Sphingobium vermicomposti sp. nov., isolated from vermicompost

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Strain VC-230^T was isolated from homemade vermicompost produced from kitchen waste. The isolate was a Gram-negative-staining, catalase- and oxidase-positive, motile rod-shaped bacterium able to grow at 15–37 °C and pH 6–8. On the basis of 16S rRNA gene sequence analysis, strain VC-230^T was determined to belong to the family *Sphingomonadaceae* by its clustering with type strains of the genus *Sphingobium*, with *Sphingobium chlorophenolicum* ATCC 33790^T (97.7 %) and *Sphingobium herbicidovorans* DSM 11019^T (97.4 %) as its closest neighbours. The polar lipid pattern, the presence of spermidine and ubiquinone 10, the predominance of the cellular fatty acids C_{18:1ω7c/9t/12t}, C_{16:1ω7c} and C_{16:0} and the G + C content of the genomic DNA supported the affiliation of this organism to the genus *Sphingobium*. The phylogenetic, chemotaxonomic, phenotypic and DNA–DNA hybridization analyses verify that strain VC-230^T represents a novel species, for which the name *Sphingobium vermicomposti* sp. nov. is proposed. The type strain is VC-230^T (=CCUG 55809^T =DSM 21299^T).

The status of the genus *Sphingomonas* has been the subject of debate (Takeuchi *et al.*, 2001; Yabuuchi *et al.*, 2002); Takeuchi *et al.* (2001) proposed the division of the genus into four genera, mainly on the basis of 16S rRNA gene sequence analysis but also according to chemotaxonomic and phenotypic traits. One of the newly proposed genera, *Sphingobium*, currently comprises 15 species (Euzéby, 1997). This paper describes the phylogenetic, phenotypic and chemotaxonomic properties of a bacterium, designated VC-230^T, isolated from homemade kitchen-refuse vermicompost produced with the earthworm *Eisenia fetida* (Vaz-Moreira *et al.*, 2008).

Vermicomposting is a method for decomposing organic residues into a humus-like product through the digestive process of an earthworm, usually *Eisenia fetida*. Unlike other composting processes, decomposition is achieved by the earthworm's digestive enzymes and, presumably, symbiotic bacteria, and does not involve a thermal phase (Epstein, 1997). Strain VC-230^T was isolated and purified by subculturing on plate count agar (PCA; Pronadisa) from a sample in which total heterotrophic bacterial counts were of the order of 10⁶ c.f.u. (g compost)^{−1}. Among the isolates

recovered from this sample, strain VC-230^T presented a unique random amplified polymorphic DNA profile (Vaz-Moreira *et al.*, 2008); subsequent analysis of the 16S rRNA gene sequence gave evidence that this organism is affiliated to the family *Sphingomonadaceae*.

Strain VC-230^T was cultured on PCA at 30 °C and stored at −80 °C in modified Luria–Bertani broth with 15 % (v/v) glycerol (Vaz-Moreira *et al.*, 2007). Colony characteristics, cell morphology, Gram-stain reaction, cytochrome *c*, oxidase and catalase activity and motility and presence of flagella were analysed following the methodologies of Murray *et al.* (1994) and Smibert & Krieg (1994). Unless otherwise stated, all biochemical and physiological tests were performed as described previously (Vaz-Moreira *et al.*, 2007). Biochemical and nutritional tests were performed using the API 20E, API 20NE, API ZYM and API 50CH strips (bioMérieux), according to the manufacturer's instructions, except for the use of mineral medium B (Barreiros *et al.*, 2003) supplemented with 4 mM (NH₄)₂SO₄ with the API 50CH strip, to test for assimilation of individual carbon sources. Antibiotic susceptibility was assayed as described by Ferreira da Silva *et al.* (2006). The ability to degrade hexachlorocyclohexane (Sigma) was tested at 30 °C in Luria–Bertani

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain VC-230^T is AM998824.

medium supplemented with 4 mg hexachlorocyclohexane l^{-1} . Hexane (Merck) extracts of culture supernatants were analysed using a Varian CP-3800 gas chromatograph equipped with a Varian Saturn 1200L triple quadrupole MS/MS mass selective detector and GC-MS workstation software, version 6.8. The internal standard was 3-octanol (Fluka).

Determination of the G+C content of genomic DNA and the respiratory quinones was done as described previously by Vaz-Moreira *et al.* (2007), using the methods of Mesbah *et al.* (1989) and Tindall (1989), respectively. Polar lipids were extracted and examined as described previously by Manaia *et al.* (2004). Whole-cell fatty acid methyl esters were analysed from cultures grown on R2A minimal medium (Difco) at 30 °C, according to a variation of the MIDI Sherlock Microbial Identification System (<http://www.ccug.se/pages/cfanew.pdf>). Polyamines were extracted as described by Hamana *et al.* (1994) and analysed by LC-MS. Spermidine was detected using a Varian LC-MS 1200L detector coupled with an SS HPLC column (100 × 20 mm; Varian) and a Pursuit 5C18 (Varian) guard column with a mobile phase of water/methanol (50:50, v/v; (Romil) in 0.1 M formic acid (Merck).

The sequence of the 16S rRNA gene was determined after PCR amplification as described by Ferreira da Silva *et al.* (2007). The 16S rRNA gene sequence was compared with others available in the GenBank database using the FASTA sequence similarity search tool of EMBL-EBI (<http://www.ebi.ac.uk>). Phylogenetic analysis was conducted using the BioNumerics software platform (version 5.1; Applied Maths). Estimates of sequence relatedness were based on the model of Jukes & Cantor (1969) and the dendrogram was created using the neighbour-joining method.

Maximum-parsimony and maximum-likelihood methods were used to assess tree stability. Non-homologous and ambiguous nucleotide positions were excluded from the calculations. Genomic DNA–DNA relatedness analyses were performed using the hybridization protocols described by Urdiain *et al.* (2008).

A comprehensive phylogenetic analysis of 16S rRNA gene sequences using neighbour-joining, maximum-likelihood and maximum-parsimony methods, which included sequences from the type strains of all species of the genera *Sphingomonas* and *Sphingobium*, provided evidence that strain VC-230^T clusters with the genus *Sphingobium* (Takeuchi *et al.*, 2001). *Sphingobium chlorophenolicum* ATCC 33790^T and *Sphingobium herbicidovorans* DSM 11019^T were the closest neighbours (97.7 and 97.4 % similarity, respectively) and the type strain of the type species of *Sphingobium*, *Sphingobium yanoikuyae* IFO 15102^T, exhibited 96.3 % similarity. A tree estimating the phylogenetic relationships between strain VC-230^T and the type strains of *Sphingobium* and *Sphingomonas* species is shown in Fig. 1.

Strain VC-230^T and the type strains of the most closely related species, *Sphingobium chlorophenolicum* CCUG 56399^T and *Sphingobium herbicidovorans* CCUG 56400^T, were analysed and compared at the phenotypic level (Table 1). Strain VC-230^T was observed to possess ubiquinone 10 (Q-10) as the major respiratory quinone (96 %). The polar lipid pattern of strain VC-230^T (Fig. 2) included phosphatidylethanolamine, phosphatidylmonomethylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylmethylethanolamine, phosphatidylcholine and a sphingoglycolipid. Similar patterns were observed for *Sphingobium chlorophenolicum* CCUG 56399^T

Fig. 1. Phylogenetic tree derived from 16S rRNA gene sequence analysis showing the relationship of strain VC-230^T with members of the genera *Sphingobium* and *Sphingomonas*. The tree was generated by the neighbour-joining method. Bootstrap values (>50%) based on 1000 resamplings are shown at branch points. The sequence of *Acetobacter acetii* DSM 3508^T was used as an outgroup. Bar, 1 substitution per 100 nucleotide positions.

Table 1. Distinctive characteristics of strain VC-230^T and type strains of closely related species

Strains: 1, *Sphingobium vermicomposti* sp. nov. VC-230^T; 2, *Sphingobium chlorophenicum* CCUG 56399^T; 3, *Sphingobium herbicidovorans* CCUG 56400^T. Data were taken from this study unless indicated. All strains were positive for oxidase, aesculin hydrolysis, assimilation of glucose and production of β -glucosidase, esterase (C4), esterase lipase (C8), leucine and valine arylamidases, trypsin, acid and alkaline phosphatases and naphthol-AS-BI-phosphohydrolase. All strains were negative for Gram-staining, citrate utilization, H₂S, indole and acetoin production, gelatin hydrolysis, nitrate reduction, glucose fermentation, oxidation of D-glucose, D-mannitol, inositol, D-sorbitol, sucrose and melibiose, assimilation of D-mannose, D-mannitol, N-acetylglucosamine, maltose, caprate, citrate, phenyl acetate, glycerol, erythritol, D- and L-arabinose, D-ribose, D- and L-xylose, adonitol, methyl β -D-xylopyranoside, D-fructose, L-sorbose, dulcitol, inositol, methyl α -D-mannopyranoside, methyl α -D-glucopyranoside, amygdalin, salicin, melibiose, sucrose, trehalose, inulin, melezitose, raffinose, starch, glycogen, xylitol, gentiobiose, turanose, D-lyxose, D-tagatose, D- and L-fucose, D- and L-arabitol, potassium gluconate, 2-ketogluconate and 5-ketogluconate and production of arginine dihydrolase, lysine and ornithine decarboxylases, urease, tryptophan deaminase, lipase (C14), cystine arylamidase, α -galactosidase, β -glucuronidase, N-acetyl- β -glucosaminidase, α -mannosidase or α -fucosidase. +, Positive; w, weak; –, negative; ND, no data available.

Characteristic	1	2	3
Isolation source	Vermicompost	Soil ^{a*}	Soil ^b
Colony colour	Yellow	Yellow	Yellow with melanin production
Cell shape	Rods	Rods	Short rods
Motility	+	–	+
Oxidation (API 20E) of:			
Amygdalin	–	+	–
L-Arabinose	+	–	+
L-Rhamnose	–	–	+
Assimilation of:			
Cellobiose	–	+	–
D-Galactose	+	–	+
Lactose	+	–	–
L-Rhamnose	+	–	+
Arbutin	+	–	–
Adipate	–	w	w
Malate	–	+	+
Enzyme production			
α -Chymotrypsin	–	+	–
β -Galactosidase	+	–	–
α -Glucosidase	–	+	+
DNA G + C content (mol%)	62	65 ^c	ND

*Data taken from: a, Nohynek *et al.* (1996); b, Zipper *et al.* (1997); c, Fujii *et al.* (2001).

and *Sphingobium herbicidovorans* CCUG 56400^T, although the unknown phosphoglycolipid was not detected in these strains. This polar lipid composition is in agreement with those observed in sphingomonads of the genera *Sphingobium* and *Sphingomonas* (Busse *et al.*, 1999). The predominant fatty acids were C_{16:1} ω 7c, C_{16:0} and C_{18:1} ω 7c/9t/12t, which comprised over 80 % of the total (Table 2). The genomic DNA G + C content for strain VC-230^T was determined to be 62.2 $\pm$ 0.3 mol%. These characteristics confirmed the inclusion of strain VC-230^T with the sphingomonads and the presence of the polyamine spermidine indicated that the strain belongs to the genus *Sphingobium* (Takeuchi *et al.*, 2001). These data were in agreement with the phylogenetic analysis based on 16S rRNA gene sequences.

Because of the high 16S rRNA gene sequence similarities between strain VC-230^T and *Sphingobium chlorophenicum* ATCC 33790^T (97.7 %) and *Sphingobium herbicido-*

vorans DSM 11019^T (97.4 %), genomic DNA–DNA relatedness values were determined between strain VC-230^T and *Sphingobium chlorophenicum* CCUG 56399^T (36 %, mean of duplicate measurements of 29 and 42 %) and *Sphingobium herbicidovorans* CCUG 56400^T (49 %, mean of 47 and 51 %). These values confirmed that strain VC-230^T represents a distinct and novel species of the genus *Sphingobium*. Such results are in agreement with the evidence given by Stackebrandt & Ebers (2006), wherein strains that show less than 98.5 % 16S rRNA gene sequence similarity present DNA–DNA reassociation values lower than 70 %, thus excluding the possibility of inclusion in these species as long as strain VC-230^T exhibits distinctive phenotypic features. In fact, when the three strains were tested concurrently, strain VC-230^T could be distinguished from *Sphingobium chlorophenicum* CCUG 56399^T and *Sphingobium herbicidovorans* CCUG 56400^T on the basis of several morphological, biochemical and physiological traits (Table 1). Furthermore, after growth under the same

Fig. 2. Polar lipid pattern of strain VC-230^T. DPG, Diphosphatidylglycerol; PC, phosphatidylcholine; PDE, phosphatidylmethylethanolamine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PGL, unidentified phosphoglycolipid; PL, unidentified phospholipid; PME, phosphatidylmonomethylethanolamine; SGL, sphingoglycolipid.

cultivation conditions, the amount of C_{18:1}ω7c/9t/12t was significantly lower in strain VC-230^T than in *Sphingobium chlorophenicum* CCUG 56399^T and *Sphingobium herbicidovorans* CCUG 56400^T (45.9 % compared with approx. 54.5 %; Table 2).

Table 2. Cellular fatty acid compositions of strain VC-230^T, type strains of closely related species and the type strain of the type species of the genus *Sphingobium*

Strains: 1, *Sphingobium vermicomposti* sp. nov. VC-230^T; 2, *Sphingobium chlorophenicum* CCUG 56399^T; 3, *Sphingobium herbicidovorans* CCUG 56400^T; 4, *Sphingobium yanoikuyae* CCUG 28380^T. Data were taken from this study. Values are percentages of total fatty acids; only those present as 1.0 % or more of the total are shown. —, Not detected or <1 %.

Fatty acid	1	2	3	4
C _{14:0}	1.2	—	—	—
C _{14:0} 2-OH	6.6	10.0	6.7	9.4
C _{16:1} ω7c	21.3	11.7	18.0	18.8
C _{16:1} ω5c	—	1.5	1.0	4.3
C _{16:0}	13.7	13.3	12.4	12.4
C _{17:1} ω6c	2.2	2.4	—	—
C _{18:1} ω7c/9t/12t	45.9	54.9	53.9	51.1
C _{18:1} ω5c	1.7	2.5	—	4.1
11-Methyl C _{18:1} ω7c	5.7	3.7	8.1	—
Summed total	99.2	100	100	100

On the basis of the polyphasic characterization presented herein, a novel species, *Sphingobium vermicomposti* sp. nov., is proposed.

Description of *Sphingobium vermicomposti* sp. nov.

Sphingobium vermicomposti (ver.mi.com.pos'ti. L. masc. n. *vermis* worm; N.L. neut. n. *compostum* compost; N.L. gen. n. *vermicomposti* from compost produced by earthworms).

Colonies are yellow, circular and convex on PCA, Luria–Bertani agar, tryptic soy agar and Mueller–Hinton agar. No growth is observed on m-faecal coliform (m-FC) medium. Cells are Gram-negative-staining rods (0.95 ± 0.23 µm long and 0.44 ± 0.07 µm wide), motile by a polar flagellum. Strictly aerobic. Nitrate is not reduced. Catalase- and oxidase-positive. Growth occurs at 15–37 °C (optimum approx. 30 °C), at pH 6–8 (optimum pH 7) and in the presence of 1 % NaCl. Aesculin is hydrolysed. Produces β-galactosidase, β-glucosidase, esterase (C4), esterase lipase (C8), leucine and valine arylamidases, trypsin, naphthol-AS-BI-phosphohydrolase and acid and alkaline phosphatases. Arbutin, D-galactose, D-glucose, lactose and L-rhamnose are used as single carbon sources in the absence of organic growth factors. L-Arabinose is oxidized (API 20E). Growth occurs with (µg per disc) sulfamethoxazole/trimethoprim (23.75/1.25) but not with amoxicillin (25), gentamicin (10), ciprofloxacin (5), tetracycline (30), cephalothin (30), meropenem (10), ceftazidime (30), ticarcillin (75) or colistin sulfate (50). The predominant fatty acids are C_{18:1}ω7c/9t/12t, C_{16:1} and C_{16:0}. The major respiratory quinone is Q-10. The major polar lipids are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylmethylethanolamine, phosphatidylcholine and a sphingoglycolipid. Spermidine is produced. Hexachlorocyclohexane is not degraded. The DNA G + C content of the type strain is 62 mol%.

The type strain is VC-230^T (=CCUG 55809^T =DSM 21299^T), isolated from homemade kitchen-waste vermicompost.

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