**Sphingobium wenxiniae** sp. nov., a synthetic pyrethroid (SP)-degrading bacterium isolated from activated sludge in an SP-manufacturing wastewater treatment facility

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A synthetic pyrethroid (SP)-degrading bacterial strain, designated JZ-1\(^\mathrm{T}\), was isolated from activated sludge of a SP-manufacturing wastewater treatment facility and studied using a polyphasic taxonomic approach. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain JZ-1\(^\mathrm{T}\) belonged to the genus *Sphingobium*, showing highest sequence similarities to *Sphingobium faniae* DSM 21829\(^\mathrm{T}\) (98.6 %), *Sphingobium cloacae* JCM 10874\(^\mathrm{T}\) (98.5 %), *Sphingobium vermiconpositi* DSM 21299\(^\mathrm{T}\) (97.4 %) and *Sphingobium ummariense* CCM 7431\(^\mathrm{T}\) (96.9 %). The polar lipid pattern, the presence of spermidine and ubiquinone Q-10, the predominance of the cellular fatty acids C\(_{18:1}\)\(\omega\)7c, C\(_{19:0}\) cyclo \(\omega\)8c, 11 methyl C\(_{18:1}\)\(\omega\)7c, C\(_{16:0}\) and C\(_{14:0}\) 2-OH, and the G+C content of the genomic DNA also supported the affiliation of the strain with the genus *Sphingobium*. Strain JZ-1\(^\mathrm{T}\) showed low DNA–DNA relatedness values with *S. faniae* DSM 21829\(^\mathrm{T}\) (30.2 %), *S. cloacae* JCM 10874\(^\mathrm{T}\) (23.3 %), *S. vermiconpositi* DSM 21299\(^\mathrm{T}\) (10.9 %) and *S. ummariense* CCM 7431\(^\mathrm{T}\) (7.9 %). Based on its phylogenetic position and its phenotypic and genotypic properties, strain JZ-1\(^\mathrm{T}\) represents a novel species of the genus *Sphingobium*, for which the name *Sphingobium wenxiniae* sp. nov. is proposed. The type strain is JZ-1\(^\mathrm{T}\) (=CGMCC 1.7748\(^\mathrm{T}\)=DSM 21828\(^\mathrm{T}\)).

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**Abbreviations**: DPG, diphosphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL phospholipid; SGL, sphingoglycolipids; SP, synthetic pyrethroid(s).

The GenBank/EMBL/DDJB accession number for the 16S rRNA gene sequence of strain JZ-1\(^\mathrm{T}\) is FJ686047.

Three supplementary figures and two supplementary tables are available with the online version of this paper.

Synthetic pyrethroids (SP) are synthetic compounds similar in structure to natural pyrethrins extracted from chrysanthemum flowers. SPs are widely used for insect control in the agriculture industry and in gardens and households as a less toxic alternative to organophosphorus and chlorinated pesticides (Katsuda, 1999). Medical studies have indicated that SPs are hazardous to health and exposure to SPs might cause endocrine disruption, lymph node and spleen damage, carcinogenesis and allergic skin reactions (Garey & Wolff, 1998; Laskowski, 2002). In addition, SPs have an acute toxicity, often at very low concentrations (<0.5 µg/Kg), on some non-target organisms, such as bees, fish and aquatic invertebrates (Smith & Stratton, 1986; Suchismita & Anilava, 2008). Microbes play significant roles in degrading and detoxifying SP residues in the environment (Maloney et al., 1988). An SP-degrading bacterium, designated strain JZ-1\(^\mathrm{T}\), was isolated from activated sludge from Yangnong Chemical Group, an SP-manufacturing wastewater treatment facility in Jiangsu Province, China, and a novel esterase gene, *pytH*, encoding an SP-hydrolysing carboxylesterase, which catalyses the initial reaction of SP metabolism, was cloned from the strain (Wang et al., 2009). The ability of the strain to degrade several SPs, including fenpropathrin, deltamethrin, fenvalerate, cypermethrin, permethrin, cyhalothrin and bifenthrin, was determined according to the methods described by Guo et al. (2009). Strain JZ-1\(^\mathrm{T}\) was able to degrade all seven SPs tested at different degradation rates (from fastest to slowest): permethrin, fenpropathrin and cypermethrin, fenvalerate, cyhalothrin, deltamethrin and bifenthrin (Supplementary Fig. S1, available in IJSEM Online). These SPs, however, did not serve as sole carbon or energy sources for growth. Strain JZ-1\(^\mathrm{T}\) was preliminarily identified as a member of the genus *Sphingobium* (Wang et al., 2009). In the present study, the taxonomic status of the strain was further characterized using a polyphasic approach.

Strain DSM 21829\(^\mathrm{T}\), the type strain of *Sphingobium faniae*, was isolated in our laboratory (Guo et al., 2010), strains
DSM 21299\textsuperscript{T} and JCM 10874\textsuperscript{T}, the type strains of *Sphingobium vernicomposti* (Vaz-Moreira et al., 2009) and *Sphingobium cloacae* (Prakash & Lal, 2006), respectively, were obtained from the DSMZ and strain CCM 7431\textsuperscript{T}, the type strain of *Sphingobium ummariense* (Singh & Lal, 2009), was obtained from the CCM (Czech Collection of Microorganisms). Several characteristics of these four type strains were compared against those of the novel organism. Unless otherwise mentioned, all strains were grown aerobically on LB agar or in LB broth (Sigma–Aldrich) at 30 °C for 3 days. The LB medium was composed of (1 L) 10 g tryptone, 5 g yeast extract and 10 g NaCl.

Cell morphology was determined by transmission electron microscopy (model H-7650; Hitachi). In preparation for electron microscopy, bacterial cells were suspended in 0.85 % NaCl. Subsequently, the cells were dried on a nickel-coated mesh, negatively stained with phosphotungstic acid and examined. Cell motility was tested by using the hanging drop method (Suzuki et al., 2001). The Gram reaction was determined using the KOH test (Buck, 1982) and further confirmed by a conventional Gram-staining method (Smibert & Krieg, 1994). Growth was tested at 4, 5, 10, 15, 25, 28, 30, 37, 40, 41 and 45 °C and at pH 5–11 (increments of 0.5 pH units) on LB broth. Tolerance of NaCl was tested on LB broth containing 0–5 % (w/v) NaCl (increments of 0.5 %). Catalase tests were carried out as described previously (Ohta & Hattori, 1983). Hydrolysis of starch and the methyl red and Voges–Proskauer tests were determined as described by Smibert & Krieg (1994). Other enzymic activities and physiological and biochemical characteristics were assayed by using API 20 NE strips (bioMérieux) according to the manufacturer’s instructions. Oxidation of various carbon compounds was determined by using the Biolog GN2 system following the manufacturer’s recommendations (Biolog). Susceptibility to antibiotics was determined on LB agar using antibiotic discs (6.35 mm in diameter; Invitrogen) containing the following antibiotics (μg): kanamycin (30), penicillin (30), tetracycline (10), gentamicin (15), erythromycin (10), chloramphenicol (30), ampicillin (100) and streptomycin (100). Zones of inhibition were recorded after incubation at 30 °C for 5 days; strains were deemed resistant when the diameter of the zone of inhibition was <9 mm.

For the determination of chemotaxonomic characteristics, cells were harvested at optimum growth by using centrifugation, washed with distilled water and freeze-dried. Analyses of cellular fatty acids, polar lipids and quinones were carried out by the identification service of the DSMZ (Braunschweig, Germany) as described by Sasser (1990), Tindall (1990a, b), Collins & Jones (1981) and Collins (1985). Bacterial polyamines were analysed by HPLC according to Busse & Auling (1988) and Busse et al. (1997).

Extraction of genomic DNA was carried out according to standard procedures (Sambrook & Russell, 2001). PCR amplification of the 16S rRNA gene sequence was performed by using the bacterial universal primer set 27f and 1492r (Lane, 1991). The purified PCR product was ligated into the linear vector pMD18-T (TaKaRa Biotechnology) and was sequenced with the vector-specific primers RV-M (5'-AGCAGTACACGAGG-3') and M13-47 (5'-CGCCAGGTTTCCAGTCAGAC-3') using an automated sequencer. Pairwise sequence similarity was calculated by using a global alignment algorithm implemented at the EzTaxon server (http://www.eztaxon.org; Chun et al., 2007). Phylogenetic analysis was performed by using the software package MEGA version 4.0 (Tamura et al., 2007) after multiple alignment of the sequence data with CLUSTAL_X (Thompson et al., 1997). Distances were calculated by using distance options according to Kimura’s two-parameter model (Kimura, 1980) and clustering was performed using the neighbour-joining method (Saitou & Nei, 1987). Statistical support for the branches of phylogenetic trees was determined by using bootstrap analyses (based on 1000 resamplings) (Felsenstein, 1985). The G+C content of the genomic DNA was determined by thermal denaturation (Mandel & Marmur, 1968) using *Escherichia coli* K-12 DNA as a reference.

The almost complete 16S rRNA gene sequence (1410 nt) was obtained for strain JZ-1\textsuperscript{T}. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain JZ-1\textsuperscript{T} was a member of the genus *Sphingobium* and showed high similarities to *S. faniae* DSM 21829\textsuperscript{T} (98.6 %), *S. cloacae* JCM 10874\textsuperscript{T} (98.5 %) and *S. vernicomposti* DSM 21299\textsuperscript{T} (97.4 %). 16S rRNA gene sequence similarities <97 % were observed between strain JZ-1\textsuperscript{T} and other species of the genus *Sphingobium*. Strain JZ-1\textsuperscript{T} showed 96.2 % sequence similarity to *Sphingobium yanoikuyae* GIFU 9882\textsuperscript{T}, the type strain of the genus *Sphingobium*. In the neighbour-joining phylogenetic tree (Fig. 1), strain JZ-1\textsuperscript{T} formed a subclade with *S. faniae* DSM 21829\textsuperscript{T}, *S. cloacae* JCM 10874\textsuperscript{T} and *S. ummariense* CCM 7431\textsuperscript{T} (96.9 %).

DNA–DNA hybridization studies were necessary to clarify the taxonomic relationship of strains since DNA–DNA hybridization has greater resolution than 16S rRNA gene sequence analysis (Tindall et al. 2010). To further clarify the taxonomic relationship of strain JZ-1\textsuperscript{T} with *S. faniae* DSM 21829\textsuperscript{T}, *S. vernicomposti* DSM 21299\textsuperscript{T}, *S. cloacae* JCM 10874\textsuperscript{T} and *S. ummariense* CCM 7431\textsuperscript{T}, DNA–DNA hybridizations were performed according to the method of Ezaki et al. (1989). Hybridizations were repeated three times; means of the resulting values were determined and reciprocal experiments were performed between strains JZ-1\textsuperscript{T} and *S. faniae* DSM 21829\textsuperscript{T}. The results indicated that strain JZ-1\textsuperscript{T} showed relatively low DNA–DNA relatedness to *S. faniae* DSM 21829\textsuperscript{T} (30.2 %; reciprocal 38.3 %), *S. vernicomposti* DSM 21299\textsuperscript{T} (10.9 %), *S. cloacae* JCM 10874\textsuperscript{T} (23.3 %) and *S. ummariense* CCM 7431\textsuperscript{T} (7.9 %). These values were well below the threshold of 70 % recommended for the delineation of bacterial species (Wayne et al., 1987).

Chemotaxonomically, strain JZ-1\textsuperscript{T} possessed chemical markers that supported its assignment to the genus

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**Sphingobium.** Cellular fatty acid analysis revealed that C14:0 2-OH (12.0 %) was the major hydroxylated fatty acid and C18:1ω7c (41.8 %), C19:0 cyclo ω8c (17.6 %), 11 methyl C18:1ω7c (10.3 %) and C16:0 (9.0 %) were the non-hydroxylated fatty acids (Supplementary Table S1). In addition, C18:1ω5c (2.5 %), summed feature 4 (C16:1ω7c and/or iso-C15:0 2-OH) (2.1 %), C17:1ω6c (1.7 %), C18:0 (0.6 %) and C15:0 2-OH (0.6 %) were identified as minor peaks, whereas C16:1ω5c (0.5 %) and 3-hydroxy fatty acids were absent. The fatty acid profile of strain JZ-1T showed the same major fatty acids characteristics as other members of the genus *Sphingobium* in that the dominant fatty acid was C18:1ω7c and the major 2-hydroxy fatty acid was C14:0 2-OH (Takeuchi et al., 2001); however, the proportions of some major fatty acids were significantly different. Compared to *S. faniiae* DSM 21829T, *S. vermicomposti* DSM 21299T, *S. cloacae* JCM 10874T and *S. yanoikuyae* GIFU 9882T, strain JZ-1T possessed a comparatively low level of C16:1ω7c and a high level of C19:0 cyclo ω8c, 11 methyl C18:1ω7c and C14:0 2-OH. The major respiratory quinone was Q-10 (95 %). The polar lipids consisted of diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylcholine (PC), phosphatidylethanolamine (PE), sphingoglycolipids (SGL) and two unidentified phospholipids (PL1 and PL2) (Supplementary Fig. S2). These polar lipids are the common polar lipids of the genus *Sphingobium*. Cells were Gram-negative, non-sporulating, rod-shaped (0.5–0.6 × 0.7–1.0 μm) and non-motile (Supplementary Fig. S3). Colonies on LB agar were circular opaque, yellow and convex with entire margins. Optimum growth was observed at 28–30 °C, at pH 7–8 and in 1 % (w/v) NaCl. The DNA G+C content of strain JZ-1T was 64.2 mol%, which fell within the range observed for other members of the genus *Sphingobium* (62–67 mol%); Takeuchi et al., 2001). Detailed phenotypic properties of strain JZ-1T are given in the species description.

The phylogenetic tree based on 16S rRNA gene sequences, the DNA G+C content, the fatty acid and polar lipid profiles and the detection of ubiquinone Q-10 clearly suggested that strain JZ-1T was a member of the genus *Sphingobium*. Comparison of the phenotypic properties of strain JZ-1T and closely related strains (Table 1 and Supplementary Table S1) demonstrated that strain JZ-1T could be differentiated from all recognised species of the genus *Sphingobium* by at least three phenotypic characteristics; for example, strain JZ-1T was negative for oxidase activity, indole production and hydrolysis of aesculin, gelatin and starch but *S. quisquiliarum* P25T was positive for these characteristics. Therefore, on the basis of its phylogenetic position and its phenotypic and genotypic properties, strain JZ-1T represents a novel species of the genus *Sphingobium*, for which the name *Sphingobium wenxiniae* sp. nov. is proposed.

**Description of Sphingobium wenxiniae** sp. nov.

*Sphingobium wenxiniae* (wen.xin’i.ae. N.L. fem. gen. n. wenxin of Wen-xin, to honour Wen-xin Chen, a respected soil microbiologist, for her great contributions to the investigation and development of rhizobia resources in China).
Cells are Gram-negative, non-spore-forming, non-motile and rod-shaped (0.5–0.6×0.7–1.0 μm). Colonies on LB agar are circular, opaque, yellow and convex with entire margins. Grows in 0–4% (w/v) NaCl (optimum 1%), at pH 6–9 (optimum pH 7–8) and at 15–40 °C (optimum 28–30 °C). Positive for catalase, glucose acidification and assimilation of malate and caprate but negative for oxidase, urease, arginine dihydrolase and β-galactosidase activities, nitrate reduction, indole reaction, Voges–Proskauer reaction, methyl red test, hydrolysis of asculin, gelatin and starch and assimilation of D-glucose, L-arabinose, D-mannose, maltose, citrate, mannitol, glucose, phenylacetate, adipate and N-acetylgalcosamine. In the Biolog GN2 system, the following compounds are oxidized: methyl pyruvate, sebacic acid, Tween 40, 2-hydroxybutyric acid, 2-ketovaleric acid, L-Arabinose, D-Fructose, glycyl-L-aspartic acid and glycyl-L-glutamic acid. The major cellular fatty acids are C_{18:1}ω7c, C_{19:0} cyclo ω8c, C_{14:0} 2-OH, 11 methyl C_{18:1}ω7c and C_{16:0}. The polar lipids are DPG, PG, PC, PE, SGL and two unidentified PL. The major cellular polyamine is spermidine. The major quinone is Q-10. Resistant to ampicillin and streptomycin.

The type strain, JZ-1^T (=CGMCC 1.7748^T=DSM 21828^T), was isolated from activated sludge from Yangnong Chemical Group, an SP-manufacturing wastewater treatment facility in Jiangsu Province, China. The DNA G+C content of the type strain is 64.2 mol%.

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