Screening for endophytic nitrogen-fixing bacteria in Brazilian sugarcane varieties used in organic farming and description of *Stenotrophomonas pavanii* sp. nov.

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Running Title: A new *Stenotrophomonas* species found in sugarcane

Summary

Strain ICB 89^T is a Gram-negative staining, rod-shaped, non-spore-forming and nitrogen-fixing bacterium and was isolated from stems of a Brazilian sugarcane variety widely used in organic farming. 16S rRNA gene sequence analysis revealed that strain ICB 89^T belongs to the genus *Stenotrophomonas* and is most closely related to *Stenotrophomonas maltophilia, S. rhizophila, S. nitritireducens, Pseudomonas geniculata, P. hibiscicola* and *P. beteli*. DNA-DNA hybridizations together with chemotaxonomic data and biochemical

characteristics allow the differentiation of strain ICB 89^T from its phylogenetically nearest neighbours. Strain ICB 89^T therefore represents a new species, for which the name *Stenotrophomonas pavanii* sp. nov. is proposed, with strain ICB 89^T (= CBMAI 564^T and LMG 25348^T) as the type strain.

In a study investigating the biodiversity of endophytic nitrogen-fixing bacteria (ENFB) in Brazilian organic cultivated sugarcane plants, 31 isolates were obtained from roots, stems and leaves. Nitrogen fixing capability was determined by the acetylene reduction assay (ARA) and the presence of the *nif*H gene sequence was detected by dot-blot hybridization. Amplified Ribosomal DNA Restriction Analysis (ARDRA) patterns and 16S rRNA gene sequence analysis revealed the presence of 11 different bacterial genera (data not shown). The strain ICB 89^T belonging to the genus *Stenotrophomonas*, was further investigated using a polyphasic taxonomic approach.

At the time of writing, the genus *Stenotrophomonas* comprises seven recognized species. The type species, *S. maltophilia*, is an important cause of nosocomial infections and is commonly found in a wide range of environmental niches (Palleroni & Bradbury, 1993). It was lately described in endophytic association with some agronomic species, exerting beneficial effects on growth (nitrogen fixation, phytohormone induction) and anti-fungal activity (Wolf *et al*., 2002; Vega *et al*., 2005; Idris *et al*., 2009). Additonally six environmental species are allocated to the genus: *S. nitritireducens*, N_2O-producing bacteria isolated from ammonia-supplied biofilters *(*Finkmann *et al*., 2000); *S. rhizophila*, a plant-associated species displaying antifungal activity (Wolf *et al*., 2002); *Stenotrophomonas acidaminiphila,* isolated from a lab scale methanogenic reactor treating industrial wastewater (Assih *et al*., 2002); *S. koreensis,* isolated from compost in Daejeon, South Korea (Yang *et al*., 2006); *S. terrae* and *S. humi,* nitrate-reducing bacteria isolated from soil samples in Ghent, Belgium (Heylen *et al*., 2007). *S. chelatiphaga*, a new species recently isolated from sewage sludge in Kazan city, Russian

Federation, by Kaparulina et al. (2009) has been formally described but so far the name has not been validated. Here we present the first report of a *Stenotrophomonas* species fixing nitrogen in sugarcane.

Strain ICB 89^T was isolated from sugarcane (*Saccharum officinarum*) variety SP80-1842 (Azevedo *et al*., 2003) and kept in organic cultivation. Leaves, stem and roots were externally decontaminated and macerated in sterile saline essentially as described by Döbereiner (1980). Aliquots of macerated plant material were inoculated into selective semi solid NFb medium (Hartmann & Baldani, 2006) and incubated at 30º C up to 10 days. Nitrogen fixation was determined by the standard assay described by Turner and Gibson (1980). Replicates of the enrichment cultures or, alternatively, vials inoculated with pure cultures, were injected with 1 ml of pure acetylene into the overhead space (10% of vial volume) and incubated at 30º C for 24 h. Acetylene reduction was detected by gas chromatographic analysis essentially as described by Liba *et al*. (2006). Single colonies of nitrogen-fixing bacteria were re-inoculated onto NFb semi-solid medium to confirm nitrogen-fixing ability by the ARA (Liba *et al*., 2006).

Genomic DNA was obtained by using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA; Cat. A 1120), according to the manufacturer's instructions. DNA was spotted onto Hybond ^{32}P N+ membranes, as recommended by the manufacturer's protocol. Dot-Blot hybridizations were carried out using a 705 bp probe for *Azospirillum brasilense* Sp7^T *nif*H gene (GenBank accession number M64344). This probe was amplified by PCR using the primers PPf (5´-GCAAGTCCACCACCTCC-3´) and PPr (5´-TCGCGTGGACCTTGTTG-3´) described by Reinhardt *et al*. (2008). Probe labeling and hybrizations were performed according to the ECL System (GE HealthCare, Chalfont St. Giles, UK) recommended protocol and hybridizations were carried out at 60ºC for 16 h, without formamide.

Strain ICB 89^T reduced acetylene in the chromatographic assay, indicating its nitrogen fixing capability and the dot-blot hybridization revealed the presence of a *nif*H-related sequence. No hybridization was detected with bovine genomic DNA, used as negative control, whereas a strong hybridization signal was obtained with *Azospirillum brasilense* Sp7^T genomic DNA, the positive control, thus confirming probe specificity. The dot-blot hybridization experiment corroborated the nitrogen fixing ability of strain ICB 89^T, as indicated by ARA.

Amplification of the 16S rRNA gene was performed using 30-50 ng of DNA in 50 *µ*l reactions containing 2 mmol l^{-1} $MgCl_2$, 200 *µ*mol l^{-1} dNTPs (each), 0.3 *µ*mol l^{-1} each of universal primers 27f (5´ AGAGTTGATCCTGGCTCAG 3´) and 1525r (5' AAGGAGGTGWTCCARCC 3´) and 2U *Taq* DNA polymerase (Invitrogen) in the recommended buffer. The reaction mixtures were incubated in a PCR device (Eppendorf Master Cycler Gradient) at 94° C for 2 min and then cycled 30 times: 94° C for 1 min, 55° C for 1 min and 72°C for 3 min. A final extension at 72° C for 10 min was used.

Sequence analysis was performed, using MegaBACE 1000 DNA sequencer (GE Healthcare, Chalfont St. Giles, UK). PCR products were purified with "GFX^{TM} PCR DNA and Gel Band Purification Kit" (cat no. 28-9034-70 GE Healthcare), according to manufacturer's instructions. Purified PCR products were eluted in 30 *µ*l sterile MilliQ water. Subsequently, 5.0 *µ*l of purified PCR product was mixed with 4.0 *µ*l DYEnamic^{TM} ET dye terminator kit (MegaBACE^{TM}, GE Healthcare), 1.0 *µ*mol l^{-1} sequencing primer (0.5 *µ*mol l^{-1}). The thermal program consisted of 30 cycles of 20 s at 95°C, 15 s at 55°C (annealing temperature) and 60 s at 60°C. Sequencing products were purified according to the manufacture's instructions. The primers used in the sequencing reactions were 27f (5´AGAGTTGATCCTGGCTCAG 3´), 782r (5' ACCAGGGTATCTAATCCTGT 3'), 530f (5' CAGCAGCCGCGGTAATAC 3'), MG5f (5' AAACTCAAAGGAATTGACGG 3') and 1525r (5' AAGGAGGTGWTCCARCC 3´). Sequencing data of the 16S rRNA gene were compared to bacterial sequences deposited at GenBank (Altschul *et al*., 1997) in order to

identify the bacteria at the genus level. Sequences with high homology scores were retrieved from GenBank and consensus sequences were aligned by Clustal*W* (Altschul *et al*., 1990) using the software MEGA 4 (http://www.megasoftware.net). A phylogenetic tree was constructed based on the neighbour-joining algorithm (Saitou & Nei, 1987), maximum-likelihood and maximum-parsimony method. The resultant tree topologies were evaluated by bootstrap analysis (Felsenstein, 1985) based on 1000 replicates.

Strain ICB 89^T showed the highest sequence similarities to *S. maltophilia* (99.9%), *S. rhizophila* (99.7%), *S. nitritireducens* (99.5%) and to the misclassified species *P. geniculata* (99.8%), *P. hibiscicola* (99.7%) and *P. beteli* (99.5%). The neighbour-joining and maximum parsimony trees, showing the taxonomic position of strain ICB 89^T within the genus *Stenotrophomonas* are given in Figure 1. The neighbour-joining and maximum likelihood trees showed quasi the same topology (ML tree in the supplementary files). Bootstrap analysis showed that the cluster encompassing ICB 89^T and all *Stenotrophomomas* type strains are maintained in 92% of the replicates. The subcluster containing *S. pavanii*, *S. maltophilia*, *P. beteli*, *P. hibiscicola* and *P. geniculata* is maintained in 100% of the replicates. EzTaxon (http://147.47.212.35:8080/index.jsp) analyses showed that ICB 89^T shares 99.7% of 16S rRNA sequence similarity with *S. maltophilia*, 99.2% with *P. geniculata*, 99.1% with *P. hibiscicola*, 99.0% with *P. beteli*, 98.1% with *S. rhizophila*, 97.5% with *S. nitritireducens* and 97.2% with *S. acidaminiphila*. In order to determine the genomic relatedness between strain ICB 89^T and its closest related phylogenetic neighbours, DNA-DNA hybridizations and phenotypic analyses were performed.

DNA–DNA hybridizations were performed at 45°C according to a modification (Goris *et al*., 1998, Cleenwerck *et al*., 2002) of the method described by Ezaki *et al*. (1989). Genomic DNA of bacterial strains was prepared according to a modification (Cleenwerck *et al*., 2002) of the procedure of Wilson (1987). Reciprocal reactions (e.g. A x B and B x A) were

performed for every pair of DNA and their variation was within the limits of this method (Goris *et al*., 1998). The DNA reassociation percentages reported are the means of a minimum of four hybridizations. The DNA G+C content of strain ICB 89^T was determined from DNA prepared for the DNA-DNA hybridizations, according to the HPLC method (Mesbah *et al.*, 1989).

Strain ICB 89^T showed a mean DNA–DNA relatedness of 60% (+/- 4.0), 59% (+/- 5.0), 51% (+/- 10.0), 35% (+/- 3.0) and 31% (+/- 0) with *S. maltophilia* LMG 958^T*, P. geniculata* LMG 2195^T*, P. beteli* LMG 978^T*, S. rhizophila* LMG 24537^T and *S. nitritireducens* LMG 22074^T, respectively, which is clearly below the 70% cut-off value for species delineation (Wayne *et al.*, 1987). DDH results for *P. hibiscicola* LMG 980^T showed 68% (+/- 3.0) DNA–DNA relatedness with ICB 89 ^{T} but type strains from these two species presented quite different phenotypic features as shown in Table 1, demonstrating that this strain belongs to a novel species. The G+C content of strain ICB 89^T is 67.5 mol%, which is consistent with the G+C content reported for the genus *Stenotrophomonas* (Assih *et al.*, 2002; Yang *et al.*, 2006; Heylen *et al.*, 2007).

Cell morphology, motility and possible sporulation were investigated using an Olympus CH-2 microscope with cells grown on TSA medium for 24 h at 28°C. Cells were found to be Gram-negative (Bergey's Manual., 2005), catalase positive and oxidase negative (Cappuccino & Sherman, 2002). Utilization of carbon sources and enzyme production was tested using the API 20E, API ZYM and API 50 CH systems (bioMérieux, Marcy l'Etoile, France), according to the manufacturer's instructions. The temperature range (4-45° C), pH range (4.0-14.0 at 30° C) and salinity range (0.5 - 6.0% w/v, at 30° C) for growth were recorded after incubation for 48 h on TSA medium. The phenotypic and biochemical characteristics of all *Stenotrophomonas* species are given in Table 1.

The cellular fatty acid patterns of the strains were determined as described by Mergaert *et al*. (2001). Cells were incubated for 24 h at 28°C-30°C on TSA medium. The MIDI system with the TSBA50 database was used for identification. The fatty acid composition of strain

ICB 89^T is given in Table 2 and the most abundant fatty acids are iso-C15:0 (32.15%), anteiso-C15:0 (17.07%), summed feature 3 (comprising C16:1ω7c and/or iso-C15:0 2-OH) (9.48%) and 16:0 (6.01%). Overall, the fatty acid profile of *S. pavanii* is similar to those of other *Stenotrophomonas* species (Assih *et al.*, 2002; Wolf *et al.*, 2002; Heylen *et al.*, 2007).

Strain ICB 89^T shows morphological and biochemical characteristics typical of the genus *Stenotrophomonas* and it can be clearly differentiated from other *Stenotrophomonas* and *Pseudomonas* species by several phenotypic properties (Table 1). Based on the results of the polyphasic taxonomic study, strain ICB 89^T represents a novel species within the genus *Stenotrophomonas*, for which the name *Stenotrophomonas pavanii* sp. nov. is proposed.

Description of *Stenotrophomonas pavanii* sp. nov.

Stenotrophomonas pavanii (pa. va' ni.i. N.L. gen. n. pavanii of Pavan, named in honour of the Brazilian geneticist Crodowaldo Pavan).

Cells stain Gram-negative and are non-motile and do not form spores. They are catalase positive and oxidase negative. Growth is observed at 20–37° C (but not at 4, 40 and 45° C), at pH 5–12 (but not at pH 4, 13, 14 and 15) and at salinity of 0.7–3 % (but not at 4, 5 and 6%). It is esterase, trypsin, β-glucosidase, aesculin, valine arylamidase, N-acetyl-β-glucosaminidase, β-glucosidase, β-galactosidase, lysine decarboxylase, citrate, tryptophan deaminase, gelatinase, Tween 80 and DNase positive.

Assimilation of acetoin, glucose, mannitol, inositol, D-sorbitol, rhamnose, sucrose, melibiose, amygdalin, arabinose, α-chymotrypsin, glycerol, erythritol, D-arabinose, L-arabinose, D-ribose, D-xylose, L-xylose, D-adonitol, methyl-βD-xylopiranoside, D-galactose, D-glucose, D-frutose, D-mannose, L-sorbose, L-rhamnose, dulcitol, D-mannitol, methyl-αD-mannopyranoside, methyl-αD-glucopyranoside, N-acetylglucosamine, arbutin, salicin, D-cellobiose, D-maltose, D-lactose, D-melibiose, D-sucrose, D-trehalose, inuline,

D-melezitose, D-rafinose, starch, glycogen, xylitol, gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate, 2-ceto-potassium gluconate, and 5-ceto-potassium gluconate are negative. Negative for urease, ornithine decarboxylase, arginine dihydrolase and cistine arylaminidase reactions.

The fatty acid composition of strain ICB 89^T is given in Table 2 and the most abundant fatty acids are iso-C15:0 (32.15%), anteiso-C15:0 (17.07%), summed feature 3 (comprising C16:1ω7c and/or iso-C15:0 2-OH) (9.48%) and 16:0 (6.01%). The G+C content of strain ICB89^T is 67.5 mol%. The type strain was isolated from stems of sugarcane variety SP80-1842 and deposited at CBMAI in Brazil (Brazilian Collection of Environmental and Industrial Microorganisms) as CBMAI 564^T and in the BCCM/LMG Bacteria Collection as LMG 25348^T.

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Table 1 Physiological characteristics of *S. pavanii* ICB 89^T and related species of the genus *Stenotrophomonas*

Strains: 1, ICB 89^T (*Stenotrophomonas pavanii* sp.nov.); 2, *S. terrae* LMG 23958^T; 3, *S. humi* LMG 23959^T ; 4, *S. nitritireducens* LMG 22074^T; 5, *S. acidaminiphila* LMG 22073^T; 6, *S. koreensis* LMG 23369^T; 7, *S. maltophilia* LMG 958^T; 8, *S. maltophilia* LMG 22072; 9, *S. rhizophila* LMG 22075^T; 10, *P. beteli* LMG 978^T; 11, *P. hibiscicola* LMG 980^T; 12, *P. geniculata* LMG 2195^T. Data presented in columns 2 to 9 were, otherwise indicated, obtained from Heylen *et al.* (2007). nd = not determined.

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323 * Data from Finkmann *et al.* (2000).

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12
Hydrolysis of Tween 80 (lipolytic activity)	+	+	-	-,+*	-,+†	+	+	-	+	nd	nd	nd
Oxidase	-	+	+	+	+	-,+ıı	-	-	+	-	-	-
ß-glucosidase (aesculin hydrolysis)	+	-	-	-	-	-	w	+	+	+	+	+
Protease (gelatin hydrolysis)	+	+	w	-	-	+	+	+	+	+	+	+
Assimilation of:												
D-Fructose	-	+	+	+	+	-	+	+	+	-	-	w
D-Glucose	-	+	-	-	+	-	+	+	+	-	w	w
D-Arabinose	-	-	-	-	-	-	-	nd	-	-	-	-
Maltose	-	+	+	-	+	-	+	+	+	w	+	w
D-Mannose	-	+	+	-	+	-	+	+	+	-	w	w
Sucrose	-	-	-	-	-	-	+	+	-	-	-	-
D-Sorbitol	-	-	-	-	-	-	-	nd	-	-	-	-
D-Galactose	-	-	-	-	-	-	-	nd	-	-	-	-
D-Xylose	-	-	-	-	-	-	-	-	-,+¶	-	-	-
D-Cellobiose	-	-	-	-	-	-	+	+	+	-	-	w
Citrate	+	+	+	+	-	-	+	+	+	+	+	+
Aesculin	+	-	-	-	-	-	+	-	-	+	+	+
Gentiobiose	-	-	-	-	-	-	+	+	-,+¶	-	-	-
D-Lactose	-	-	-	-	-	-	-,+#	w,+#	-	-	+	-
D-Melibiose	-	-	-	-	-	-	-	+	-	-	-	-
D-Turanose	-	-	-	-	-	-	-	+	-,+¶	-	-	-
Inositol	-	w	-	-	-	-	-	-	-	-	-	-
N-Acetylglucosamine	-	+	+	+	+	-	+	+	+	-	-	-
Amygdalin	-	-	-	-	-	-	+	+	-	-	-	-
Arbutin	-	-	-	-	-	-	+	+	-	-	w	w
Salicin	-	-	-	-	-	-	+	-	-	-	w	w
Lipase	-	+	+	-	-	-	-	-	-	-	-	+
Leucine Arylamidase	+	+	-	-	-	-	-	+	+	+	+	+
Valine Arylamidase	+	-	-	-	-	-	-	+	+	+	-	-
N-acetyl-ß-glucosamidase	+	-	+	+	+	-	-	-	-	-	-	-
Glycogen	-	-	-	-	-	-	-	-	-,+¶	-	-	-
Growth at 4 °C	-	-	-	-	-	-	-	nd	+	-	-	-
Growth at 40 °C	-	-	-	-	+	-	-	-	-	+	+	+
Growth in the presence of 4.5% NaCl	-	+	-	-	-	-	+	+	+	-	-	+
Growth at pH 12	+	-	-	-	-	-	-	-	-	+	-	-

324 † Data from Assih *et al.* (2002).
325 ıı Data from Yang *et al.* (2006).
326 # Data from Drancourt *et al.* (1997).
327 ¶ Data from Wolf *et al.* (2002).
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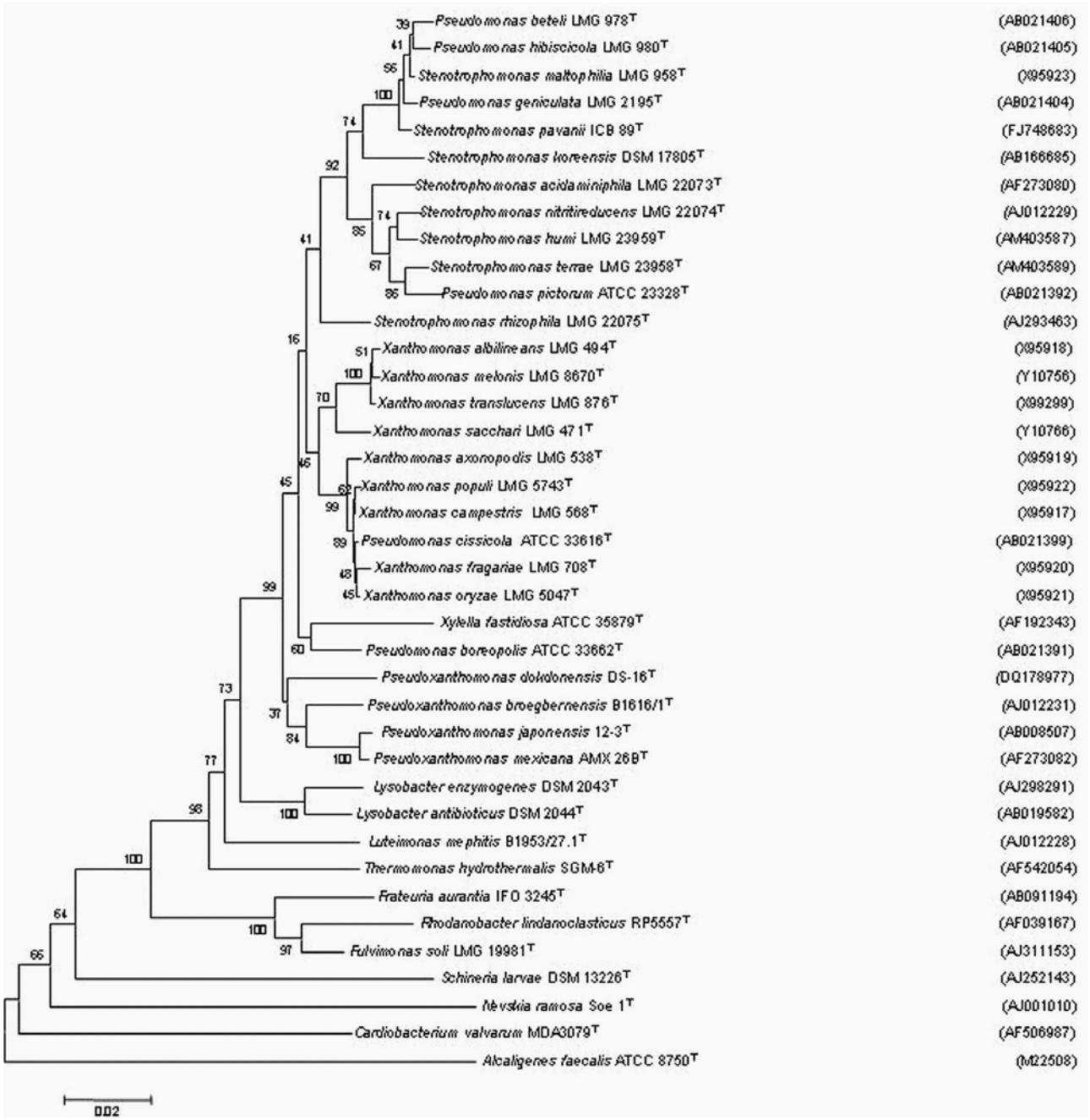
Table 2 Cellular fatty acid composition of *Stenotrophomonas pavanii* sp. nov. and its closest phylogenetic neighbours

Values are percentages of the total fatty acid content. Strains: 1, *Stenotrophomonas pavanii* sp. nov. ICB 89^T; 2, *Stenotrophomonas terrae* LMG 23958^T; 3, *Stenotrophomonas humi* LMG 23959^T; 4, *Stenotrophomonas nitritireducens* LMG 22074^T; 5, *Stenotrophomonas acidaminiphila* LMG 22073^T; 6, *Stenotrophomonas koreensis* LMG 23369^T; 7, *Stenotrophomonas maltophilia* LMG 958^T; 8, *Stenotrophomonas maltophilia* LMG 22072; 9, *Stenotrophomonas rhizophila* LMG22075^T; 10, *Pseudomonas beteli* LMG 978^T; 11, *Pseudomonas hibiscicola* LMG 980^T; 12, *Pseudomonas geniculata* LMG 2195^T. Fatty acids accounting for less than 1.0 % of the total fatty acids in all strains are not shown. Summed feature 3 contains C16:1ω7c and/or iso-C15:0 2-OH. Data presented in columns 2 to 9 were obtained from Heylen *et al.* (2007). nd, not detected; ECL, equivalent chain-length.

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12
$C_{10:0}$	0.57	0.4	0.6	0.3	0.3	1.4	0.6	0.9	0.7	1.03	0.77	1.15
$C_{10:0}$ 3-OH	0.17	0.1	0.1	0.1	0.1	0.4	0.2	0.3	0.2	0.33	0.26	nd
iso-$C_{10:0}$	nd	0.8	0.9	0.6	1.7	1.3	nd	nd	nd	nd	nd	nd
$C_{11:0}$ 3-OH	nd	0.2	0.3	0.4	0.2	2.2	0.1	0.1	0.1	0.11	0.13	nd
iso-$C_{11:0}$	3.81	6.6	6.5	6.4	5.2	13.5	4.0	5.35	4.6	6.14	5.0	4.96
iso-$C_{11:0}$ 3-OH	1.59	2.0	1.9	2.1	2.7	14	1.9	3.0	2.1	3.24	2.25	2.94
$C_{12:0}$	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	2.41
$C_{12:0}$ 3-OH	2.21	1.4	2.6	1.2	1.3	2.2	3.0	4.7	3.6	5.08	3.81	5.12
iso-$C_{12:0}$ 3-OH	0.27	4.1	5.2	3.0	3.1	1.0	0.2	0.5	0.3	0.35	0.29	nd
$C_{13:0}$	nd	nd	nd	nd	nd	1.0	nd	nd	nd	nd	nd	nd
$C_{13:0}$ 2-OH	0.75	nd	nd	nd	nd	nd	nd	nd	nd	0.91	1.02	nd
iso-$C_{13:0}$	0.21	0.6	0.6	0.8	0.6	9.5	0.5	0.6	0.6	0.51	0.36	nd
iso $C_{13:0}$ 3-OH	4.35	2.6	2.0	2.4	2.8	8.5	4.2	5.4	4.9	5.81	4.93	4.59
$C_{14:0}$	2.28	2.5	2.1	1.2	0.9	1.7	3.1	3.6	3.8	3.41	2.84	4.73
iso-$C_{14:0}$	0.91	14.2	15.7	8.7	7.0	2.3	0.7	1.4	1.3	0.96	0.91	1.47
iso-$C_{15:0}$	32.15	23	20.5	30.6	32.0	15.4	38.5	33.0	36.0	30.52	32.59	29.69
iso-$C_{15:1}$	0.54	4.6	2.0	2.8	2.7	16	0.9	1.2	1.0	0.78	0.72	1.11
anteiso-$C_{15:0}$	17.07	4.6	5.1	5.8	5.4	0.9	9.3	11.3	12.3	13.94	15.43	10.35
$C_{16:0}$	6.01	3.0	2.1	3.4	2.5	0.3	6.4	3.8	4.9	3.75	5.23	6.98
iso-$C_{16:0}$	2.11	8.0	12.7	10.2	10.1	0.2	1.2	1.3	1.4	1.37	1.52	1.40
$C_{16:1}$ω9c	2.10	1.0	0.8	0.8	0.8	nd	2.6	2.5	2.5	2.21	2.06	3.30
iso-$C_{17:0}$	3.69	0.8	0.9	1.9	2.0	0.3	3.2	1.5	1.9	1.71	2.36	1.92
iso-$C_{17:1}$ω9c	3.97	7.2	4.6	11.4	11.6	1.4	4.3	3.5	3.3	3.32	3.33	3.19
cyclo-$C_{17:0}$	0.20	nd	nd	1.8	nd	nd	nd	nd	0.1	nd	0.24	nd
$C_{18:1}$ω9c	1.45	nd	nd	nd	nd	nd	nd	nd	nd	0.49	0.74	0.81
summed feature 3	9.48	9.5	9.4	1.7	4.6	1.7	10.2	10.3	9.4	9.81	9.34	11.45
Unknown (ECL 11.799)	0.38	nd	0.50	nd	nd	nd	1.80	2.70	2.00	2.60	1.94	2.43

LEGENDS

Figure 1 Neighbour-joining dendrogram of 16S rRNA gene sequences showing the estimated phylogenetic relationships between *Stenotrophomonas pavanii* sp. nov. and closely related *Stenotrophomonas* and *Pseudomonas* species. Bootstrap values (percentages of 1000 replicates) are shown. Bar represents 0.02 % estimated sequence divergence.


Pseudomonas beteli LMG 978T (AB021406)
Pseudomonas hibiscicola LMG 980T (AB021405)
Stenotrophomonas maltophilia LMG 958T (X95923)
Pseudomonas geniculata LMG 2195T (AB021404)
Stenotrophomonas pavanii ICB 89T (FJ748683)
Stenotrophomonas koreensis DSM 17805T (AB166685)
Stenotrophomonas acidaminiphila LMG 22073T (AF273080)
Stenotrophomonas nitritireducens LMG 22074T (AJ012229)
Stenotrophomonas humi LMG 23959T (AM403587)
Stenotrophomonas terrae LMG 23958T (AM403589)
Pseudomonas pictorum ATCC 23328T (AB021392)
Stenotrophomonas rhizophila LMG 22075T (AJ293463)
Xanthomonas albilineans LMG 494T (X95918)
Xanthomonas melonis LMG 8670T (Y10756)
Xanthomonas translucens LMG 876T (X99299)
Xanthomonas sacchari LMG 471T (Y10766)
Xanthomonas axonopodis LMG 538T (X95919)
Xanthomonas populi LMG 5743T (X95922)
Xanthomonas campestris LMG 568T (X95917)
Pseudomonas cissicola ATCC 33616T (AB021399)
Xanthomonas fragariae LMG 708T (X95920)
Xanthomonas oryzae LMG 5047T (X95921)
Xylella fastidiosa ATCC 35879T (AF192343)
Pseudomonas boreopolis ATCC 33662T (AB021391)
Pseudoxanthomonas dokdonensis DS-16T (DQ178977)
Pseudoxanthomonas broegbernensis B1616/1T (AJ012231)
Pseudoxanthomonas japonensis 12-3T (AB008507)
Pseudoxanthomonas mexicana AMX 26BT (AF273082)
Lysobacter enzymogenes DSM 2043T (AJ298291)
Lysobacter antibioticus DSM 2044T (AB019582)
Luteimonas mephitis B1953/27.1T (AJ012228)
Thermomonas hydrothermalis SGM-6T (AF542054)
Frateuria aurantia IFO 3245T (AB091194)
Rhodanobacter lindanoclasticus RP5557T (AF039167)
Fulvimonas soli LMG 19981T (AJ311153)
Schineria larvae DSM 13226T (AJ252143)
Nevskia ramosa Soe 1T (AJ001010)
Cardiobacterium valvarum MDA3079T (AF506987)
Alcaligenes faecalis ATCC 8750T (M22508)
0.02

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