Devosia limi sp. nov., isolated from a nitrifying inoculum

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A Gram-negative, rod-shaped, non-spore-forming bacteria was isolated from a nitrifying inoculum. On the basis of 16S rRNA gene sequence similarity, this strain, designated LMG 22951^T, was shown to belong to the 'Alphaproteobacteria' and to be related to *Devosia neptuniae* (97.4 %) and *Devosia riboflavina* (97.0 %). The results of DNA–DNA hybridization, analysis of fatty acid composition, SDS-PAGE, physiological and biochemical tests allowed genotypic and phenotypic differentiation of LMG 22951^T from the two recognized *Devosia* species. LMG 22951^T therefore represents a novel species within this genus, for which the name *Devosia limi* is proposed. The type strain is LMG 22951^T (=DSM 17137^T).

The genus *Devosia* (family *Hyphomicrobiaceae*, order *Rhizobiales*) was first described by Nakagawa *et al.* (1996), and at present the genus comprises two species, *Devosia riboflavina* (Nakagawa *et al.*, 1996), formerly *Pseudomonas riboflavina* (Foster, 1944), comprising one soil isolate, and *Devosia neptuniae* (Rivas *et al.*, 2003), comprising two isolates from a dinitrogen-fixing root-nodule from the aquatic legume *Neptunia natans* in India. Furthermore, a hitherto uncultured endosymbiont of the marine ciliated protozoon *Euplotes magnicirratus* was shown to belong to *Devosia* and given the provisional name '*Candidatus Devosia euplotis*' (Vannini *et al.*, 2004). Three closely related sequences of this provisional species are available in the EMBL database.

Among organisms isolated from an enriched nitrifying community, strain LMG 22951^T was recovered on a mineral salts medium (Stanier *et al.*, 1966) with 1.69 mM succinate incubated at 22 °C for 3 weeks. Enrichment of the autotrophic nitrifying community from activated sludge was stimulated by adding a daily load of nitrogen [58.7 g total ammoniacal nitrogen (TAN) l⁻¹ day⁻¹ as ammonium chloride and 29.3 g NO₂⁻ N l⁻¹ day⁻¹ as sodium nitrite] to a 500 litre reactor. The purpose of the enriched nitrifying community is to enhance removal of ammonia and nitrite in aquaria and aquacultures. For more detailed information refer to Grommen *et al.* (2002).

The nearly complete 16S rRNA gene sequence of LMG 22951^T was determined as described below. DNA was extracted according to Pitcher *et al.* (1989) as modified by Heyndrickx *et al.* (1996). PCR amplification was performed

as described by Heyrman & Swings (2001). The PCR-amplified 16S rRNA gene products were purified using the Nucleofast 96 PCR system (Machery-Nagel). For each sequence reaction a mixture of 3 µl purified product, 1 µl BigDye Termination RR mix version 3.1 (Perkin Elmer), 1.5 µl BigDye buffer (5 ×) and 3 µl (20 ng µl⁻¹) of one of the six sequencing primers was used. MilliQ water was added to a total volume of 10 µl. The primers and PCR program were as described by Heyrman & Swings (2001). The sequence was determined using an Applied Biosystems 3100 DNA Sequencer following the manufacturer's protocols (Perkin-Elmer). Sequence assembly was performed with the software KODON version 2.0 (Applied Maths). Phylogenetic analysis using BIONUMERICS version 3.5 (Applied Maths) showed that the closest related sequences of LMG 22951^T were *D. neptuniae* (97.4 % 16S rRNA gene sequence similarity) and *D. riboflavina* (97.0 %). Furthermore, LMG 22951^T showed 97.5 % similarity to the three sequences of '*Candidatus D. euplotis*' (Fig. 1).

The DNA G+C content of LMG 22951^T, determined by HPLC (Mesbah *et al.*, 1989) according to the further specifications given by Logan *et al.* (2000), was 61.9 mol%. This is within the range of 61–63 mol% given in the emended genus description of *Devosia* (Rivas *et al.*, 2003). DNA–DNA hybridization experiments were performed between LMG 22951^T and the type strains of *D. neptuniae* and *D. riboflavina* using a modification of the microplate method of Ezaki *et al.* (1989) as described by Willems *et al.* (2001). A hybridization temperature of 45 °C (calculated with correction for the presence of 50 % formamide) was used. LMG 22951^T showed DNA–DNA relatedness of 24 % to *D. neptuniae* LMG 21357^T and 17 % to *D. riboflavina* LMG 2277^T, suggesting that the strain represents a novel genospecies.

After incubation for 48 h at 28 °C on trypticase soy agar

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain LMG 22951^T is AJ786801.

Fig. 1. Phylogenetic dendrogram obtained by neighbour-joining clustering of 16S rRNA gene sequences showing the position of LMG 22951^T among the available sequences of *Devosia* species. Accession numbers are shown in parentheses. Relevant bootstrap values (expressed as percentages of 1000 replications) are shown at branch points.

(TSA), a loopful of well-grown cells was harvested and fatty acid methyl esters were prepared as described by Vandamme *et al.* (1992), separated and then identified using the Sherlock Microbial Identification System (version 3.0; MIDI). Dominant fatty acids of LMG 22951^T were C_{18:1}ω7c (36.32%), 11-methyl-C_{18:1}ω7c (30.42%), C_{16:0} (11.34%) and C_{18:0} (9.68%). LMG 22951^T differs from the other *Devosia* species in containing smaller amounts of C_{16:0} and larger amounts of C_{18:0} and C_{18:1}ω7c (Table 1). Neighbour-joining clustering (data not shown) with the Canberra metric distance coefficient using BIONUMERICS version 3.5 (Applied Maths) indicated 76.3% similarity of LMG 22951^T to *D. neptuniae* LMG 21357^T and 70.3% to *D. riboflavina* LMG 2277^T.

For SDS-PAGE of whole-cell proteins, isolates were grown on phosphate-buffered nutrient agar (NA) (pH 6.8) and incubated aerobically at 28 °C for 40 h. The preparation of whole-cell proteins and SDS-PAGE were performed as described by Pot *et al.* (1994). Densitometric analysis, normalization and interpolation of the protein profiles were performed using GELCOMP v4.2 (Applied Maths). UPGMA clustering of the protein profiles (data not shown)

Table 1. Cellular fatty acid compositions of *Devosia limi* sp. nov. LMG 22951^T, *D. neptuniae* LMG 21357^T and *D. riboflavina* LMG 2277^T

tr, Trace (<1.0%); —, not detected.

Fatty acid	<i>D. limi</i> LMG 22951 ^T	<i>D. neptuniae</i> LMG 21357 ^T	<i>D. riboflavina</i> LMG 2277 ^T
C _{10:0} 3-OH	1.68	tr	4.09
C _{14:0}	tr	tr	—
C _{16:0}	11.34	24.65	22.56
C _{17:0}	tr	—	—
cyclo-C _{17:0}	—	3.13	1.60
C _{18:1} ω7c	36.32	14.35	26.80
C _{18:0}	9.68	4.95	2.92
11-methyl C _{18:1} ω7c	30.42	36.15	34.88
cyclo-C _{19:0} ω8c	tr	7.85	4.19
C _{18:0} 3-OH	4.59	4.79	—
C _{20:1} ω7c	tr	—	tr
Summed feature 3*	2.96	2.27	1.54

*Summed feature 3 comprises C_{16:1}ω7c and/or iso-C_{15:0} 2-OH.

revealed a similarity below 70% between LMG 22951^T and *D. neptuniae* LMG 21357^T and *D. riboflavina* LMG 2277^T. This observation supports the conclusion that LMG 22951^T belongs to a novel species within the genus *Devosia*.

Cell morphology was investigated by light microscopy at ×1000 magnification of cells grown on TSA for 48 h at 28 °C. Cells were Gram stained and examined for catalase and oxidase activity. The presence of the nitrogen-fixing genes *nifH* and *nifD* was tested using the primers and PCR conditions described by Rivas *et al.* (2002) after plasmid DNA extraction with a Fast Plasmid Mini kit (Eppendorf). Strains were tested for the ability to denitrify by anaerobic incubation at 22 or 37 °C in trypticase soy broth (TSB) supplemented with 10 mM NO₃⁻ as KNO₃ and 10 μM phenol red. The presence of nitrite and nitrate was tested after 24 and 48 h and 7 days by using the procedure described by Smibert & Krieg (1994). *Escherichia coli* LMG 2029^T was included as a control. Utilization of carbon sources and enzyme production (Table 2) were tested using API 20NE, API 50CH, API ZYM strips (bioMérieux) and Biolog MicroPlates according to the manufacturers' instructions. *D. riboflavina* LMG 2277^T and *D. neptuniae* LMG 21357^T were included as controls. Strain LMG 22951^T produced negative results for all API 20NE test strip carbon assimilation tests. The same result was achieved using a mineral salts medium (Stanier *et al.*, 1966) as well as using denser cell suspensions, extended the incubation time (up to 14 days) and the addition of 0.01% yeast extract. API 50CH strips and the Biolog system did not give reproducible results, even with prolonged incubation (up to 7 days). Antimicrobial susceptibilities (Table 2) were determined with the disc diffusion method using an ST6090 Disc Dispenser (Oxoid). Antibigrams were determined according to the conventional Kirby-Bauer method (Bauer *et al.*, 1966). Isolates were classified into three categories based on the quantitative interpretation criteria recommended by the NCCLS (1993).

Based on the polyphasic data presented, strain LMG 22951^T represents a novel species in the genus *Devosia*, for which the name *Devosia limi* sp. nov. is proposed.

Description of *Devosia limi* sp. nov.

Devosia limi (li'mi. L. gen. n. *limi* of sludge).

After 48 h of incubation at 28 °C on TSA or NA, colonies are circular with smooth margins, light yellow to light brown

Table 2. Physiological characteristics of the type strains of *Devosia* species

+, Positive; (+), weakly positive; –, negative. All strains were positive for oxidase, catalase, phosphatase alkaline, leucine arylamidase, phosphatase acid, naphthol-AS-BI-phosphohydrolase, β -galactosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase and aesculin hydrolysis. All strains were negative for denitrification at 22 and 37 °C, lipase, cystine arylamidase, chymotrypsin, β -glucuronidase, α -glucosidase, indole production, glucose fermentation, arginine dihydrolase and gelatinase. In contrast to previous publications, all strains gave negative results for nitrate reductase and urease. All strains were negative for assimilation of gluconate, caprate, adipate, malate, citrate and phenylacetate. R, Resistant; S, sensitive; I, intermediate. All strains were resistant to erythromycin (15 µg), ciprofloxacin (5 µg), polymyxin B (300 U), gentamicin (10 µg), chloramphenicol (30 µg), rifampicin (5 µg), amikacin (30 µg) and kanamycin (30 µg). All strains were sensitive to ampicillin (10 µg) and vancomycin (30 µg).

Characteristic	<i>D. limi</i> LMG 22951 ^T	<i>D. neptuniae</i> LMG 21357 ^T	<i>D. riboflavina</i> LMG 2277 ^T
Enzyme activities			
Esterase lipase	(+)	–	+
α -Galactosidase	–	+	+
α -Mannosidase	–	+	+
α -Fucosidase	–	+	+
Assimilation of:			
Glucose	–	+	+
Arabinose	–	+	+
Mannose	–	+	+
Mannitol	–	+	+
Maltose	–	+	+
<i>N</i> -Acetylglucosamine	–	–	+
Sensitivity to antibiotics			
Methicillin (5 µg)	I	S	I
Tetracycline (30 µg)	I	R	S
Presence of <i>nifH</i> and <i>nodD</i>	–	+	–

and 1–4 mm in diameter. Cells are rod-shaped (0.5–1 µm wide and 1–3 µm long), Gram-negative, obligately aerobic, non-spore-forming and non-motile. No formation of filaments is observed. Cells are oxidase and catalase positive. Enzyme activities, carbon source utilization and resistance to antibiotics are given in Table 2.

The type strain, LMG 22951^T (= DSM 17137^T), was isolated from a commercial nitrifying inoculum in Gent, Belgium.

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