Devosia chinhatensis sp. nov., isolated from a hexachlorocyclohexane (HCH) dump site in India

Mukesh Kumar, Mansi Verma and Rup Lal

Correspondence

Rup Lal

duzdel@vsnl.com

Molecular Biology Laboratory, Department of Zoology, University of Delhi, Delhi 110007, India

A Gram-negative, motile, rod-shaped and non-spore-forming bacterium was isolated from a soil sample collected from the area adjoining an India Pesticide Limited plant, Lucknow, India. Strain IPL18^T was characterized on the basis of phenotypic and genotypic data. Based on 16S rRNA gene sequence analysis, this strain was shown to belong to genus *Devosia*, with highest sequence similarity of 97.5% to *Devosia riboflavina* DSM 7230^T. A neighbour-joining phylogenetic tree based on 16S rRNA gene sequence analysis revealed that strain IPL18^T shows an evolutionary relationship with *Devosia neptuniae* J1^T. Strain IPL18^T contains C_{18:1}ω7c, 11-methyl C_{18:1}ω7c and C_{16:0} as the major fatty acids along with 3-OH C_{18:0}, characteristic of the genus *Devosia*. The branched-chain fatty acid iso-H C_{18:1} (4.69%), not present in any of the *Devosia* species known so far, has been reported in strain IPL18^T. The results of DNA–DNA hybridization experiments (13% relatedness with *D. riboflavina* DSM 7230^T and 11% with *D. neptuniae* J1^T), phylogenetic analysis and biochemical tests confirm that strain IPL18^T represents a novel species of the genus *Devosia* for which the name *Devosia chinhatensis* sp. nov. is proposed. The type strain is IPL18^T (=CCM 7426^T=MTCC 8593^T).

The genus *Devosia* was first described by Nakagawa *et al.* (1996) with the transfer of '*Pseudomonas riboflavina*' (Foster, 1944) to *Devosia riboflavina*. The genus *Devosia* belongs taxonomically to the family *Hyphomicrobiaceae* and the order *Rhizobiales*. Since then, *Devosia neptuniae* (Rivas *et al.*, 2002, 2003), '*Candidatus Devosia euplotis*' (Vannini *et al.*, 2004), *Devosia limi* (Vanparys *et al.*, 2005), *Devosia soli* (Yoo *et al.*, 2006) and *Devosia insulae* (Yoon *et al.*, 2007) have been described within the genus.

In the course of screening of culturable microbial diversity from a hexachlorocyclohexane (HCH) dump site, soil samples collected from 0–20 cm depth from an India Pesticide Limited plant, Lucknow, India, were analysed and many bacterial strains were isolated. While most of the bacterial strains were sphingomonads, strain IPL18^T was found to belong to genus *Devosia*. This strain was further characterized by using a polyphasic approach. Based on the results of phylogenetic, genotypic, phenotypic and chemotaxonomic characterization, strain IPL18^T represents a novel species of genus *Devosia*.

The 16S rRNA gene was amplified by colony PCR using universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-TACGGTTACCTTGTTACGACTT-3'). The conditions used for PCR amplification were as follows:

denaturation of the target DNA at 96 °C for 5 min, followed by 30 cycles consisting of denaturation at 96 °C for 1 min, primer annealing at 55 °C for 1 min and primer extension at 72 °C for 1.5 min. After the last cycle, final extension was done at 72 °C for 10 min. The amplified fragment of approximately 1.5 kb was separated by agarose gel electrophoresis and purified by using an E.Z.N.A. gel extraction kit (Omega Bio-Tek). The purified fragment was sequenced using various combinations of universal primer sets and sequencing was done with an ABI 3100 Avant Genetic analyser (Applied Biosystems) at the Department of Zoology, University of Delhi, Delhi, India.

A nearly complete 16S rRNA gene sequence of 1394 nt was obtained for strain IPL18^T. A standard nucleotide–nucleotide BLAST search (Altschul *et al.*, 1997) against the GenBank database using this sequence showed that strain IPL18^T belongs to genus *Devosia*, with maximum similarity to the 16S rRNA gene sequence of *D. riboflavina* DSM 7230^T. Phylogenetic analysis was then carried out to establish the position of strain IPL18^T within the evolutionary radiation encompassed by genus *Devosia*. Nearly full-length 16S rRNA gene sequences of the species of the genus *Devosia* with validly published names were retrieved from the GenBank database at NCBI (<http://www.ncbi.nlm.nih.gov>). The sequence of *Rhizobium leguminosarum* USDA 2370^T was used as an outgroup. These sequences as well as that of strain IPL18^T were aligned using the CLUSTAL_X program (version 1.81b; Thompson *et al.*, 1997). The alignment was checked manually for

Abbreviation: HCH, hexachlorocyclohexane.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain IPL18^T is EF433462.

quality and the terminal nucleotides not common to all sequences were removed. Phylogenetic analysis was carried out with TREECON for Windows software package (version 1.3b; Van de Peer & De Wachter, 1994). The evolutionary distance matrix was calculated by the distance model of Jukes & Cantor (1969). A phylogenetic tree (Fig. 1) was constructed using the neighbour-joining method (Saitou & Nei, 1987) and the resultant tree topologies were evaluated by bootstrap analysis based on 1000 resamplings. Phylogenetic analysis showed that strain IPL18^T formed a distinct phyletic line in the clade of *D. neptuniae* J1^T, *D. limi* LMG 22951^T and 'Candidatus *D. euplotis*' LIV5, separating it from *Devosia* species with validly published names. Phylogenetic trees were also constructed based upon 1000 resamplings by means of SEQBOOT, followed by neighbour-joining and DNA parsimony methods within the PHYLIP package (version 3.63; Felsenstein, 1993). All these analyses resulted in similar tree topology (data not shown), further supporting the distinct lineage of the isolate IPL18^T as shown in Fig. 1. These results clearly indicated that strain IPL18^T represents a novel species of genus *Devosia*. Thus, further studies that included 16S rRNA gene signature sequences within genus *Devosia*, DNA–DNA hybridization, fatty acid analysis and phenotypic characterization were conducted to ascertain the taxonomic status of strain IPL18^T.

The 16S rRNA gene sequence of strain IPL18^T was checked for the presence of signature sequences as reported by Nakagawa *et al.* (1996). The 16S rRNA gene sequence of *Escherichia coli* was taken as reference (Brosius *et al.*, 1978). During this analysis, 16S rRNA gene sequence of IPL18^T, type strains of all *Devosia* species and *E. coli* were aligned using the CLUSTAL_X program (version 1.81b; Thompson *et al.*, 1997) and checked manually for the presence of nucleotides at the corresponding positions. Variations in signature sequence compared with those described by Nakagawa *et al.* (1996) are summarized in Table 1. The

Fig. 1. Phylogenetic tree based on nearly complete 16S rRNA gene sequences (1382 aligned positions) showing relationships between strain IPL18^T and other species of genus *Devosia*. Numbers at nodes represent bootstrap values (based on 1000 resamplings). Bar, 2 nt substitutions per 100 nt positions. GenBank accession numbers are shown in parentheses.

presence of signature sequence A:U at positions 445:489 and 1419:1481 was observed only in *D. riboflavina* DSM 7230^T and *D. soli* GH2-10^T, but was replaced by G:C in other *Devosia* species with validly published names, including strain IPL18^T. In addition, signature sequence G:C at position 155:166 was observed in all species of genus *Devosia* along with IPL18^T, except for *D. insulae* DS-56^T, where G:C was replaced by C:G.

16S rRNA gene sequence similarity of IPL18^T with other type strains of genus *Devosia* was assessed using the CLUSTAL_X program. Considering the IPL18^T sequence as reference, 1379 nt common to all species of genus *Devosia* were manually observed for percentage similarity. Strain IPL18^T showed highest sequence similarity of 97.5 % with *D. riboflavina* DSM 7230^T, followed by 96.9 % (*D. limi* LMG 22951^T), 96.7 % (*D. insulae* DS-56^T), 96.4 % (*D. neptuniae* J1^T), 96.3 % (*D. soli* GH2-10^T) and 96.1 % with 'Candidatus *D. euplotis*' CO, 'Candidatus *D. euplotis*' LIV5 and 'Candidatus *D. euplotis*' CAMP4.4. Since the 16S rRNA gene sequence analysis of strain IPL18^T showed more than 97 % similarity with *D. riboflavina* DSM 7230^T and phylogenetic data predicted its evolutionary relationship with *D. neptuniae* J1^T, DNA–DNA hybridization was done between these strains for further characterization of the strain IPL18^T.

The 16S rRNA gene sequence analysis does not have enough discriminatory power for the delineation of closely related species. Therefore, the gold-standard method for species delineation is DNA–DNA hybridization (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994). In order to clarify further the taxonomic status of strain IPL18^T, DNA–DNA hybridization was carried out by the membrane filter method (Tourova & Antonov, 1987). DNA (10 µg) of each strain was transferred onto positively charged nylon membrane (Hybond-N; Amersham Pharmacia) using dot-blot apparatus (Bio-Rad). The membrane was air-dried and UV cross-linked. The DNA probe of each strain was labelled with [α -P³²]ATP (Board of Radiation and Isotope Technology, India) using a nick translation kit (Amersham Pharmacia) and hybridization was done overnight at 65 °C. After hybridization, the filters were

Table 1. Variations in 16S rRNA gene signature sequence of genus *Devosia* compared with those described by Nakagawa *et al.* (1996)

The *E. coli* numbering system was used.

Position(s)	Nakagawa <i>et al.</i> (1996)	This study
155:166	G:C	G/C:C/G
240:286	A:U	A:U
445:489	A:U	G/A:C/U
681:709	U:A	U:A
694	G	G
1419:1481	A:U	G/A:C/U

washed twice, first with $0.5 \times \text{SSC}$ (0.075 M sodium chloride, 0.0075 M sodium citrate, $\text{pH } 7$) and 0.1% SDS for 10 min at room temperature and then with $0.1 \times \text{SSC}$ and 0.5% SDS for 20 min at 65°C to remove the unbound probe. The amount of DNA probe hybridized was estimated by using a β scintillation counter (Perkin Elmer) and hybridization values (means of four replicates) obtained were expressed as the percentage of probe bound relative to the homologous reaction. The DNA–DNA hybridization data showed that strain IPL18^T had low DNA relatedness values of 13% with *D. riboflavina* DSM 7230^T and 11% with *D. neptuniae* J1^T. Since all hybridization values were below the threshold value (70%) that has been recommended for the delineation of genomic species (Stackebrandt & Goebel, 1994), this confirms that strain IPL18^T represents a novel species of the genus *Devosia* different from *D. riboflavina* DSM 7230^T and *D. neptuniae* J1^T.

Cellular fatty acids analysis of strain IPL18^T was carried out at the Institute of Microbial Technology, Chandigarh, India, using the following procedure. Fatty acid methyl esters from 40 mg cells were analysed by saponification, methylation and extraction using the methods of Miller (1982) and Kuykendall *et al.* (1988). The fatty acid methyl esters mixture was separated using the Sherlock microbial identification system (Microbial ID), which consisted of an Agilent model 5980 gas chromatograph fitted with a 5% phenyl-methyl silicon column ($0.2 \text{ mm} \times 25 \text{ m}$) and flame ionization detector. Identification and comparison was made with the TSBA 50 5.00 database of the Sherlock microbial identification system. The fatty acid profile of strain IPL18^T showed the presence of $\text{C}_{18:1\omega 7c}$, 11-methyl $\text{C}_{18:1\omega 7c}$ and $\text{C}_{16:0}$ as the major fatty acids along with 3-hydroxy $\text{C}_{18:0}$, characteristic of genus *Devosia*. Even though the presence of branched-chain fatty acids has been reported in *D. insulae* DS-56^T, iso-H $\text{C}_{18:1}$ (4.69%) branched-chain fatty acid (not present in any of the *Devosia* species known so far) has been reported in strain IPL18^T (Table 2). The difference in percentage

composition of $\text{C}_{18:1\omega 7c}$, $\text{C}_{16:0}$ and 11-methyl $\text{C}_{18:1\omega 7c}$ and presence of iso-H $\text{C}_{18:1}$ (4.69%) further substantiate that strain IPL18^T represents a novel species of genus *Devosia*.

Morphological characteristics (colour, shape and size of colonies) of strain IPL18^T and its growth at different temperatures, pH and salt concentrations were studied on Luria–Bertani (LB) agar medium. The colonies were cream-coloured, circular with entire margin and 0.5 – 2 mm in size when grown on LB agar plates for 3 days . The optimum range of temperatures, pH and NaCl concentrations for its growth were 5 – 40°C , $\text{pH } 5$ – 9 and 0 – 3% , respectively. All phenotypic and biochemical characterization were done at 28°C unless otherwise mentioned. Strain IPL18^T grew rapidly on nutrient agar and slow growth was observed on trypticase soy agar. Cells in the early exponential growth phase were used for detecting flagella using a transmission electron microscope. For transmission electron microscopy, the cells were negatively stained with 1% (w/v) phosphotungstic acid and air-dried grids were examined under a Philips CM-10 transmission electron microscope at the All India Institute of Medical Sciences, India. The cells were rod-shaped and motile with a single flagellum at one end (Fig. 2). The presence of a single flagellum at one end, in comparison with several polar flagella in *D. riboflavina* IFO 13584^T, strongly demarcated strain IPL18^T from *D. riboflavina* IFO 13584^T. Strain IPL18^T was further subjected to a series of biochemical tests. The strain was tested for oxidase activity using oxidase discs (Himedia). Catalase activity was determined by bubble production in a 3% hydrogen peroxide solution. Urease activity was checked using the method of Christensen (1946). The strain was found to be positive for the presence of oxidase, catalase and urease enzymes. Assimilation of various carbohydrates was tested in mineral medium as described by Gordon *et al.* (1974). Strain IPL18^T was found to be positive for assimilation of glucose, L-arabinose, mannitol, D-mannose and maltose, as was

Table 2. Cellular fatty acid compositions (%) of strain IPL18^T and type strains of species of the genus *Devosia*

Strains: 1, strain IPL18^T; 2, *D. riboflavina* DSM 7230^T; 3, *D. neptuniae* LMG 21357^T; 4, *D. limi* LMG 22951^T; 5, *D. soli* KACC 11509^T; 6, *D. insulae* DSM 17955^T. –, Not detected. 2–5, Data obtained from Yoo *et al.* (2006); 6, data obtained from Yoon *et al.* (2007).

Fatty acid	1	2	3	4	5	6
$\text{C}_{10:0}$ 3-OH	–	2.7	–	1.3	3.5	–
$\text{C}_{16:0}$	20.64	14.7	19.0	9.7	12.7	12.7
$\text{C}_{18:1\omega 7c}$	52.41	68.3	43.9	49.2	67.2	22.5
$\text{C}_{18:0}$	8.12	2.6	5.2	10.0	3.0	3.2
iso-H $\text{C}_{18:1}$	4.69	–	–	–	–	–
11-Methyl $\text{C}_{18:1\omega 7c}$	10.27	6.8	18.8	19.4	5.8	30.2
$\text{C}_{18:0}$ 3-OH	2.22	–	2.0	2.4	5.2	–
Cyclo- $\text{C}_{19:0}$ $\omega 8c$	–	–	1.6	–	–	1.1
Summed feature 3*	–	3.0	4.7	2.8	2.8	0.6
Unknown 14.959	1.65	–	2.4	2.0	–	–

*Summed feature 3 comprises $\text{C}_{16:1\omega 7c}$ and/or iso- $\text{C}_{15:0}$ 2-OH.

Fig. 2. Transmission electron micrograph of strain IPL18^T grown on LB agar for 72 h.

observed for *D. riboflavina* IFO 13584^T (Nakagawa *et al.*, 1996) and *D. neptuniae* J1^T (Rivas *et al.*, 2003). The isolate hydrolysed aesculin but did not hydrolyse starch, casein, DNA or gelatin. Lipase production was checked using 1% tributyrin and Tween 80 and was found to be negative. β -Galactosidase activity was observed using ONPG discs (Himedia). Strain IPL18^T was tested for denitrification

under anaerobic conditions using nutrient broth containing 1% (w/v) potassium nitrate. The presence of nitrate and nitrite was tested within 24 to 48 h as described by Smibert & Krieg (1994). The isolate showed β -galactosidase activity and was negative for nitrate reductase. Indole production and citrate assimilation tests showed negative results. Antibiotic-sensitivity tests were performed on LB agar medium using antibiotic discs (Himedia). Strain IPL18^T was found to be sensitive to ciprofloxacin (5 μ g), gentamicin (10 μ g), chloramphenicol (30 μ g), rifampicin (5 μ g), amikacin (30 μ g), ampicillin (10 μ g), vancomycin (30 μ g) and tetracycline (30 μ g), and resistant to polymyxin B (300 U) and kanamycin (30 μ g). Strain IPL18^T showed significant differences in its response to antibiotic susceptibility in comparison with *D. riboflavina* IFO 13584^T and *D. neptuniae* J1^T. The comparison of phenotypic and biochemical characterization between strain IPL18^T and all recognized species of genus *Devosia* has been summarized in Table 3.

Based on the results described above, we conclude that strain IPL18^T represents a novel species of the genus *Devosia*, for which the name *Devosia chinhatensis* sp. nov. is proposed.

Table 3. Differential phenotypic characteristics of strain IPL18^T and other *Devosia* species

Taxa: 1, strain IPL18^T; 2, *D. riboflavina* (data from Nakagawa *et al.*, 1996; Rivas *et al.*, 2003; Vanparys *et al.*, 2005; Yoo *et al.*, 2006); 3, *D. neptuniae* (Rivas *et al.*, 2003; Vanparys *et al.*, 2005; Yoo *et al.*, 2006); 4, *D. limi* (Vanparys *et al.*, 2005; Yoo *et al.*, 2006); 5, *D. soli* (Yoo *et al.*, 2006); 6, *D. insulae* (Yoon *et al.*, 2007). All strains are positive for catalase, β -galactosidase and aesculin hydrolysis. All are negative for gelatin, indole production, lipase and citrate assimilation and are resistant to polymyxin B. +, Positive; –, negative; s, sensitive; R, resistant; I, intermediate; ND, no data available.

Character	1	2	3	4	5	6
Colony colour	Cream	Cream	Pearl white	Light yellow to light brown	Light beige	Ivory
Cell morphology	Rods	Rods	Rods	Rods	Rods	Ovals or rods
Motility	+	+	+	–	ND	+
Oxidase	+	+	+	+	–	–
Nitrate reduction	–	+*	+*	–	–	–
Urease	+	+*	+*	–	+	+
Starch hydrolysis	–	+†	–	–	+	–
Casein	–	–	+	–	–	–
Assimilation of:						
Glucose	+	+	+	–	–	–
L-Arabinose	+	+	+	–	–	–
Mannitol	+	+	+	–	–	–
D-Mannose	+	+	+	–	–	–
Maltose	+	+	+	–	–	–
Susceptibility to:						
Ciprofloxacin	S	R	R	R	S	ND
Gentamicin	S	R	R	R	R	R
Chloramphenicol	S	R	R	R	S	S
Rifampicin	S	R	R	R	S	ND
Amikacin	S	R	R	R	S	ND
Kanamycin	R	R	R	R	S	R
Tetracycline	S	S	R	I	S	S

*Reported as positive by Nakagawa *et al.* (1996) and Rivas *et al.* (2003) but negative by Vanparys *et al.* (2005).

†Reported as negative by Nakagawa *et al.* (1996) but positive by Yoo *et al.* (2006).

Description of *Devosia chinhatensis* sp. nov.

Devosia chinhatensis (chin.hat.en'sis. N.L. fem. adj. *chinhatensis* pertaining to Chinhat, India, from where the type strain was isolated).

Cells are Gram-negative, aerobic, rod-shaped, non-spore-forming and motile with a single flagellum. Colonies are cream in colour, circular, with entire margin and 0.5–2 mm in size when grown on LB agar plates for 3 days. Growth ranges of temperature, pH and NaCl concentration are 5–40 °C, pH 5–9 and 0–3 %, respectively. Positive for assimilation of glucose, L-arabinose, mannitol, D-mannose and maltose. Cells are positive for catalase, oxidase, urease, β -galactosidase and aesculin hydrolysis. The major cellular fatty acids are C_{18:1} ω 7c, C_{16:0} and 11-methyl C_{18:1} ω 7c. Branched-chain fatty acid iso-H C_{18:1} (4.69 %) is present. Sensitive to ciprofloxacin, gentamicin, chloramphenicol, rifampicin, amikacin, ampicillin, vancomycin and tetracycline and resistant to polymyxin B and kanamycin.

The type strain is IPL18^T (=CCM 7426^T =MTCC 8593^T).

Acknowledgements

We thank Dr Encarna Velázquez, Departamento de MicroBiología y Genética, Universidad de Salamanca, Salamanca, Spain, for providing strains *D. riboflavina* DSM 7230^T and *D. neptuniae* J1^T. This work was supported by grants from the NBAIM (ICAR) and DBT, Government of India. We would like to thank J. P. Euzéby for etymological advice. M.K. acknowledges ICMR, Government of India, for providing a research scholarship.

References

- Altschul, S. F., Maden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**, 3389–3402.
- Bala, S., Khanna, R., Dadhwal, M., Prabhakaran, S. R., Shivaji, S., Cullum, J. & Lal, R. (2004). Reclassification of *Amycolatopsis mediterranei* DSM 46095^T as *Amycolatopsis rifamycinica* sp. nov. *Int J Syst Evol Microbiol* **54**, 1145–1149.
- Brosius, J., Palmer, M. L., Kennedy, P. J. & Noller, H. F. (1978). Complete nucleotide sequence of a 16S ribosomal RNA gene from *Escherichia coli*. *Proc Natl Acad Sci U S A* **75**, 4801–4805.
- Christensen, W. B. (1946). Urea decomposition as a means of differentiating *Proteus* and paracolon cultures from each other and from *Salmonella* and *Shigella* types. *J Bacteriol* **52**, 461–466.
- Felsenstein, J. (1993). PHYLIP (phylogeny inference package), version 3.5c. Department of Genome Sciences, University of Washington, Seattle, USA.
- Foster, J. W. (1944). Microbiological aspects of riboflavin. I. Introduction. II. Bacterial oxidation of riboflavin to lumichrome. *J Bacteriol* **47**, 27–41.
- Gordon, R. E., Barnett, D. A., Handerman, J. E. & Pang, C. H.-N. (1974). *Nocardia coeliaca*, *Nocardia autotrophica*, and the nocardin strain. *Int J Syst Bacteriol* **24**, 54–63.
- Jukes, T. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, vol. 3, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.
- Kuykendall, L. D., Roy, M. A., O'Neill, J. J. & Devine, T. E. (1988). Fatty acids, antibiotic resistance, and deoxyribonucleic acid homology groups of *Bradyrhizobium japonicum*. *Int J Syst Bacteriol* **38**, 358–361.
- Miller, L. T. (1982). A single derivatization method for routine analysis of bacterial whole-cell fatty acid methyl esters, including hydroxyl acids. *J Clin Microbiol* **16**, 584–586.
- Nakagawa, Y., Sakane, T. & Yokota, A. (1996). Transfer of '*Pseudomonas riboflavina*' (Foster 1944), a gram-negative, motile rod with long-chain 3-hydroxy fatty acids, to *Devosia riboflavina* gen. nov., sp. nov., nom. rev. *Int J Syst Bacteriol* **46**, 16–22.
- Rivas, R., Velázquez, E., Willems, A., Vizcaino, N., Subba-Rao, N. S., Mateos, P. F., Gillis, M., Dazzo, F. B. & Martínez-Molina, E. (2002). A new species of *Devosia* that forms a unique nitrogen-fixing root-nodule symbiosis with the aquatic legume *Neptunia natans* (L.f.) Druce. *Appl Environ Microbiol* **68**, 5217–5222.
- Rivas, R., Willems, A., Subba-Rao, N. S., Mateos, P. F., Dazzo, F. B., Kroppenstedt, R. M., Martínez-Molina, E., Gillis, M. & Velázquez, E. (2003). Description of *Devosia neptuniae* sp. nov. that nodulates and fixes nitrogen in symbiosis with *Neptunia natans*, an aquatic legume from India. *Syst Appl Microbiol* **26**, 47–53.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- Tourova, T. P. & Antonov, A. S. (1987). Identification of microorganisms by rapid DNA-DNA hybridization. *Methods Microbiol* **19**, 333–355.
- Van de Peer, Y. & De Wachter, Y. (1994). TREECON for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. *Comput Appl Biosci* **10**, 569–570.
- Vannini, C., Rosati, G., Verni, F. & Petroni, G. (2004). Identification of the bacterial endosymbionts of the marine ciliate *Euplotes magnicirrus* (Ciliophora, Hypotrichia) and proposal of '*Candidatus Devosia euplotis*'. *Int J Syst Evol Microbiol* **54**, 1151–1156.
- Vanparrys, B., Heylen, K., Lebbe, L. & De Vos, P. (2005). *Devosia limi* sp. nov., isolated from a nitrifying inoculum. *Int J Syst Evol Microbiol* **55**, 1997–2000.
- Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.
- Yoo, S. H., Weon, H. Y., Kim, B. Y., Hong, S. B., Kwon, S. W., Cho, Y. H., Go, S. J. & Stackebrandt, E. (2006). *Devosia soli* sp. nov., isolated from greenhouse soil in Korea. *Int J Syst Evol Microbiol* **56**, 2689–2692.
- Yoon, J.-H., Kang, S.-J., Park, S. & Oh, T.-K. (2007). *Devosia insulae* sp. nov., isolated from soil, and emended description of the genus *Devosia*. *Int J Syst Evol Microbiol* **57**, 1310–1314.