Sphingobium faniae sp. nov., a pyrethroid-degrading bacterium isolated from activated sludge treating wastewater from pyrethroid manufacture

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A bacterial strain capable of degrading pyrethroid, designated JZ-2^T, was isolated from activated sludge treating pyrethroid-contaminated wastewater. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain JZ-2^T belongs to the genus *Sphingobium*. It showed the highest levels of 16S rRNA gene sequence similarity to *Sphingobium cloacae* JCM 10874^T (98.3 %) and *Sphingobium ummariense* CCM 7431^T (97.1 %), and 94.8–96.9 % similarity to the type strains of other members of the genus *Sphingobium*. Strain JZ-2^T contained C_{18:1ω7c} as the predominant fatty acid, C_{14:0} 2-OH as the major 2-hydroxy fatty acid, ubiquinone Q-10 as the main respiratory quinone, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, phosphatidylmonomethylethanolamine, phosphatidylethanolamine and two sphingoglycolipids as the predominant polar lipids and spermidine as the major polyamine. DNA–DNA hybridization results showed that strain JZ-2^T had low genomic relatedness with *S. cloacae* JCM 10874^T (34 %) and *S. ummariense* CCM 7431^T (23 %). Based on the phenotypic, genotypic and phylogenetic data presented, strain JZ-2^T is considered to represent a novel species of the genus *Sphingobium*, for which the name *Sphingobium faniae* sp. nov. is proposed. The type strain is JZ-2^T (=CGMCC 1.7749^T =DSM 21829^T).

Pyrethroids, highly active neurotoxic pesticides, have been widely used to control insects domestically and in agriculture (Katsuda, 1999). Although pyrethroids generally have lower toxicity to mammals than other classes of insecticides (e.g. organochlorines or organophosphates), they affect the central nervous system, cause allergic skin reactions and eye irritation and are highly toxic to some non-target organisms such as bees, fish and aquatic invertebrates (Bradbury & Coats, 1989; Kidd & James, 1991; Tripathi & Singh, 2004; Kumar *et al.*, 2008). Microbes play significant roles in degrading and detoxifying pyrethroid residues in the environment, and many pyrethroid-degrading bacteria have been isolated and characterized (Maloney *et al.*, 1988; Sakata *et al.*, 1992; Grant *et al.*, 2002; Yu & Fan, 2003; Lee *et al.*, 2004; Tallur *et al.*, 2008).

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JZ-2^T is FJ373058.

Figures showing the time course of degradation of various pyrethroids by strain sp. JZ-2^T, colonies of strain sp. JZ-2^T grown with fenpropathrin, a TEM of a negatively stained cell of strain JZ-2^T and two-dimensional TLC of polar lipids of JZ-2^T and a table comparing the cellular fatty acid profiles of strain JZ-2^T and recognized *Sphingobium* species are available as supplementary material with the online version of this paper.

Recently, a conventional enrichment culture was carried out in 10-fold-diluted Luria–Bertani (LB/10) liquid medium supplemented with fenpropathrin (100 mg l⁻¹) as an additional carbon source. A sample was taken from an aerobic pyrethroid-contaminated wastewater at the Yangnong Chemical Group Co. Ltd (Jiangsu, China). The enrichment culture was spread on LB/10 agar plates supplemented with fenpropathrin. Colonies producing a clear zone by degradation of fenpropathrin were picked and purified. A pyrethroid-degrading strain, designated JZ-2^T, was isolated. This strain was capable of degrading fenpropathrin at 100 mg l⁻¹ (or the same amount of deltamethrin, fenvalerate, cypermethrin, permethrin or cyhalothrin) within 3 days in liquid culture (see Supplementary Figs S1 and S2, available in IJSEM Online).

The results of preliminary BLAST searches based on 16S rRNA gene sequences in the NCBI database revealed that strain JZ-2^T showed highest similarities to species in the genus *Sphingobium*. At the time of writing, this genus comprised 15 recognized species. Members of the genus *Sphingobium* include environmental isolates that are able to degrade many toxic organic pollutants such as phenanthrene (Prakash & Lal, 2006), nonylphenol (Ushiba *et al.*, 2003) and polycyclic aromatic compounds (Wittich, 1998).

In the present study, the taxonomic position of strain JZ-2^T was characterized by using a polyphasic approach.

For investigation of morphological features, strain JZ-2^T was cultivated aerobically on LB agar plates for 3 days at 30 °C. Cell morphology was examined by light microscopy (BH-2; Olympus) and transmission electron microscopy (H-7650; Hitachi). For the latter, cells were stained with 2 % potassium phosphotungstate. The Gram stain was performed according to the classical Gram procedure (Buck, 1982). Growth at 4, 30 and 42 °C on LB agar was monitored for 10 days. Salt tolerance was determined by cultivating the organism in liquid LB supplemented with NaCl at final concentrations in the range 0–5.0 %. Oxidase and catalase tests were carried out as described by Ohta & Hattori (1983). Urease production and citrate utilization were determined according to Cowan & Steel (1965). Hydrolysis of starch, the methyl red test, indole production and the Voges–Proskauer reaction were determined as described by Smibert & Krieg (1994). The phenotypic characteristics of the new isolate were determined by using the API 20NE system (bioMérieux) according to the manufacturer's instructions. Tests for biodegradation of several pyrethroids (fenprothrin, deltamethrin, fenvalerate, cypermethrin, permethrin and cyhalothrin) were carried out according to the methods described by Maloney *et al.* (1988). Polar lipid, quinone and cellular fatty acid analyses of strain JZ-2^T were carried out by the Identification Service of the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany) as described by Tindall (1990a, b). Fatty acid profiles of strain JZ-2^T were obtained by using the Sherlock Microbial Identification System (Microbial ID, Inc.). For polyamine analysis, strain JZ-2^T was grown on PYE medium (0.3 % peptone from casein, 0.3 % yeast extract, pH 7.2) and harvested at approximately 70 % of the maximum optical density and, after lyophilization, polyamines were extracted and analysed by HPLC according to Busse & Auling (1988) and Busse *et al.* (1997).

Genomic DNA from strain JZ-2^T was extracted and purified according to standard procedures (Sambrook & Russell, 2001). The purified PCR product was sequenced by using an automated sequencer (model 3730; Applied Biosystems). The 16S rRNA gene sequence was compared with known sequences found in the GenBank database by using the BLAST program of the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/BLAST/>). The alignment of the 16S rRNA gene sequences of strain JZ-2^T and recognized species of the genus *Sphingobium* was compared by using CLUSTAL X software (Thompson *et al.*, 1997), and phylogenetic analysis was performed with MEGA version 3.1 (Kumar *et al.*, 2004). An evolutionary distance matrix was calculated by using Kimura's two-parameter distance model (Kimura, 1980), and a phylogenetic tree was reconstructed with the neighbour-joining method; the robustness of the tree was examined by bootstrap analysis of 1000 replications (Saitou & Nei, 1987). The G + C content of the DNA of strain JZ-2^T was determined by thermal denaturation (Mandel &

Marmur, 1968), for which *Escherichia coli* K-12 was used as a standard. DNA–DNA hybridization experiments were performed fluorometrically according to the method of Ezaki *et al.* (1989).

The almost-complete 16S rRNA gene sequence (1401 nt) of strain JZ-2^T was determined in this study. A tree depicting the phylogenetic affinity of this strain within the genus *Sphingobium* and other related taxa is shown in Fig. 1. Evaluation of the tree topology revealed that strain JZ-2^T clustered with species of the genus *Sphingobium* and formed a subclade with *Sphingobium cloacae* JCM 10874^T and *Sphingobium ummariense* RL-3^T. Strain JZ-2^T showed levels of 16S rRNA gene sequence similarity of 98.3 and 97.1 % to *S. cloacae* JCM 10874^T and *S. ummariense* RL-3^T, respectively, and 94.8–96.9 % to the type strains of other species of the genus *Sphingobium*. In order to clarify the taxonomic relationship of strain JZ-2^T with *S. cloacae* JCM 10874^T and *S. ummariense* RL-3^T, DNA–DNA hybridization experiments were performed. Levels of DNA–DNA relatedness between strain JZ-2^T and *S. cloacae* JCM 10874^T and *S. ummariense* RL-3^T were 34 and 23 %, respectively, well below the 70 % cut-off point recommended for the delineation of bacterial species (Wayne *et al.*, 1987).

Strain JZ-2^T possessed chemical markers that supported its assignment to the genus *Sphingobium*. Cellular fatty acid analysis revealed the presence of C_{14:0} 2-OH (5.12 % of the total) and C_{18:1}ω7c (57.34 %) as the major hydroxylated and non-hydroxylated components, respectively. Other main fatty acids (exceeding 4 % of the total) detected were C_{16:0} (13.76 %), 11-methyl C_{18:1}ω7c (4.96 %) and C_{17:1}ω6c (4.77 %). The detailed fatty acid profiles of strain JZ-2^T and recognized *Sphingobium* species are shown in Supplementary Table S1. Polar lipid analysis showed that strain JZ-2^T contained diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, phosphatidylmonomethylethanolamine, phosphatidylethanolamine and two sphingoglycolipids (Supplementary Fig. S3). The major respiratory quinone was ubiquinone Q-10 (92 %); Q-9 (8 %) was also detected as a minor component. Strain JZ-2^T contained spermidine as the predominant polyamine [25–36 μmol (g dry weight)^{−1}], with trace amounts of spermine [0.14–0.19 μmol (g dry weight)^{−1}]. This polyamine pattern is in agreement with data for recognized species of the genus *Sphingobium*. The DNA G + C content of strain JZ-2^T was 63.8 ± 0.8 mol% (mean ± SD of 6 determinations), which fell within the range observed for other members of the genus *Sphingobium* (62–67 mol%; Takeuchi *et al.*, 2001).

Cells of strain JZ-2^T were strictly aerobic, Gram-negative, non-sporulating, rod-shaped (0.5–0.6 × 1.1–1.2 μm) and non-motile (Supplementary Fig. S4). Colonies on LB agar were circular, smooth with entire margins, semi-transparent and pale yellow and formed brown water-soluble pigment after 2 days incubation at 30 °C. Strain JZ-2^T was able to tolerate up to 1.5 % NaCl, whereas *S. cloacae* JCM 10874^T was able to tolerate 3 % NaCl. The novel strain

Fig. 1. Phylogenetic tree constructed by the neighbour-joining method based on 16S rRNA gene sequences of strain JZ-2^T and the type strains of related species. Bootstrap values, expressed as percentages of 1000 replications, are given at branch points. Bar, 0.005 substitutions per nucleotide position.

assimilated D-mannose and malate, characteristics which differentiated it from *S. cloacae* JCM 10874^T and *S. ummariense* RL-3^T. Strain JZ-2^T was capable of degrading efficiently various pyrethroids tested, such as fenprothrin, deltamethrin, fenvalerate, cypermethrin, permethrin and cyhalothrin, whereas *S. cloacae* JCM 10874^T and *S. ummariense* RL-3^T could not. Differential phenotypic characteristics between strain JZ-2^T and recognized species

of the genus *Sphingobium* are given in Table 1. Based on this unique phenotypic profile, strain JZ-2^T was clearly differentiated from recognized species of the genus *Sphingobium*.

On the basis of its phenotypic, genotypic and phylogenetic characteristics, strain JZ-2^T is considered to represent a novel species of the genus *Sphingobium*, for which the name *Sphingobium faniae* sp. nov. is proposed.

Table 1. Differential phenotypic characteristics between strain JZ-2^T and recognized *Sphingobium* species

Strains: 1, JZ-2^T; 2, *S. cloacae* JCM 10874^T; 3, *S. ummariense* CCM 7431^T (data in columns 1–3 from the present study unless indicated otherwise); 4, *S. chlorophenolicum* DSM 8671^T (Ushiba *et al.*, 2003); 5, *S. japonicum* UT26^T (Pal *et al.*, 2005); 6, *S. francense* Sp+^T (Pal *et al.*, 2005); 7, *S. indicum* B90A^T (Pal *et al.*, 2005); 8, *S. amiense* YT^T (Ushiba *et al.*, 2003); 9, *S. herbicidovorans* DSM 11019^T (Ushiba *et al.*, 2003); 10, *S. fuliginis* DSM 18781^T (Prakash & Lal, 2006); 11, *S. xenophagum* BN6^T (Stolz *et al.*, 2000); 12, *S. olei* IMMIB HF-1^T (Young *et al.*, 2007); 13, *S. rhizovicinum* CC-FH12-1^T (Young *et al.*, 2008); 14, *S. yanoikuyae* JCM 7371^T (Ushiba *et al.*, 2003); 15, *S. chungbukense* KCTC 2955^T (Kim *et al.*, 2000); 16, *S. aromaticiconvertens* DSM 12677^T (Wittich *et al.*, 2007). ND, No data available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Motility	—	— ^{a*}	— ^b	—	—	—	—	ND	+	—	ND	ND	+	+	+	—
Assimilation of:																
D-Glucose	+	—	+	+	+	+	+	+	+	+	+	+	+	+	+	—
L-Arabinose	+	—	+	—	+	+	+	—	+	+	+	+	+	+	+	—
D-Mannose	+	—	—	—	—	—	—	—	—	ND	—	—	+	—	+	—
Malate	+	—	—	—	ND	ND	ND	—	+	ND	+	+	+	+	—	—
Maltose	—	—	+	—	ND	ND	ND	+	+	+	+	+	+	+	+	—
Citrate	—	—	—	—	—	—	ND	—	—	ND	+	—	+	+	—	—
Aesculin hydrolysis	—	—	+	+	+	+	—	—	+	—	—	+	+	+	+	—
Activity of:																
Catalase	+	+	—	+	+	+	+	+	+	+	+	—	—	+	+	+
Urease	+	+	—	+	ND	ND	+	—	—	—	—	—	—	+	ND	ND
β-Galactosidase	+	—	+	—	ND	ND	ND	—	—	—	—	—	+	+	—	+
Nitrate reductase	—	—	—	—	ND	ND	ND	—	—	+	—	—	+	—	ND	—

*Data from: a, Ushiba *et al.* (2003); b, Singh & Lal (2009).

Description of *Sphingobium faniae* sp. nov.

Sphingobium faniae (fan'i.æ. N.L. fem. n. *faniae* of Fan, to honour Yun-Liu Fan, a respected molecular biologist, for her contributions to the development of agricultural genetic engineering in China).

Cells are Gram-negative, aerobic, non-sporulating, rod-shaped ($0.5\text{--}0.6 \times 1.1\text{--}1.2\ \mu\text{m}$) and non-motile. Colonies on LB agar are circular, smooth, entire, semi-transparent and pale yellow and form brown, water-soluble pigment after 2 days incubation at $30\ ^\circ\text{C}$. Optimum growth at $30\ ^\circ\text{C}$ and pH 7.5; growth is not observed at 4 or $42\ ^\circ\text{C}$. Able to tolerate 1.5% NaCl. Positive for oxidase, urease and catalase, but negative for hydrolysis of gelatin and starch, citrate utilization, the methyl red test, indole production and the Voges–Proskauer reaction. The major non-polar fatty acid is $\text{C}_{18:1\omega7\text{c}}$ and the major 2-hydroxy fatty acid is $\text{C}_{14:0}$ 2-OH. Polar lipids are diphosphatidylglycerol, phosphatidylmonomethylethanolamine, phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine and two sphingoglycolipids. The major cellular polyamine is spermidine, and the major quinone is ubiquinone Q-10. The DNA G+C content of the type strain is $63.8 \pm 0.8\ \text{mol}\%$. Positive for urease, β -galactosidase, hydrolysis of arginine and assimilation of glucose, arabinose, mannose, mannitol, potassium gluconate, capric acid and malic acid; negative for nitrate reduction, hydrolysis of gelatin and aesculin, glucose fermentation and assimilation of L-tryptophan, maltose, phenylacetic acid, adipic acid, N-acetylglucosamine and trisodium citrate. Sensitive to penicillin ($30\ \mu\text{g}$), tetracycline ($10\ \mu\text{g}$), gentamicin ($15\ \mu\text{g}$), erythromycin ($10\ \mu\text{g}$), chloramphenicol ($30\ \mu\text{g}$); resistant to ampicillin ($50\ \mu\text{g}$) and streptomycin ($50\ \mu\text{g}$).

The type strain, JZ-2^T (=CGMCC 1.7749^T =DSM 21829^T), was isolated from activated sludge of an aerobic, pyrethroid-contaminated wastewater of the Yangnong Chemical Group Co., Ltd (Jiangsu, China).

Acknowledgements

We are grateful to Dr Susanne Verbarb and Dr B. J. Tindall (DSMZ) for analysis of polar lipids, quinones and cellular fatty acids. We would also like to thank Dr Bing-jun Yu (College of Life and Science, Nanjing Agricultural University) for analysis of polyamines. This work was supported by the National High Technology Research and Development Program of China (2006AA10Z402), the Natural Science Foundation of Jiangsu Province, China (BK2008331), and the Chinese National Technology Platform Programs (2005DKA21201-2).

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