Brevundimonas viscosa sp. nov., isolated from saline soil

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A Gram-negative, rod-shaped bacterial strain, designated F3^T, was isolated from a saline soil sample in China and studied by using a polyphasic taxonomic approach. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain F3^T was affiliated with the genus *Brevundimonas*, with *Brevundimonas kwangchunensis* KSL-102^T (98.4 % similarity) and *Brevundimonas alba* DSM 4736^T (98.2 %) as its closest relatives. Strain F3^T contained ubiquinone-10 (Q-10) as the predominant ubiquinone and C_{18:1}ω7c, C_{17:1}ω8c and C_{16:0} as the major fatty acids. The DNA G + C content of strain F3^T was 66.7 mol%. Levels of DNA–DNA relatedness between strain F3^T and the type strains of closely related *Brevundimonas* species were below 22 %. On the basis of phenotypic characteristics and genotypic distinctiveness, strain F3^T should be classified as representing a novel species of the genus *Brevundimonas*, for which the name *Brevundimonas viscosa* sp. nov. is proposed. The type strain is F3^T (=CGMCC 1.10683^T=JCM 17426^T).

The genus *Brevundimonas* was proposed by Segers *et al.* (1994) based on the reclassification of two *Pseudomonas* species. Several *Caulobacter* species were subsequently transferred to the genus *Brevundimonas* (Abraham *et al.*, 1999), and novel species of the genus were isolated from diverse habitats (Abraham *et al.*, 2010; Choi *et al.*, 2010; Estrela & Abraham, 2010; Fritz *et al.*, 2005; Kang *et al.*, 2009; Li *et al.*, 2004; Yoon *et al.*, 2006a, b, 2007). At the time of writing, the genus comprises 21 recognized species (<http://www.bacterio.cict.fr/b/brevundimonas.html>). Members of the genus *Brevundimonas* are Gram-negative and have relatively high DNA G + C contents. Chemotaxonomically, members of the genus contain C_{18:1}ω7c and C_{16:0} as the major fatty acids and ubiquinone-10 (Q-10) as the predominant ubiquinone.

In this study, we describe the morphological, biochemical and phylogenetic characteristics of a *Brevundimonas*-like strain, F3^T. The organism was considered to be *Brevundimonas*-like on the basis of preliminary 16S rRNA gene sequence comparisons. Accordingly, the aim of the present study was to elucidate the taxonomic position of strain F3^T by means of phenotypic, genetic and chemotaxonomic analyses.

Strain F3^T was originally isolated from a saline soil sample collected from a coastal area of Hainan Province. It was isolated by the standard dilution-plating technique at 30 °C on nutrient agar (NA; Difco). To investigate its morphological, physiological and biochemical characteristics, strain F3^T was routinely cultivated at 30 °C on trypticase soy agar (TSA; Difco). Cell morphology was observed under a Nikon light microscope with cells grown for 3 days at 30 °C on TSA. Motility of cells was studied on Luria–Bertani swarming agar (0.3 %, w/v), and the presence of flagella was examined by transmission electron microscopy of cells from exponentially growing cultures. Colony morphology was observed on TSA at 30 °C for 3 days. The Gram reaction was determined by using the bioMérieux Gram stain kit according to the manufacturer's instructions. The pH range for growth was determined in nutrient broth (NB; Difco) at pH 4.5–11.5 (intervals of 0.5 pH units) prior to sterilization by the addition of HCl or Na₂CO₃ (below pH 10.5) and KOH (above pH 10.5). Growth at 4, 10, 15, 20, 25, 30, 37 and 40 °C was examined on TSA. Tolerance to NaCl was studied in trypticase soy broth (TSB; Difco) supplemented with 0–10 % (w/v) NaCl. Growth under anaerobic conditions was determined after incubation in an anaerobic chamber on TSA and on TSA supplemented with nitrate, both of which had been prepared anaerobically by using a nitrogen atmosphere. Catalase and oxidase activities, and hydrolysis of casein, gelatin, hypoxanthine, starch, Tweens 20, 40, 60 and 80, tyrosine, urea and xanthine were determined as described

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain F3^T is HM777012.

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

by Cowan & Steel (1965). Hydrolysis of aesculin and nitrate reduction were studied as described by Lányi (1987). Assimilation of various substrates was determined by using the Biolog GN2 MicroPlate assay as recommended by the manufacturer. Susceptibility to antibiotics was tested by placing antibiotic-impregnated discs on TSA plates that were seeded with suspensions of the test strain. The following antibiotics were used: polymyxin B (100 U), streptomycin (50 µg), penicillin G (20 U), chloramphenicol (100 µg), ampicillin (10 µg), cephalothin (30 µg), gentamicin (30 µg), novobiocin (5 µg), tetracycline (30 µg), kanamycin (30 µg), lincomycin (15 µg) and oleandomycin (15 µg). Enzyme activities and other physiological and biochemical properties were tested by using the API ZYM and API 20E systems (bioMérieux). Physiological and biochemical characteristics and the morphology of strain F3^T are shown in Table 1 and in Table S1 and Fig. S1

Table 1. Differential phenotypic characteristics between strain F3^T and the type strains of phylogenetically related species of the genus *Brevundimonas*

Strains: 1, F3^T; 2, *B. aveniformis* EMB102^T; 3, *B. diminuta* LMG 2089^T (data from Ryu *et al.*, 2007); 4, *B. kwangchunensis* KSL-102^T. Data were taken from this study unless indicated otherwise. ND, Not determined; w, weakly positive. All strains were positive for oxidase and catalase and negative for arginine dihydrolase, β-galactosidase and hydrolysis of starch.

Characteristic	1	2	3	4
Urease	–	+	–	–
Nitrate reduction	+	–	–	–
Hydrolysis of:				
Gelatin	+	+	–	–
Tween 40	+	–	+	–
Tween 80	+	–	–	–
Utilization of:				
Cellobiose	–	–	–	+
D-Fructose	–	–	–	+
D-Glucose	+	–	–	+
Maltose	–	–	–	+
D-Mannose	–	–	–	+
Sucrose	–	–	–	+
Trehalose	–	–	–	+
Turanose	–	–	–	+
Acetic acid	–	–	+	+
Lactic acid	–	–	–	+
Succinic acid	–	w	+	+
D-Alanine	–	w	+	+
L-Alanine	–	+	+	+
L-Aspartic acid	–	+	+	+
L-Leucine	–	–	+	–
L-Serine	–	+	+	+
Glucose 1-phosphate	–	–	+	+
Glucose 6-phosphate	–	–	+	+
DNA G + C content (mol%)	66.7	64.1	66.0–68.0	68.7

available in IJSEM Online. Additional characteristics are given in the species description below.

Cell biomass for DNA extraction was obtained after cultivation in TSB at 30 °C. Chromosomal DNA was isolated and purified according to the method described by Yoon *et al.* (1996), with the exception that RNase T1 was used in combination with RNase A to minimize contamination with RNA. The 16S rRNA gene was amplified by PCR by using two universal primers as described by Yoon *et al.* (1998). Sequencing of the amplified 16S rRNA gene was performed as described by Yoon *et al.* (2003). The EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007) was used for determining the closest known relatives of strain F3^T and calculating levels of 16S rRNA gene sequence similarity. The 16S rRNA gene sequences of strain F3^T and related taxa (obtained from the NCBI database) were aligned by using the CLUSTAL X 1.8 program (Thompson *et al.*, 1997). Phylogenetic trees were constructed with the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Kluge & Farris, 1969) and maximum-likelihood methods using the MEGA4 program (Tamura *et al.*, 2007), with bootstrap values based on 1000 replications. The phylogenetic tree constructed on the basis of 16S rRNA gene sequences indicated that strain F3^T was most closely affiliated to the genus *Brevundimonas*. In the neighbour-joining phylogenetic tree, strain F3^T joined the cluster comprising *Brevundimonas kwangchunensis* KSL-102^T and *Brevundimonas aveniformis* EMB102^T (Fig. 1). The topologies of the phylogenetic trees constructed with the maximum-parsimony and maximum-likelihood (Fig. 1) algorithms also supported the notion that the isolate belongs to the genus *Brevundimonas*. Strain F3^T shared 98.4–95.5% 16S rRNA gene sequence similarity with members of the genus *Brevundimonas*, and showed highest similarity to *B. kwangchunensis* KSL-102^T (98.4%), *Brevundimonas alba* DSM 4736^T (98.2%), *Brevundimonas vesicularis* ATCC 11426^T (97.9%), *Brevundimonas lenta* DS-18^T (97.7%), *Brevundimonas aurantiaca* CB-R^T (97.7%), *Brevundimonas nasdae* W1-2B^T (97.7%), *Brevundimonas intermedia* DSM 4732^T (97.6%), *Brevundimonas basaltis* J22^T (97.5%), *B. aveniformis* EMB102^T (97.1%), *Brevundimonas mediterranea* V4.BO.10^T (97.1%) and *Brevundimonas poindexteriae* FWC40^T (97.0%). Levels of 16S rRNA gene sequence similarity to the type strains of species of other genera included in the phylogenetic analysis were less than 95.6% (Fig. 1).

Cell biomass for isoprenoid quinone analysis was obtained after cultivation in TSB at 30 °C. Isoprenoid quinones were extracted according to the method of Komagata & Suzuki (1987) and were analysed by using reversed-phase HPLC and a YMC ODS-A (250 × 4.6 mm) column. For cellular fatty acid analysis, cell mass of strain F3^T was harvested from TSA plates after incubation for 3 days at 30 °C. The fatty acids were extracted and fatty acid methyl esters were prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990); the database used was TSBA40. The DNA G + C content was determined according to the method of

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the positions of strain F3^T, *Brevundimonas* species and some other related taxa. Filled circles and asterisks indicate that the corresponding branches were recovered in the neighbour-joining, maximum-parsimony and maximum-likelihood trees, and both neighbour-joining and maximum-parsimony trees, respectively. Bootstrap values (expressed as percentages of 1000 replications) $\geq 50\%$ are shown at branch points. GenBank accession numbers are given in parentheses. Bar, 0.005 substitutions per nucleotide position.

Tamaoka & Komagata (1984), with the modification that DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC. Chemotaxonomic properties confirmed the phylogenetic affiliation of strain F3^T to the genus *Brevundimonas*. The predominant isoprenoid quinone detected in strain F3^T was Q-10. The major fatty acids ($>10\%$ of the total) were C_{18:1}ω7c (39.3%), C_{17:1}ω8c (14.8%) and C_{16:0} (11.5%). This fatty acid profile was

similar to those of recognized *Brevundimonas* species, although there were differences in the proportions of some components between the taxa (Table S2). The DNA G+C content of strain F3^T was 66.7 mol%.

For polar lipid analyses, strain F3^T was grown for 3 days in TSB at 30 °C. Polar lipids extracted according to the method of Minnikin *et al.* (1979) were identified by

two-dimensional TLC as described by Collins & Jones (1980). The polar lipid profile comprised phosphatidylglycerol, diphosphatidylglycerol, two unknown glycolipids and one unknown phospholipid (Fig. S2).

DNA–DNA hybridization was performed fluorometrically according to the method of Ezaki *et al.* (1989) by using photobiotin-labelled DNA probes and microdilution wells. Hybridization was performed with five replications for each sample. The highest and lowest values obtained in each sample were excluded and the means of the remaining three values were quoted as DNA–DNA relatedness values. DNA–DNA hybridization experiments were carried out to evaluate the genomic DNA relatedness between strain F3^T and its most closely related *Brevundimonas* species. Strain F3^T exhibited levels of DNA–DNA relatedness of less than 22% to the type strains of *B. kwangchunensis* (21.7%), *B. alba* (20.9%), *B. vesicularis* (18.6%), *B. lenta* (17.5%), *B. aurantiaca* (15.9%), *B. nasdae* (16.2%), *B. intermedia* (13.9%), *B. basaltis* (12.3%), *B. aveniformis* (11.1%), *B. mediterranea* (9.7%) and *B. poindexteriae* (8.6%). These values were significantly lower than the threshold of 70% that is commonly accepted for the definition of bacterial species (Wayne *et al.*, 1987).

The major cellular fatty acids of strain F3^T were C_{18:1}ω7c and C_{16:0}, as with recognized *Brevundimonas* species, in particular *Brevundimonas diminuta*, the type species of the genus. The DNA G + C content of strain F3^T was 66.7 mol%, consistent with that of *B. diminuta* strains, which ranged from 66.0 to 68.0 mol%. Phylogenetically, strain F3^T formed a distinct clade within the genus *Brevundimonas* and clustered with *B. kwangchunensis* KSL-102^T and *B. aveniformis* EMB102^T. In addition, it was closely related to *B. kwangchunensis* KSL-102^T based on 16S rRNA gene sequences (98.4% similarity). Therefore, strain F3^T should be assigned to the genus *Brevundimonas*. However, levels of DNA–DNA relatedness between strain F3^T and its closest relatives in the genus *Brevundimonas* were less than 22%. Phylogenetic distinctiveness together with genetic data and differential phenotypic properties were sufficient to allocate strain F3^T to a species that is separate from recognized *Brevundimonas* species. On the basis of the data presented, strain F3^T is therefore considered to represent a novel species of the genus *Brevundimonas*, for which the name *Brevundimonas viscosa* sp. nov. is proposed.

Description of *Brevundimonas viscosa* sp. nov.

Brevundimonas viscosa (vis.co'sa. L. fem. adj. *viscosa* sticky).

Cells are Gram-negative, aerobic rods (0.4–0.6 × 0.9–2.5 μm). Motile by means of a single polar flagellum. Colonies on TSA are circular, smooth, slightly convex, white and 1–2 mm in diameter after 3 days of incubation at 30 °C. Grows at 10–36 °C (optimum 28–30 °C), with 0–3% (w/v) NaCl (optimum 0%) and at pH 5.5–11.0 (optimum pH 7.0–8.0). Anaerobic growth does not occur on TSA or on TSA supplemented with nitrate. Hydrolyses casein, aesculin, tyrosine and Tweens 20 and 40, weakly hydrolyses Tween

60, but does not hydrolyse urea, hypoxanthine or xanthine. H₂S and indole are not produced. Lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase are absent. Positive for activity of alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase and α-glucosidase. Negative for activity of lipase (C14), cystine arylamidase, α-chymotrypsin, α-galactosidase, β-glucuronidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. Sensitive to chloramphenicol, cephalothin, novobiocin, tetracycline, kanamycin, polymyxin B, ampicillin and gentamicin. The predominant ubiquinone is Q-10. Major fatty acids are C_{16:0}, C_{17:1}ω8c and C_{18:1}ω7c. The polar lipid profile comprises phosphatidylglycerol, diphosphatidylglycerol, two unknown glycolipids and one unknown phospholipid. The DNA G + C content of the type strain is 66.7 mol%. Other phenotypic characteristics are given in Tables 1 and S1.

The type strain, F3^T (=CGMCC 1.10683^T=JCM 17426^T), was isolated from saline soil in China.

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