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NOTE

Devosia lucknowensis sp. nov., a Bacterium Isolated from Hexachlorocyclohexane (HCH) Contaminated Pond Soil[§]

Ankita Dua[1], Jaya Malhotra[1], Anjali Saxena[1], Fazlurrahman Khan[2], and Rup Lal[1]*

[1]*Department of Zoology, University of Delhi, Delhi-110007, India*
[2]*IMTECH- Institute of Microbial Technology, Sector-39A, Chandigarh, India*

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Strain L15^T, a Gram-negative, motile, orange colored bacterium was isolated from pond soil in the surrounding area of a hexachlorocyclohexane (HCH) dump site at Ummari village in Lucknow, India. Phylogenetic analysis based on 16S rRNA gene sequence showed that strain L15^T belongs to the family *Hyphomicrobiaceae* in the order *Rhizobiales*. Strain L15^T showed highest 16S rRNA gene sequence similarity to *Devosia chinhatensis* IPL18^T (98.0%). Chemotaxonomic data revealed that the major fatty acids were summed feature 8 ($C_{18:1}$ *ω7c* and/or $C_{18:1}$ *ω6c*), $C_{18:1}$ *ω7c* 11-methyl, $C_{16:0}$ and $C_{18:0}$. The major polar lipids of strain L15^T were diphosphatidylglycerol and phosphatidylglycerol. The genomic DNA G+C content of strain L15^T was 59.8%. Polyamine profile showed the presence of sym-homospermidine with traces of putrescine. Ubiquinone Q-10 was the major respiratory quinone present. Based on these data, strain L15^T (=CCM 7977^T =DSM 25398^T) was classified as a type strain of a novel species, for which the name *Devosia lucknowensis* sp. nov. is proposed.

Keywords: *Devosia lucknowensis*, hexachlorocyclohexane dumpsite, taxonomy

The genus *Devosia* was established by the reclassification of *Pseudomonas riboflavina* as *Devosia riboflavina* (Nakagawa *et al*., 1996). Presently, the genus *Devosia* contains thirteen validly published species: *Devosia riboflavina* (Nakagawa *et al*., 1996), *Devosia neptuniae* (Rivas *et al*., 2003), *Devosia limi* (Vanparys *et al*., 2005), *Devosia soli* (Yoo *et al*., 2006), *Devosia insulae* (Yoon *et al*., 2007), *Devosia subaequoris* (Lee, 2007), *Devosia chinhatensis* (Kumar *et al*., 2008), *Devosia geojensis* (Ryu *et al*., 2008), *Devosia crocina* (Verma *et al*., 2009), *Devosia albogilva* (Verma *et al*., 2009), *Devosia yakushimensis* (Bautista *et al*., 2010), *Devosia psychrophila* (Zhang *et al*., 2012), and *Devosia glacialis* (Zhang *et al*., 2012). We have previously studied several bacterial strains from the hexachlorocyclohexane (HCH) contaminated dumpsite located at Ummari Village, Lucknow, India (Verma *et al*., 2009; Garg *et al*., 2011; Jindal *et al*., 2012; Saxena *et al*., 2012). Here we describe strain L15^T, that was isolated from soil collected from a pond near the HCH dumpsite. Though majority of strains isolated from this site were from the family *Sphingomonadaceae*, strain L15^T belonged to the genus *Devosia* (family *Hyphomicrobiaceae*, order *Rhizobiales*, class *Alphaproteobacteria*). On the basis of the polyphasic approach, strain L15^T was found to represent a novel species in the genus *Devosia*, for which the name *Devosia lucknowensis* sp. nov. is proposed.

A soil sample collected from the pond near the HCH dumpsite was serially diluted in 1× phosphate buffer saline, pH 7.2 containing NaCl (8.5 g/L), $Na_2HPO_4{\cdot}2H_20$ (1.91 g/L), and KH_2PO_4 (0.38 g/L) and plated on nystatin (100 U/ml) and streptomycin (10 μg/ml) amended Luria-Bertani (LB) plates (Vanbroekhoven *et al*., 2004; Dadhwal *et al*., 2009). An orange-colored colony that appeared within 72 h of incubation at 28°C was picked up and purified by streaking on LB agar plate. The isolate was routinely cultured on R2A agar and also stored in glycerol suspension (20% v/v) at -80°C. The bacterium was then submitted to Deutsche Sammlung von Mikroorganismen and Zellkulturen (=DSM 25398^T) and Czech Collection of Microorganisms (=CCM 7977^T). Reference strains, *Devosia chinhatensis* IPL18^T and *Devosia crocina* IPL20^T were available from laboratory stocks. *Devosia psychrophila* Cr7-05^T and *Devosia glacialis* Cr4-44^T were obtained from Institute of Microbiology, University of Innsbruck, Innsbruck, Austria. *Devosia riboflavina* DSM 7230^T, *Devosia soli* KACC 11509^T, *Devosia subaequoris* JCM 14206^T, and *Devosia yakushimensis* LMG 24299^T were obtained from Universidad de Salamanca, Spain, Korean Agricultural Culture Collection (KACC), Korea, Japan Collection of Microorganisms and BCCM^{TM}/LMG Bacteria Collection respectively.

PCR amplification and 16S rRNA gene sequencing were carried out using 3100 Avant^{TM} Genetic Analyzer (Applied Biosystems, USA) at the Department of Zoology, University of Delhi, Delhi (Kumar *et al*., 2008). The sequence thus obtained (1,457 nt) using 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1542R (5′-AAGGAGGTGATCCAGCCGCA-3′) universal primers was assembled manually using Clone Manager software, version 5. The 16S rRNA gene sequence

*For correspondence. E-mail: ruplal@gmail.com; Tel.: +91-11-27666254; Fax: +91-11-27666254
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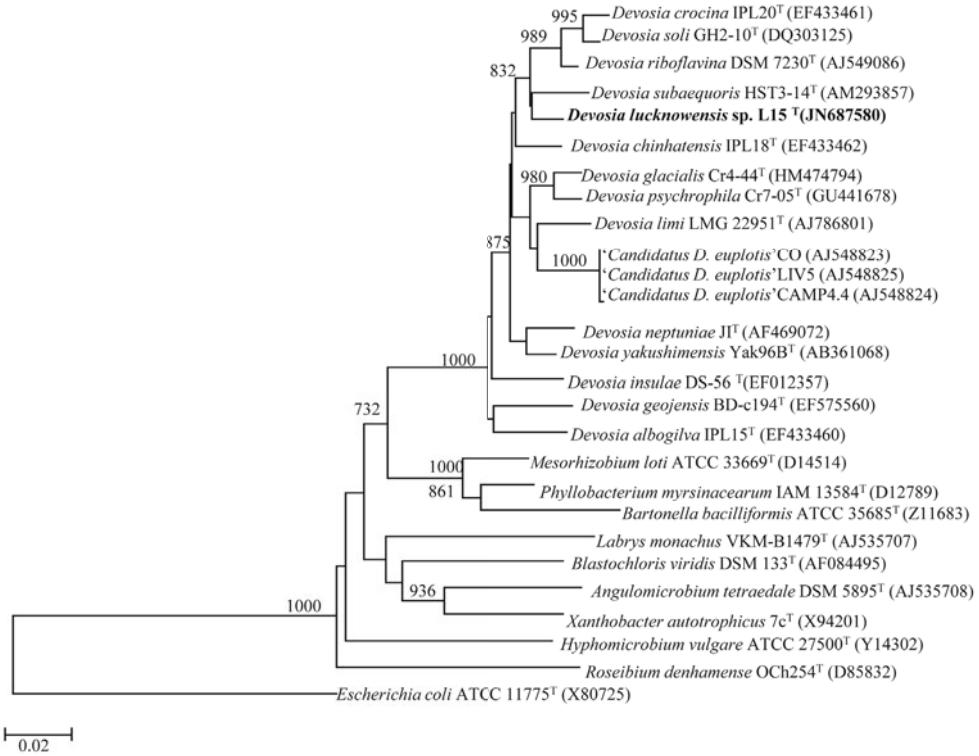

Fig. 1. Phylogenetic tree based on nearly complete 16S rRNA gene sequences showing the relationship among the strain L15^T and other *Devosia* species. The tree was constructed by the distance model of Jukes & Cantor of TREECONW program version 1.3b and rooted by using *E. coli* ATCC 11775^T (X80725) as an out group. Number at nodes represents bootstrap values (based on 1,000 re-samplings). Scale bar represents 0.02 nucleotide substitutions per 1,000 nucleotide position. The GenBank accession numbers for 16S rRNA gene sequences are shown in parentheses.

of strain L15^T was subjected to similarity searches by using sequence matching tool of NCBI BLAST program (http: www.ncbi.nlm.nih.gov) and the EzTaxon Server (http://ez-taxon-e.ezbiocloud.net/; Kim *et al.*, 2012). The nearly full-length 16S rRNA gene sequences of species closely related to strain L15^T were retrieved from the GenBank for the construction of a phylogenetic tree and rooting was done by using 16S rRNA gene sequence of *Escherichia coli* (X80725^T). The selected sequences were aligned using CLUSTALX version 1.81b (Thompson *et al.*, 1997). The evolutionary distance matrix was calculated using the distance model of Jukes and Cantor (1969) available within the TREECONW software package, version 1.3b by Van de Peer and De Wachter (1994). The tree topology was also validated with maximum-likelihood method based on 100 re-samplings using the SEQBOOT, DNAMLK, and CONSENSE program in the PHYLIP package (Felsenstein, 1981).

The colony morphology and growth of strain L15^T on various standard bacteriological media (HiMedia, India) were studied on R2A agar, LB agar, nutrient agar (NA), and tryptic soya agar (TSA) according to the manufacturer's instructions. Characteristic physiological and biochemical tests were performed on strain L15^T, that was grown on R2A broth/agar at 28°C. Cell morphology and motility were examined using the electron microscope (Transmission Electron microscope, TEM 269D, Morgagni, Fei) (Fig. 2) as well as by the hanging drop method. Susceptibility towards antibiotics was determined on R2A agar using readymade susceptibility test discs (HiMedia).

Growth at various NaCl concentrations (0–10%, w/v, using increments of 1.0%) was investigated in R2A broth. The pH range for growth was also determined in R2A broth and the adjustment to various pH values (pH 1–10, using increments of 1 pH units) was done by the addition of 1 N HCl and 1 N NaOH. Growth temperature (4–45°C) was checked using R2A agar in one week incubation time as described by Arden-Jones *et al.* (1979). Catalase activity was determined by adding 3% (v/v) hydrogen peroxide solution to the colonies grown on R2A agar (McCarthy and Cross, 1984) and bacteriological differentiation discs (HiMedia) were used for testing oxidase activity. Antibiotic resistance was tested with disc diffusion method using commercial antibiotic impregnated discs (HiMedia) against the following: amikacin (30 µg/disc), ampicillin (10 µg/disc), chloramphenicol (30 µg/disc), ciprofloxacin (5 µg/disc), gentamicin (10 µg/disc), kanamycin (30 µg/disc), oxytetracycline (30 µg/disc), poly-

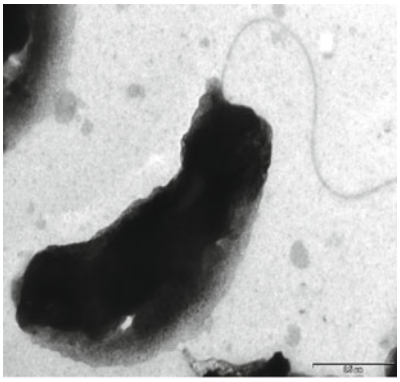

Fig. 2. Transmission electron micrograph of a negatively stained cell of strain L15^T grown on R2A agar at 28°C showing polar flagellation; bar =0.5 µm.

myxin-B (300 µg/disc), rifampicin (5 µg/disc), tetracycline (30 µg/disc), and vancomycin (30 µg/disc). Biochemical and physiological traits as well as sugar assimilation patterns were tested according to the manufacturer's guidelines using the HiMedia's Biochemical test kits and API 20NE test strips (bioMèrieux, France). Hydrolysis of Tween 20 and Tween 80 was tested according to the Arden-Jones *et al.* (1979). Urease activity was tested as described by Christensen (1946). Hydrolysis of casein, gelatin, starch, and aesculin were tested as described by Cowan and Steel (1965). β-Galactosidase activity was observed using ONPG discs of HiMedia. Nitrate reduction test was done by the method described by Smibert and Kreig (1994).

Fatty acid methyl ester (FAME) analysis was carried out at Disha Life Sciences Ltd., Ahmedabad, India. The cultures were grown on TSA media for 24–72 h at 28°C (with the exception of *D. psychrophila* and *D. glacialis* which were grown at 8°C). Fatty acid methyl esters were analyzed from 2–4 loops of inoculum (in log phase) scraped from the third quadrant of a petri dish and subjected to saponification, methylation and extraction using the method of Miller (1982) and Kuykendall *et al.* (1988). Fatty acid methyl ester mixtures were separated using the Sherlock Microbial Identification System (MIDI, USA) and identification of the fatty acid was done by using the Aerobe (TSBA, 6.0 version) database.

Quinones were extracted from 200 mg dry cell mass with a 10% aqueous solution of 0.3% (w/v) NaCl in methanol and petroleum ether (60–80°C boiling point) at a ratio of 1:1. The upper phase was collected and dried in a rotavapor (Buchi, Switzerland) and residue was dissolved in 100 µl acetone and analyzed on a TLC plate (Silica gel 60 F254, 20×20 cm, Merck, Germany, 1.05554.0007) using petroleum ether (boiling point 60–80°C) and diethyl ether (85:15, v/v). Purified ubiquinones were dissolved in 2-propanol and analysed by reverse phase TLC according to Collins and Jones (1980).

Polar lipid analysis of strain L15^T was carried out by two dimensional thin layer chromatography (TLC) as described by Bligh and Dyer (1959). Total lipid profile was detected by spraying with 5% ethanolic molybdatophosphoric acid solution (0.5 g molybdatophosphoric acid in 10 ml ethanol) followed by heating at 120°C.

Table 1. Differential morphological and physiological characteristics of strain L15^T and phylogenetically closely related members of the genus *Devosia*
1, Strain L15^T; 2, *Devosia chinhatensis* IPL18^T; 3, *Devosia riboflavina* DSM 7230^T; 4, *Devosia subaequoris* HST3-14^T; 5, *Devosia soli* GH2-10^T; 6, *Devosia crocina* IPL20^T; 7, *Devosia psychrophila* Cr7-05^T; 8, *Devosia glacialis* Cr4-44^T; 9, *Devosia yakushimensis* Yak96B^T.
All strains are positive for aesculin hydrolysis and production of β-galactosidase; negative for hydrolysis of Tween 20, gelatin, DNA, citrate, indole production, reduction of nitrite and for assimilation of raffinose, sodium gluconate, dulcitol, sorbose, inositol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, phenylacetic acid, fermentation of glucose and production of arginine dihydrolase.
+, positive ; w, weak reaction;-, negative.

Characteristic	1	2	3	4	5	6	7	8	9
Colony colour	orange	cream	cream	light yellow	light beige	orange	white	pink	shiny beige
Production of:									
Catalase	+	+	+	+	+	-	-	+	+
Oxidase	+	+	+	+	-	+	+	+	+
Urease activity	-	+	+	+	+	+	-	+	+
Assimilation of:									
D-Glucose	+	+	+	-	-	-	+	-	+
L-Arabinose	+	+	+	-	-	+	+	-	+
D-Arabinose	+	+	+	-	-	+	+	-	+
D-Mannose	+	+	+	w	-	+	+	-	+
D-Mannitol	+	+	+	w	-	+	+	-	+
N-Acetyl-glucosamine	+	+	+	-	-	-	+	-	-
D-Maltose	+	+	+	w	-	+	+	-	+
Lactose	+	-	+	-	-	+	+	-	+
Xylose	+	-	-	-	-	+	+	w	+
Fructose	+	+	+	+	-	-	-	+	-
Galactose	+	-	-	-	-	+	+	+	+
Trehalose	-	-	+	+	-	-	+	w	+
Melibiose	-	-	-	-	-	-	-	-	+
Sucrose	+	+	-	+	-	+	-	-	-
Inulin	+	-	-	+	+	+	+	w	-
Glycerol	-	+	+	+	-	+	+	w	-
Salicin	+	-	+	+	-	+	+	-	-
Sorbitol	-	-	-	+	+	-	-	-	-
Arabitol	-	-	+	-	-	+	+	+	-
Erythritol	-	-	-	-	-	-	-	+	+
Rhamnose	+	-	+	-	-	+	+	-	+
Cellobiose	+	-	+	+	-	+	+	-	+
Xylitol	+	-	-	-	-	+	+	+	+

Table 2. Cellular fatty acid profile of strain L15^T and related *Devosia* species. 1, Strain L15^T; 2, *D. chinhatensis* IPL18^T; 3, *D. riboflavina* DSM 7230^T; 4, *D. subaequoris* HST3-14^T; 5, *D. soli* GH2-10^T; 6, *D. crocina* IPL20^T; 7, *D. psychrophila* Cr7-05^T; 8, *D. glacialis* Cr4-44^T; 9, *D. yakushimensis* Yak96B^T. ND, not detected. Values are percentages of total fatty acids. All data has been collected from this study only.

Fatty acid	1	2	3	4	5	6	7	8	9
$C_{12:0}$	0.5	ND	0.3	0.2	1.0	0.4	1.0	1.0	0.4
$C_{14:0}$	0.7	0.4	0.6	1.1	0.8	0.8	2.9	0.9	0.9
$C_{16:0}$	12.1	9.0	24.3	16.7	11.5	12	17.3	8.9	20.4
$C_{17:0}$	0.9	1.2	0.4	3.2	0.5	0.6	0.5	ND	3.8
$C_{16:1}$ *ω7c*-OH	3.0	ND	ND	ND	ND	ND	ND	ND	ND
$C_{18:0}$	6.2	2.7	2.2	2.0	6.2	2.2	3.0	ND	3.1
$C_{8:0}$3-OH	0.4	1.6	ND	ND	ND	ND	1.2	ND	ND
$C_{10:0}$3-OH	0.2	ND	1.9	0.1	2	0.7	ND	2.6	8.3
$C_{18:0}$3-OH	2.6	4.3	0.3	ND	4.4	ND	0.5	ND	0.6
$C_{18:1}$*ω9c*	1.7	ND	ND	ND	1.1	ND	ND	ND	ND
11-Methyl $C_{18:1}$*ω7c*	16.3	10.7	30.5	0.6	18.6	2	2.5	3.3	25.5
Summed feature 3	1.0	2.9	2.9	4	1.2	5.4	23.5	16.6	1.4
Summed feature 8	22.0	59.8	21	53.4	18.6	67.5	37.5	24.2	22.6

*Summed features consist of one or more fatty acids that could not be separated. Summed feature 3 contained one or more of the following isomers: (16:1 ω7*c* and/or 16:1 *ω6c*), Summed feature 8: (18:1 *ω7c* and/or 18:1 *ω6c*).

Polyamines were extracted as described by Busse and Auling (1988) and analyzed by one dimensional thin layer chromatography. The extracted sample was applied on a TLC plate (Silica gel 60 F_{254}, 20×20 cm, Merck, Germany, 1.05554.0007) and the running solvent used was ethylacetate/cyclohexane (2:3, v/v).

The G+C content of strain L15^T was calculated by the method described by Gonzalez and Saiz-Jimenez (2002) using Applied Biosystems 7500 Real-Time PCR system.

DNA-DNA hybridization was carried out between strain L15^T and eight closely related strains, which showed more than 97% sequence similarity of 16S rRNA gene sequence. Total genomic DNA of all the nine strains was extracted and purified and hybridization was performed as described by Tourova and Antonov (1987). The amount of bound probe DNA was calculated by using scintillation counter (1450 LSC & Luminescence counter Wallac Microbeta Trilux, PerkinElmer, USA).

Strain L15^T showed highest sequence similarity with *Devosia chinhatensis* IPL18^T (98.0%) followed by *Devosia riboflavina* DSM 7230^T (97.8%), *Devosia subaequoris* HST3-14^T (97.7%), *Devosia soli* GH2-10^T (97.4%), *Devosia crocina* IPL 20^T (97.3%), *Devosia psychrophila* Cr7-05^T (97.3%), *Devosia glacialis* Cr4-44^T (97.3%), and *Devosia yakushimensis* Yak96B^T (97.1%).

Phylogenetic analysis of the nucleotide sequence of the 16S rRNA gene revealed that strain L15^T belonged to the genus *Devosia.* Trees constructed using the two different methods are shown in Fig. 1 and Supplementary data Fig. S1. Both the trees showed similar topology, with the strain L15^T forming a distinct cluster with its closest neighbors.

Strain L15^T grew well under aerobic conditions on R2A, NA, and LB agar at 28°C within 48–72 h of incubation. It was aerobic, Gram-negative, rod-shaped (0.46–1.09 μm), and motile with a polar flagellum (Fig. 2). Colonies were orange colored, circular, and smooth (size varied from 0.5–1 mm in diameter). Strain L15^T grew well between 25–28°C, pH range of 7–9 and NaCl concentration range of 0–3%. Optimal growth occurred at 28°C.

There were both qualitative and quantitative physiological, and biochemical differences in the characteristics of Strain L15^T and other related species of *Devosia* (Table 1). For example, strain L15^T was negative for urease activity, while the other species were not. Morphological, physiological, and biochemical data clearly highlighting major differences between the novel strain and the related species used in the study are shown in Table 1.

Strain L15^T contained sym-homospermidine and traces of putrescine, which have already been reported among members of the genus *Devosia* (Hamana *et al.*, 2003). DNA G+C ratio was found to be 62.7 mol% which is in accordance with the range defined for the genus (Zhang *et al.*, 2012). Q-10 was identified as the major ubiquinone. The major fatty acids

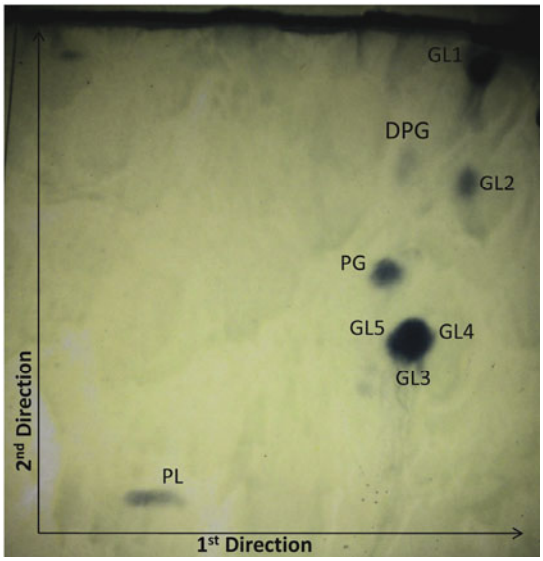

Fig. 3. Two dimensional thin-layer chromatography of polar lipids of strain L15^T; total lipids stained with 5% ethanolic molybdatophosphoric acid. DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; GL, unidentified glycolipid; PL, unidentified polar lipid.

Table 3. DNA-DNA relatedness between strain L15^T and phylogenetically related *Devosia* species. 1, Strain L15^T; 2, *Devosia chinhatensis* IPL18^T; 3, *Devosia riboflavina* DSM 7230^T; 4, *Devosia subaequoris* HST3-14^T; 5, *Devosia soli* GH2-10^T; 6, *Devosia crocina* IPL20^T; 7, *Devosia psychrophila* Cr7-05^T; 8, *Devosia glacialis* Cr4-44^T; 9, *Devosia yakushimensis*Yak96B^T.

Radio labelled DNA (probes)	Percent hybridization of strains								
	1	2	3	4	5	6	7	8	9
1	100	54.3	52.1	43.2	36.1	43.8	42.2	37.6	40.4
2	53	100	34.1	38.4	39.1	34.5	38.3	38.8	34.0
3	53.2	36	100	39.4	47	44.3	38.9	40.4	37.3
4	37.1	37.9	41.3	100	47.2	37.3	53.2	35.1	35
5	35	57.9	55.1	36.7	100	40.4	38.0	38.0	34
6	36.6	36.2	48.0	37.9	39.9	100	31.0	36.9	35.1
7	35.4	46.6	44.6	44.5	43.7	35.3	100	30.6	37.2
8	35.2	39.0	37.2	40	42.1	37.2	35.2	100	40.2
9	39.3	35.9	38.7	38.8	30.1	37.1	37.1	38.0	100

of strain L15^T consisted of summed feature 8 (18:1 *ω7c* and/or 18:1 *ω6c*) (22%), 18:1 *ω7c* 11-methyl (16.3%), 16:0 (12.1%), and 18:0 (6.2%) (Table 2). The isolated strain showed a similar major fatty acid composition to the related type strains of the genus *Devosia*, but there were significant quantitative differences when cultivated under the same conditions (Table 2). Furthermore, the polar lipid analysis of strain L15^T exhibited the presence of diphosphatidylglycerol, phosphatidylglycerol, unknown glycolipids, and unknown phospholipids (Fig. 3). These polar lipids have been reported in closely related *Devosia* species (Bautista *et al*., 2010; Zhang *et al*., 2012).

DNA-DNA hybridization (Table 3) revealed that strain L15^T showed low levels of DNA-DNA relatedness with *Devosia chinhatensis* IPL18^T (54.3%), *Devosia riboflavina* DSM 7230^T (52.1%), *Devosia subaequoris* HST3-14^T (43.2%), *Devosia soli* GH2-10^T (36.1%), *Devosia crocina* IPL20^T (43.8%), *Devosia psychrophila* Cr7-05^T (42.2%), *Devosia glacialis* Cr4-44^T (37.6%), and *Devosia yakushimensis* Yak96B^T (40.4%). In accordance with the recommendations of the *ad hoc* committee (Wayne *et al*., 1987), these values, which are <70%, confirm that the type strain L15^T is a novel species of the genus *Devosia*.

On the basis of these results, including phenotypic and chemotaxonomic characteristics, phylogenetic analysis and DNA-DNA relatedness, strain L15^T represents a member of a novel species, for which the name *Devosia lucknowensis* sp. nov. is proposed.

Description of *Devosia lucknowensis* sp. nov.

D. lucknowensis: lucknowensis. N.L. fem. adj. *lucknowensis*, of or belonging to Lucknow.

Gram-negative, aerobic, motile, non spore-forming, rod-shaped bacterium (0.46–1.09 μm) with a polar flagellum. Colonies are orange pigmented, small (diameter 0.5–1 mm on R2A agar after 48 h of incubation at 28°C), entire, smooth, circular, convex, and opaque. Optimum growth occurs at 28°C. The type strain grows well at pH and NaCl concentration ranges 7.0–9.0 and 0–3%, respectively. Strain L15^T is positive for catalase and oxidase and negative for urease activity. Nitrate is not reduced to nitrite. The bacterium can hydrolyze aesculin, but not Tween 20, Tween 80, gelatin, starch, citrate and DNA. Positive for production of β-galactosidase activity. Indole production is negative. The bacterium assimilates D-glucose, L-arabinose, D-arabinose, D-mannose, D-mannitol, N-acetyl glucosamine, D-maltose, lactose, xylose, fructose, galactose, sucrose, inulin, salicin, rhamnose, cellobiose, xylitol but not trehalose, melibiose, glycerol, sorbitol, erythritol and arabitol. Strain L15^T is sensitive to amikacin (30 μg/disc), chloramphenicol (30 μg/disc), ciprofloxacin (5 μg/disc), gentamicin (10 μg/disc), kanamycin (30 μg/disc), oxytetracycline (30 μg/disc), polymyxin-B (300 μg/disc), rifampicin (5 μg/disc), tetracycline (30 μg/disc), vancomycin (30 μg/disc) but resistant to ampicillin (30 μg/disc). Sym-homospermidine and traces of putrescine are present in strain L15^T. The major polar lipids of strain L15^T were diphosphatidylglycerol, phosphatidylglycerol, unknown glycolipids, and unknown phospholipids. Respiratory pigment ubiquinone Q-10 is present. The predominant cellular fatty acids of strain L15^T were summed feature 8 (18:1 *ω7c* and/or 18:1 *ω6c*) (22%), 18:1 *ω7c* 11-methyl (16.3%), 16:0 (12.1%), and 18:0 (6.2%). The G+C content of genomic DNA is 59.8%. The type strain, L15^T (=CCM 7977^T=DSM 25398^T) was isolated from HCH contaminated pond soil at Ummari village, Lucknow, India.

The GenBank accession number for 16S rRNA gene sequence of strain L15^T (=CCM 7977^T =DSM 25398^T) is JN 687580.

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