C14:0 2-OH (6.97 %) as the major hydroxylated fatty acid. Other fatty acids detected were C16:0ω5c (7.45 %), C16:0 2-OH (5.42 %), C16:0 (3.62 %), C17:0 (3.48 %), summed feature 4 (C16:1ω7c and/or iso-C15:0 2-OH; 2.86 %) and C18:0 (1.22 %). The summed amount of unknown fatty acids was 5.34 %. The detailed fatty acid profiles of strain X23T and other species of the genus Sphingobium are shown in Supplementary Table S1. The respiratory quinone of strain X23T was ubiquinone Q-10 and the polar lipids consisted of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, sphingoglycolipid and unknown amnolipids and phospholipids (Supplementary Fig. S2a). These polar lipids are also commonly found in other species of the genus Sphingobium, e.g. S. amienae VT1 (Supplementary Fig S2b), S. fuliginis TKP1, S. xenaphagum BN65, S. cloacae S-3T, S. olei IMMIB HF-1T and S. ummariensis RL-3T. Strain X23T contained 3.09 μmol spermidine (g wet cells)⁻¹ as the major polyamine. The genomic DNA G+C content of strain X23T was 62.7 ± 1 mol%, which fell within the range...
of 62–67 mol% observed for other members of the genus *Sphingobium*.

Phylogenetic analysis (Fig. 1) showed that strain X23T formed a monophyletic clade within the genus *Sphingobium*; this relationship was also maintained in the tree based on the neighbour-joining algorithm (Supplementary Fig. S3). The 16S rRNA sequence similarity value between strain X23T and *Sphingobium* of 62–67 mol% observed for other members of the genus *Sphingobium* (95.5 %) and DNA–DNA relatedness between strain X23T and other type strains, i.e. a U–A pair at position 990:1215 and the presence of U at position 593 (Takeuchi et al., 2001) have shown that the inability to reduce nitrate and the presence of spermidine as the major polyamine are good diagnostic markers for the genus *Sphingobium*. Comparison of phenotypic properties between strain X23T and closely related type strains (Table 1 and Supplementary Table S1) revealed significant differences, supporting the conclusion that strain X23T was distinct from all known species of the genus *Sphingobium*. Hydrolysis of urea and acid production from glucose in strain X23T were positive and negative respectively, in contrast to *S. amiense* YT^T^, *S. herbicidovorans* MBIC 3166^T^, *S. fuliginis* TKP^T^ and *S. yanoikuyae* Gifu 9882^T^. Unlike these type strains, strain X23T did not possess C_{17:1} \alpha \text{cis-10} and the quantity of summed feature 4 was much lower. On the basis of phylogenetic analysis, phenotypic differences and chemotaxonomic data, strain X23T is suggested to represent a novel species of the genus *Sphingobium*, for which the name *Sphingobium qiguonii* sp. nov. is proposed.

### Description of *Sphingobium qiguonii* sp. nov.

*Sphingobium qiguonii* (qi.guo’ni.i. N.L. masc. gen. n. qiguonii of Qi-Guo, to honour Qi-Guo Zhao, a respected Chinese pedologist, for his enormous contributions to pedology and soil biology in China).

Cells are aerobic, Gram-staining-negative, catalase- and oxidase-positive, non-motile, non-sporulating rods with rounded ends, approximately 0.6–0.8 μm wide and 1.0–1.2 μm long without flagellum. Colonies on MLB agar are circular (0.5–0.8 cm in diameter after 5 days), entire, low yellow; CV, cream–yellow.

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**Table 1.** Differential biochemical characteristics of strain X23^T^ and the type strains of other species of the genus *Sphingobium*

Strains: 1, *Sphingobium qiguonii* sp. nov. X23^T^; 2, *S. amiense* YT^T^; 3, *S. herbicidovorans* MBIC 3166^T^; 4, *S. fuliginis* TKP^T^ (Prakash & Lal, 2006); 5, *S. yanoikuyae* Gifu 9882^T^ (Ushiba et al., 2003); 6, *S. cloacae* S-3^T^; 7, *S. xenophagum* BN6^T^; 8, *S. olei* IMMIB HF-1^T^ (Young et al., 2007); 9, *S. ummariense* RL-3^T^ (Singh & Lal, 2009); 10, *S. aromaticiconvertens* RW16^T^ (Wittich et al., 2007); 11, *S. rhizovicum* CC-FH12-1^T^; 12, *S. chlorophenolicum* ATCC 33790^T^ (Ushiba et al., 2003); 13, *S. chungbukense* DJ77^T^ (Kim et al., 2000); 14, *S. japonicum* UT26^T^ (Pal et al., 2005); 15, *S. francense* SP+^T^ (Pal et al., 2005). ++, Positive reaction; −, negative reaction; ND, no data available. Data for strains 1, 2, 3, 6, 7 and 11 from this study; data for other strains from references given. GW, Greyish-white; Y, yellow; CW, cream–white; LY, light yellow; CV, cream–yellow.
convex, opaque and greyish-white. Optimum temperature and pH for growth are 28 °C and 6.0–7.0, respectively. Positive for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, gelatinase and urease (API 20E and 20NE). Negative for nitrate reduction, β-galactosidase, β-glucosidase, tryptophan deaminase, Voges–Proskauer reaction, H₂S and indole production. Acid is not produced from glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin or arabinose. Utilizes glucose, maltose, arabinose, gluconate and citrate, but not mannitol, N-acetylglucosamine, mannotriol, capric acid, adipic acid, malate and phenylacetic acid. The following carbon sources are oxidized (Biolog GN2 system): L-arabinose, D-arabinose, D-fructose, dextrin, Tween 40 and 80, α-D-glucose, malate, succinic acid, sodium mononethyl ester, acetic acid, citric acid, DL-lactic acid, α-ketoglutaric acid, α-ketovaleric acid, propionic acid, D-saccharic acid, sebacic acid, glycolic acid, glycol-1, L-glutamic acid, aspartic acid, pyruvic acid, l-malic acid, succinic acid, adipic acid, succinic, bromosuccinic acid, L-aspartagine, L-proline, L-ornithine, glycogen, N-acetyl-d-glucosamine, N-acetyl-d-galactosamine, cellobiose, i-erythritol, gentiobiose, α-lactose, lactulose, melibiose, methyl β-D-glucoside, raffinose, trehalose, turanose, cis-aconitic acid, D-galactonic acid, L-galacturonic acid, D-galacturonic acid, DL-gluconic acid, p-hydroxyphenylacetic acid, itaconic acid, malonic acid, quinic acid, succinic acid, succinamide, L-histidine, L-hydroxyproline, L-leucine, L-phenylalanine, L-pyroglutamic acid, D-serine, L-serine, L-threonine, DL-carnitine, γ-aminobutyric acid, urocanic acid, thymidine, 2-aminoethanol, 2,3-butanediol and DL-z-glycerol phosphate. Ubiquinone 10 (Q-10) is the predominant respiratory quinone. Major cellular fatty acids are C₁₈:₁ω₇c (within summed feature 7), C₁₆:₁ω₅c, C₁₄:₀2-OH, and C₁₆:₀2-OH and minor fatty acids are C₁₆:₀, C₁₇:₀, C₁₈:₀ and summed feature 4 (C₁₆:₁ω₇c and/or iso-C₁₅:₀2-OH). The polar lipids consist of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, sphingoglycolipid and unknown aminolipids and phospholipids. Spermidine is the major polyamine. The genomic DNA G+C content of the type strain is 62.7 mol%.

The type strain, X23(T) (=DSM 3183(T) =CCCTCC AB 208221T) was isolated from a wastewater treatment system in a pesticide manufacturing company in Xuzhou, Jiangsu province, PR China.

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References


