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Brevundimonas vancanneytii sp. nov., isolated from blood of a patient with endocarditis

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A Gram-negative, rod-shaped, non-spore-forming bacterial strain, designated LMG 2337^T, was isolated from the blood of a patient with endocarditis and characterized. The strain was affiliated with the alphaproteobacterial genus *Brevundimonas*, with *Brevundimonas diminuta* LMG 2089^T (98.3 % 16S rRNA gene sequence similarity) and *Brevundimonas terrae* KSL-145^T (97.5 %) as its closest relatives. This affiliation was supported by chemotaxonomic data: the G+C content was 66.3 mol %, the major polar lipids were phosphatidyl diacylglycerol, sulfoquinovosyl diacylglycerol and phosphatidyl glucopyranosyl diacylglycerol and the major fatty acids were summed feature 7 (one or more of $C_{18:1}\omega7c$, $C_{18:1}\omega9t$ and $C_{18:1}\omega12t$) and $C_{16:0}$. Strain LMG 2337^T displayed an unusually broad substrate spectrum. The results from DNA–DNA hybridization and physiological and biochemical tests allowed the genotypic and phenotypic differentiation of strain LMG 2337^T from all of the type strains of hitherto-described *Brevundimonas* species. The strain therefore represents a novel species, for which the name *Brevundimonas vancanneytii* sp. nov. is proposed, with type strain LMG 2337^T (=CCUG 1797^T =ATCC 14736^T).

The genus *Brevundimonas* was proposed by Segers *et al.* (1994) to harbour strains previously assigned to *Pseudomonas diminuta* and *Pseudomonas vesicularis*. At that time, *Brevundimonas* included only species that form short rods that are motile by means of one polar flagellum with a short wavelength. This changed when caulobacteria from a broad range of freshwater, brackish water, marine and soil habitats were studied using a polyphasic approach (Anast & Smit, 1988; MacRae & Smit, 1991; Segers *et al.*, 1994). The descriptions of the genera *Caulobacter* and *Brevundimonas* were emended and a number of *Caulobacter* species were transferred to the genus *Brevundimonas* (Abraham *et al.*, 1999). Today, *Brevundimonas* species are differentiated from *Caulobacter* mainly by the lack of loop 46 in the V8 region of the 16S rRNA gene (Abraham *et al.*, 2008), the presence of $C_{12:0}$ 3-OH, the glycophospholipid composition and higher salt tolerances. We report here on a novel species within the genus *Brevundimonas* that was isolated from blood of a patient with endocarditis and preliminarily identified as belonging to *Brevundimonas diminuta* (Segers *et al.*, 1994).

The reference strains for this study were obtained from the ATCC, DSMZ and LMG culture collections. The strains were grown in the freshwater *Caulobacter* medium PYEM [per litre MQ-water (Biocel A 10; Millipore): 2 g peptone, 2 g yeast extract, 0.5 g NH_4Cl]. After autoclaving and cooling, 5 ml filter-sterilized 0.2 mg riboflavin ml^{-1}, 2 ml 50 % glucose, 1 ml 20 % $MgSO_4$ and 1 ml 10 % $CaCl_2$ (all sterilized) were added. The strains were grown in 2 l flasks at 30 °C with shaking at 100 r.p.m. and biomass was harvested in the late exponential phase after 72 h.

For the determination of DNA base composition, genomic DNA was isolated from two loopfuls of bacterial cells using the DNeasy Blood and Tissue kit for purification of total DNA (Qiagen) with the addition of RNase A (Sigma), according to the manufacturers' instructions. DNA was enzymically digested as described by Gehrke *et al.* (1984) and the mean G+C content was determined by HPLC (Tamaoka & Komagata, 1984). Calculations were performed according to Mesbah *et al.* (1989), with non-methylated lambda phage DNA (Sigma) as the standard. The G+C content of strain LMG 2337^T was 66.3±0.1 mol %, which is in the range of values already reported for species of the genus *Brevundimonas* (Segers *et al.*, 1994; Vancanneyt *et al.*, 2005; Yoon *et al.*, 2006).

The phylogenetic position of strain LMG 2337^T was determined by analysis of the 16S rRNA gene sequence (Abraham *et al.*, 1999) using CLUSTAL W (Thompson *et al.*, 1997). An alignment of sequences from the EMBL database (Kanz *et al.*, 2005) was used to construct a maximum-parsimony tree (Fig. 1) with MEGA version 3.1 (Kumar *et al.*, 2004) and *Hirschia baltica* DSM 5838^T as an outgroup. The 16S rRNA gene sequence from strain LMG

Abbreviation: DGL, 1,2-di-*O*-acyl-3-*O*-[β-D-glucopyranosyl-(1→4)-α-D-glucopyranuronosyl]glycerol.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain LMG 2337^T is AJ227779.

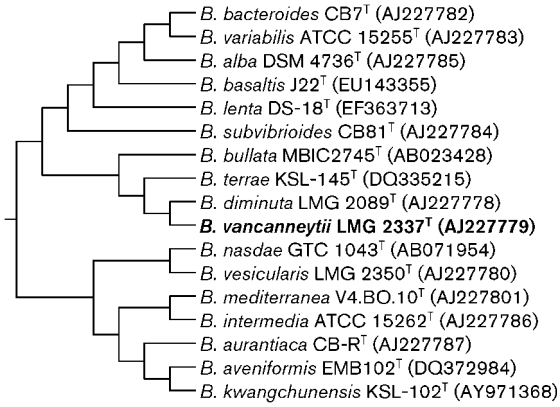

Fig. 1. Maximum-parsimony dendrogram of the phylogenetic relationships between strain LMG 2337^T and strains of the genus *Brevundimonas*, based on a distance-matrix analysis of 16S rRNA gene sequences. The sequence of *Hirschia baltica* DSM 5838^T (GenBank accession no. AJ421782) was used as an outgroup (not shown).

2337^T lacked loop 46 in the V8 region, which is characteristic for *Brevundimonas* species, and had the highest similarities with *B. diminuta* LMG 2089^T (98.3 %) and *Brevundimonas terrae* KSL-145^T (97.5 %).

To determine whether strain LMG 2337^T belonged to the species *B. diminuta* or *B. terrae* or whether it represented a novel species, DNA–DNA hybridizations between strain LMG 2337^T and *B. diminuta* LMG 2089^T and *B. terrae* DSM 17329^T were performed. DNA was isolated using a French pressure cell and purified by chromatography on hydroxyapatite as described by Cashion *et al.* (1977). DNA–DNA hybridization was carried out twice as described by De Ley *et al.* (1970) using the modification described by Huß *et al.* (1983) in a Cary 100 Bio UV/Vis spectrophotometer equipped with a Peltier-thermostatted 6×6 multicell changer and a temperature controller with an *in situ* temperature probe. The DNA–DNA relatedness between strain LMG 2337^T and *B. diminuta* LMG 2089^T and *B. terrae* DSM 17329^T was 62 and 16 %, respectively. It has been shown that DNA–DNA relatedness between two strains of 70 % or more qualifies them as members of the same species (Wayne *et al.*, 1987). Hence, the results indicated that strain LMG 2337^T indeed belonged to a novel species.

Table 1. Fatty acid contents of whole-cell hydrolysates of strain LMG 2337^T and some type strains of the genus *Brevundimonas*

Strains: 1, *Brevundimonas vancanneytii* sp. nov. LMG 2337^T; 2, *B. diminuta* LMG 2089^T; 3, *B. bullata* DSM 7126^T; 4, *B. subvibrioides* LMG 14903^T; 5, *B. alba* ATCC 15265^T; 6, *B. variabilis* ATCC 15255^T; 7, *B. bacteroides* LMG 15096^T; 8, *B. aurantiaca* ATCC 15266^T; 9, *B. intermedia* ATCC 15262^T; 10, *B. vesicularis* LMG 2350^T. Values are percentages of total fatty acids; data were obtained in this study. ECL, Equivalent chain-length; tr, trace (<1.0 %); −, not detected.

Fatty acid	1	2	3	4	5	6	7	8	9	10
$C_{12:0}$ 3-OH	1.8	1.5	1.3	2.8	1.1	2.3	2.3	2.3	1.7	1.9
$C_{12:1}$ 3-OH	−	tr	tr	−	tr	−	−	tr	−	−
$C_{14:0}$	1.3	tr	tr	4.6	1	2.5	3	3.4	1.5	2.4
$C_{15:0}$	2.5	7.6	5.1	1.5	5.4	5.4	tr	3	2.8	4
ECL 15.275	−	−	−	−	2	−	−	−	−	−
$C_{16:0}$	20.8	10.1	18.9	15.9	16.8	13.5	12.8	21.3	24.3	20.7
Summed feature 4*	4.6	1	4.5	11.2	5.9	6.2	6.7	4	7.5	5.1
$C_{16:1}\omega 9c$	−	−	−	−	2.7	−	−	−	−	−
$C_{17:0}$	1.0	8.5	1.6	tr	2.5	2.2	tr	1.7	1.6	2.4
$C_{17:1}\omega 6c$	tr	6.2	1.5	tr	1.5	2.5	tr	1	1.1	1.4
$C_{17:1}\omega 8c$	1.6	10.8	3.3	1.8	7.7	5.7	1.4	1.6	2	2.6
ECL 17.897†	1.0	1.1	1.1	tr	1.4	tr	tr	1.3	tr	1.1
$C_{18:0}$	−	tr	tr	1.4	tr	tr	tr	tr	tr	tr
Summed feature 7*	53.5	38.7	55.5	56.7	43.2	55.8	69.4	56.5	49.4	53.7
$C_{18:1}\omega 9c$	−	−	−	−	1	−	−	−	−	−
11-Methyl $C_{18:1}\omega 5t$	1.3	−	1.6	tr	2.7	−	tr	3.5	6.3	4.5
ECL 18.797	−	3.1	tr	−	tr	1	−	−	−	−
$C_{19:0}$ cyclo $\omega 8c$	7.4	6.2	1.1	−	2.4	−	−	−	−	−
$C_{20:2}\omega 6,9c$	−	−	−	1.6	−	−	−	−	−	−

*Summed features represent groups of two or three fatty acids that cannot be separated by the Microbial Identification System. Summed feature 4 consisted of one or more of iso-$C_{15:0}$ 2-OH, $C_{16:1}\omega 7c$ and $C_{16:1}\omega 7t$. Summed feature 7 consisted of one or more of $C_{18:1}\omega 7c$, $C_{18:1}\omega 9t$ and $C_{18:1}\omega 12t$.

†11-Methyl-12-*trans*-octadecanoic acid has ECL 18.080.

For whole-cell fatty acid analysis, cells were grown at 30 °C for 48 h on PYEM agar plates (1.5 % agar). Cells were saponified (15 %, w/v, NaOH at 100 °C for 30 min), methylated to fatty acid methyl esters (methanolic HCl at 80 °C for 10 min) and extracted (hexane/methyl *tert*-butyl ether, 1 : 1, v/v) as described in detail by Osterhout *et al.* (1991). Fatty acid methyl esters were analysed on a gas chromatograph equipped with a fused-silica capillary column (25 m × 0.2 mm) with cross-linked 5 % phenyl methyl silicone (film thickness 0.33 μm; HP Ultra 2) using the protocol of Osterhout *et al.* (1991). For all strains, the main fatty acids were summed feature 7 (one or more of $C_{18:1}\omega7c$, $C_{18:1}\omega9t$ and $C_{18:1}\omega12t$) and $C_{16:0}$ and the main hydroxy fatty acid was $C_{12:0}$ 3-OH, all of which are characteristic of the genus *Brevundimonas* (Table 1).

Polar lipid analysis with fast atom bombardment mass spectrometry was performed in the negative mode on the first of two mass spectrometers of a tandem high-resolution instrument in an $E_1B_1E_2B_2$ configuration (JMS-HX/HX110A; JEOL) using conditions described by Abraham *et al.* (1997). All strains contained phosphatidylglycerol, 1,2-di-*O*-acyl-3-*O*-[β-D-glucopyranosyl-(1→4)-α-D-glucopyranuronosyl] glycerol (DGL), lyso-phosphoglucolipid and 3-*O*-[6′-(*sn*-glycero-3′-phosphoryl)-α-D-glucopyranosyl]-*sn*-glycerol. The almost exclusive occurrence of $C_{19:1}$ DGL and the almost exclusive absence of $C_{18:1}$ DGL in strain LMG 2337^T are remarkable. Furthermore, sulfoquinovosyl diacylglycerol was detected in strain LMG 2337^T (Table 2).

For phenotypic characterization, strains were grown at 30 °C in 20 ml PYEM amended with 0, 5, 10, 20, 30, 40, 60, 80 or 100 g NaCl l^{-1}. The OD_{600} of each cell suspension was determined at the beginning of the experiment and after 2 days and the difference between the two measurements was used to determine salt tolerance. Strain LMG 2337^T grew best with 5–30 g NaCl l^{-1} and grew slowly with 60 g NaCl l^{-1} but not with 80 g NaCl l^{-1}.

Table 2. Phospholipids and sulfolipids in strain LMG 2337^T and some type strains of the genus *Brevundimonas*

Strains: 1, *B. vancanneytii* sp. nov. LMG 2337^T; 2, *B. diminuta* LMG 2089^T; 3, *B. bullata* DSM 7126^T; 4, *B. subvibrioides* LMG 14903^T; 5, *B. variabilis* ATCC 15255^T; 6, *B. bacteroides* LMG 15096^T; 7, *B. aurantiaca* DSM 4731^T; 8, *B. intermedia* ATCC 15262^T; 9, *B. vesicularis* LMG 2350^T. All data were obtained in this study. DGL, 1,2-di-*O*-acyl-3-*O*-[β-D-glucopyranosyl-(1→4)-α-D-glucopyranuronosyl]glycerol; LPGL, lyso-phosphoglucolipid; PGL, 3-*O*-[6′-(*sn*-glycero-3′-phosphoryl)-α-D-glucopyranosyl]-*sn*-glycerol; PG, phosphatidylglycerol; SQDG, sulfoquinovosyl diacylglycerol.

Mass (Da)	Polar lipid	COOH		Present in strain:								
		R_1	R_2	1	2	3	4	5	6	7	8	9
734	PG	$C_{18:1}$	$C_{15:0}$	−	−	−	−	−	−	+	+	−
746	PG	$C_{18:1}$	$C_{16:1}$	+	+	+	+	+	−	−	−	−
748	PG	$C_{18:1}$	$C_{16:0}$	+	+	+	+	+	+	+	+	+
750	PG	$C_{19:0}$	$C_{15:0}$	−	−	−	−	−	−	−	−	+
760	PG	$C_{18:1}$	$C_{17:1}$	+	−	+	−	−	−	−	+	−
762	PG	$C_{19:1}$	$C_{16:0}$	+	+	+	−	−	+	+	+	−
774	PG	$C_{18:1}$	$C_{18:1}$	+	+	+	+	+	+	+	+	+
788	PG	$C_{19:1}$	$C_{18:1}$	+	+	+	−	+	−	−	−	−
820	SQDG	$C_{18:1}$	$C_{16:0}$	+	−	+	+	+	+	+	+	−
834	SQDG	$C_{18:1}$	$C_{17:0}$	−	−	+	+	−	−	−	−	−
846	SQDG	$C_{18:1}$	$C_{18:1}$	−	−	+	+	+	+	+	+	−
930	DGL	$C_{18:1}$	$C_{16:1}$	−	−	−	−	+	−	+	−	+
932	DGL	$C_{18:1}$	$C_{16:0}$	−	+	+	+	+	+	+	+	+
944	DGL	$C_{18:1}$	$C_{17:1}$	−	−	−	−	−	−	−	−	−
958	DGL	$C_{18:1}$	$C_{18:1}$	−	+	+	−	+	−	+	−	+
972	DGL	$C_{19:1}$	$C_{18:1}$	+	−	−	−	−	−	−	−	−
986	DGL	$C_{19:1}$	$C_{19:1}$	+	+	−	−	−	−	+	−	−
1172	LPGL			−	−	+	−	−	−	−	−	−
1174	LPGL			+	−	+	+	−	+	−	−	−
1411	PGL			−	−	+	+	+	+	−	−	−
1413	PGL			+	+	+	+	+	−	+	+	+
1425	PGL			−	−	−	−	−	+	−	+	−
1427	PGL			+	+	+	+	−	−	+	+	+
1439	PGL			+	+	+	+	+	+	+	+	+
1441	PGL			−	−	−	−	−	−	−	−	−
1453	PGL			+	+	+	−	−	+	+	+	+
1465	PGL			+	+	−	−	+	+	−	−	+

Substrate specificity tests were conducted with API 20NE test strips (bioMérieux) and GN2 MicroPlates (Biolog), according to the manufacturers' instructions, at 30 °C for 24 h. A test was considered positive if the interface between the sample well and the air was visibly turbid due to bacterial growth (Rüger & Krambeck, 1994). The results are given in the species description and in Table 3. It should be noted that strain LMG 2337^T showed an exceptionally broad substrate usage, which differed sharply from that of the reference strains. Enzyme activity tests were conducted with API ZYM test strips (bioMérieux), according to the manufacturer's instructions. The test for leucine arylamidase activity was weakly positive in strain LMG 2337^T but positive in *B. diminuta* LMG 2089^T. The characters that discriminate between strain LMG 2337^T and its closest phylogenetic neighbours are summarized in Table 3.

Table 3. Characteristics that are useful for distinguishing strain LMG 2337^T from closely related type strains of the genus *Brevundimonas*

Strains: 1, *B. vancanneytii* sp. nov. LMG 2337^T; 2, *B. terrae* DSM 17329^T; 3, *B. diminuta* LMG 2089^T; 4, *B. bullata* DSM 7126^T. All data were obtained in this study unless indicated otherwise. ++, Strongly positive; +, positive; (+), weakly positive; −, negative.

Characteristic	1	2	3	4
Enzyme activity				
Leucine arylamidase	(+)	(+)	+	++
Valine arylamidase	(+)	(+)	(+)	++
Acid phosphatase	++	(+)	++	+
Protease	−	+	−	−
Substrate utilization				
Arabinose	+	(+)	(+)	(+)
D-Galactose	+	−	−	−
Gentiobiose	+	−	−	−
Glycogen	−	−	+	(+)
Glycerol	(+)	−	++	−
Maltose	(+)	−	−	−
D-Mannose	+	−	+	−
Adonitol	+	−	−	−
Caprate	++	(+)	+	−
Malate	++	(+)	(+)	(+)
α-Ketobutyric acid	++	−	++	−
α-Ketovaleric acid	++	−	+	−
α-Ketoglutaric acid	+	−	−	−
Propionic acid	++	−	++	(+)
Malonic acid	(+)	−	+	−
Quinic acid	(+)	−	+	−
Bromosuccinic acid	++	−	+	−
Succinic acid monomethyl ester	++	−	++	−
Pyruvic acid methyl ester	++	−	++	+
Citric acid	(+)	−	−	−
p-Hydroxyphenylacetic acid	+	−	−	−
D-Alanine	++	−	(+)	−
L-Alanyl glycine	++	−	++	−
L-Asparagine	++	−	++	−
L-Aspartic acid	++	−	++	−
L-Histidine	++	−	++	−
L-Serine	++	−	+	−
L-Threonine	++	−	++	−
Phenylethylamine	−	−	+	−
N-Acetyl-D-galactosamine	−	−	+	+
Alaninamide	++	−	++	−
α-Cyclodextrin	−	−	+	−
DNA G+C content (mol%)	66.3	61.8[a*]	65.9[b]	66.7[c]

*Data from: *a*, Yoon *et al.* (2006); *b*, Segers *et al.* (1994); *c*, Fritz (2000).

Strain LMG 2337^T could be differentiated from all hitherto-described *Brevundimonas* species. In particular, strain LMG 2337^T differed from *B. terrae* DSM 17329^T by having strong acid phosphatase activity and the ability to use α-keto-fatty acids and many amino acids, from *B. diminuta* LMG 2089^T by having smaller amounts of $C_{17:1}\omega6c$ and $C_{17:1}\omega8c$, the ability to use *p*-hydroxyphenylacetic acid, D-gentiobiose, D-alanine and adonitol and from *Brevundimonas bullata* DSM 7126^T by the ability to use many amino acids. As a consequence, we propose a novel species, *Brevundimonas vancanneytii* sp. nov.

Description of *Brevundimonas vancanneytii* sp. nov.

Brevundimonas vancanneytii (van.can.ney′ti.i. N.L. masc. gen. n. *vancanneytii* of Vancanneyt, named after Marc Vancanneyt, a microbiologist in Ghent, Belgium, who has contributed to the chemotaxonomy of many genera).

Cells are short rods and motile by one polar flagellum with no prosthecae. Cell division is by fission. Colonies are whitish yellow. Grows on peptone yeast extract medium with 0–60 g NaCl l^{-1} but not with 80 g NaCl l^{-1}. Utilizes α-(+)-D-glucose, β-(+)-D-fructose, (+)-D-galactose, (+)-trehalose, (+)-D-mannose, (+)-melibiose, sucrose, (+)-raffinose, maltose, lactose, D-psicose, (+)-gentiobiose, (+)-L-arabinose, α-L-rhamnose, α-L-fucose, (+)-turanose, (+)-D-arabitol, glycerol, *myo*-inositol, D-mannitol, D-sorbitol, adonitol, D-saccharic acid, malic acid, *cis*-aconitic acid, citric acid, D-glucuronic acid, D-galacturonic acid, D-gluconic acid, *p*-hydroxyphenylacetic acid, (−)-quinic acid, 4-aminobutyric acid, DL-lactic acid, succinic acid, capric acid, itaconic acid, pyruvic acid methyl ester, succinic acid monomethyl ester, α-, β- and γ-hydroxybutyric acids, propionic acid, acetic acid, bromosuccinic acid, α-ketobutyric acid, α-ketoglutaric acid, α-ketovaleric acid, formic acid, malonic acid, sebacic acid, D-galactonic acid lactone, D-glucosaminic acid, urocanic acid, succinamic acid, L-pyroglutamic acid, L-aspartic acid, L-glutamic acid, glycyl L-glutamic acid, glycyl L-aspartic acid, L-alanyl glycine, hydroxy-L-proline, L-threonine, L-leucine, L-histidine, L-asparagine, L-proline, D- and L-alanine, D- and L-serine, L-ornithine, L-phenylalanine, *N*-acetyl-D-glucosamine, putrescine, 2-aminoethanol, alaninamide, Tweens 40 and 80, methyl D-glucoside, dextrin, glucuronamide, uridine, 2,3-butanediol, DL-α-glycerol phosphate, glucose 1-phosphate and glucose 6-phosphate. Does not utilize glycogen, inosine, thymidine, aesculin, arginine, urea or tryptophan. Nitrate is not reduced. Produces alkaline and acid phosphatases, esterase (C4), esterase lipase (C8), leucine arylamidase, trypsin, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase and oxidase, but not catalase. The polar lipids are α-D-glucopyranosyl-, α-D-glucuronopyranosyl-, β-D-glucopyranosyl-(1→4)-α-D-glucopyranuronosyl-, sulfoquinovosyl-, phosphatidyl- and 6-phosphatidyl-α-D-glucopyranosyl diacylglycerols. The major fatty acids (>10 %) are summed feature 7 (one or more of $C_{18:1}\omega7c$, $C_{18:1}\omega9t$ and $C_{18:1}\omega12t$) and $C_{16:0}$. The minor fatty acids are $C_{19:0}$ cyclo $\omega8c$, summed feature 4 (one or more of iso-$C_{15:0}$ 2-OH, $C_{16:1}\omega7c$ and $C_{16:1}\omega7t$), $C_{15:0}$, $C_{12:0}$ 3-OH, $C_{17:1}\omega8c$, $C_{14:0}$, 11-methyl $C_{18:1}\omega5t$ and $C_{17:0}$. The G+C content of the type strain is 66.3 mol%.

The type strain is LMG 2337^T (=CCUG 1797^T =ATCC 14736^T), isolated from blood of a patient with endocarditis.

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