Devosia albobogilva sp. nov. and *Devosia crocina* sp. nov., isolated from a hexachlorocyclohexane dump site

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Two bacterial strains, IPL15^T and IPL20^T, isolated from a hexachlorocyclohexane dump site in India, were characterized by using a polyphasic approach. Based on 16S rRNA gene sequence analysis, both strains belonged to the genus *Devosia*; highest sequence similarities of strain IPL15^T were observed with *Devosia neptuniae* J1^T and *Devosia geojensis* BD-c194^T (96.2 % in each case) and the highest sequence similarity of strain IPL20^T was observed with *Devosia soli* GH2-10^T (98.6 %). Phylogenetic analysis showed the distinct lineages of strains IPL15^T and IPL20^T among members of the genus *Devosia*. The presence of C_{18:0} 3-OH and C_{10:0} 3-OH fatty acids supported their respective positions within the genus *Devosia*. On the basis of phenotypic characteristics, phylogenetic analysis and DNA–DNA hybridization results, it is concluded that strains IPL15^T and IPL20^T represent two distinct species of the genus *Devosia* for which the names *Devosia albobogilva* sp. nov. and *Devosia crocina* sp. nov., respectively, are proposed. The type strains are *Devosia albobogilva* IPL15^T (=CCM 7427^T=MTCC 8594^T) and *Devosia crocina* IPL20^T (=CCM 7425^T=MTCC 8590^T).

The genus *Devosia* was first described by Nakagawa *et al.* (1996) with the transfer of *Pseudomonas riboflavina* (Foster, 1944) to *Devosia riboflavina* and currently comprises the following species: *D. riboflavina* (Nakagawa *et al.*, 1996), *D. neptuniae* (Rivas *et al.*, 2002, 2003), ‘*Candidatus Devosia euplotis*’ (Vannini *et al.*, 2004), *D. limi* (Vanparys *et al.*, 2005), *D. soli* (Yoo *et al.*, 2006), *D. insulae* (Yoon *et al.*, 2007), *D. subaequoris* (Lee, 2007), *D. geojensis* (Ryu *et al.*, 2008) and *D. chinhatensis* (Kumar *et al.*, 2008).

In our attempts to study the bacterial diversity of a hexachlorocyclohexane (HCH)-contaminated site in Chinhat, Lucknow, India, strains IPL15^T and IPL20^T were recovered on Luria–Bertani (LB) agar at 28 °C (Kumar *et al.*, 2008). To check the HCH-degrading abilities of these isolates, each one of them was inoculated into 5 ml LB medium in 20 ml test tubes supplemented with technical HCH (5 µg ml⁻¹) as described previously (Kumari *et al.*,

2002). Neither strain degraded the HCH isomers, in spite of their tolerance to very high levels of HCH. These two strains were found to belong to the genus *Devosia* and have been characterized in the present study using a polyphasic taxonomic approach (Prakash *et al.*, 2007b).

On LB agar, colonies of strain IPL15^T were creamish, whereas those of strain IPL20^T were orange. Both strains formed circular colonies with entire margins and sizes varied from 0.5 to 2.0 mm when grown on LB agar at 28 °C for 3 days. Both strains were tested for their growth on tryptic soy agar (TSA) and nutrient agar (NA). Growth at different temperatures, pH and NaCl concentrations was determined as described by Arden Jones *et al.* (1979).

Gram stain was assessed using a HiMedia Gram Stain kit. The presence of flagella was detected using cells from the early exponential growth phase with a Philips CM-10 transmission electron microscope (TEM) at the All India Institute of Medical Sciences, New Delhi, India. Cells were negatively stained with 1 % (w/v) phosphotungstic acid and air-dried grids were examined by TEM. Each strain had a single polar flagellum (see Supplementary Fig. S1, available in IJSEM Online), in contrast to *D. riboflavina* DSM 7230^T, which possesses several polar flagella.

16S rRNA gene sequences of strains IPL15^T (1345 bp) and IPL20^T (1361 bp) were determined as described previously (Kumar *et al.*, 2008) and sequence analysis indicated that

Abbreviations: HCH, hexachlorocyclohexane; TEM, transmission electron microscope.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *Devosia albobogilva* IPL15^T and *Devosia crocina* IPL20^T are EF433460 and EF433461, respectively.

Transmission electron micrographs of cells of the two novel strains, DNA–DNA hybridization data for strain IPL20^T and related strains, and fatty acid profiles of the two novel strains and related species are available as supplementary material with the online version of this paper.

strains IPL15^T and IPL20^T belonged to the genus *Devosia*. Sequence alignment was done using the program CLUSTAL_X v.1.81b (Thompson *et al.*, 1997). The evolutionary distance matrix was calculated using the distance model of Jukes and Cantor (1969) within the TREECONW software package v.1.3b (Van de Peer & De Wachter, 1994) and the resultant tree topology was evaluated using bootstrap analysis of 1000 datasets. A phylogenetic tree was constructed using the neighbour-joining method of Saitou & Nei (1987) and the resultant tree topology showed that strains IPL15^T and IPL20^T formed distinct phyletic lines among all previously reported *Devosia* species (Fig. 1). Similar tree topologies were obtained when phylogenetic trees were constructed by using the Kimura two-parameter matrix of the TREECONW software and the DNA parsimony method of the PHYLIP package v.3.5 (Felsenstein, 1993) (data not shown). Results confirmed that although strains IPL15^T and IPL20^T clustered in two different clades, they both belonged to the genus *Devosia*.

The 16S rRNA gene sequence similarity between strains IPL15^T and IPL20^T was only 96.1 %. 16S rRNA gene sequence similarities between strain IPL15^T and the following strains are given in parentheses: *D. neptuniae* J1^T (96.2 %); *D. geojensis* BD-c194^T (96.2 %); *D. chinhatensis* IPL18^T (96.0 %); *D. limi* LMG 22951^T (96.0 %); *D.*

riboflavina DSM 7230^T (95.9 %); *D. subaequoris* HST3-14^T (95.9 %); *D. insulae* DS-56^T (95.5 %); *D. soli* GH2-10^T (95.2 %); 'Candidatus *D. euplotis*' CO (94.7 %); 'Candidatus *D. euplotis*' LIV5 (94.7 %); and 'Candidatus *D. euplotis*' CAMP4.4 (94.6 %).

As low sequence similarities (<97 %) were found between strain IPL15^T and all species of the genus *Devosia* with validly published names (Stackebrandt & Goebel, 1994), DNA–DNA hybridization studies of strain IPL15^T with its close relatives were not performed. On the other hand, strain IPL20^T showed highest 16S rRNA gene sequence similarity of 98.6 % with *D. soli* GH2-10^T, followed by *D. riboflavina* DSM 7230^T (97.8 %), *D. subaequoris* HST3-14^T (96.8 %), *D. limi* LMG 22951^T (96.2 %), *D. neptuniae* J1^T (95.9 %), ‘*Candidatus D. euplotis*’ CO (95.5 %), ‘*Candidatus D. euplotis*’ LIV5 (95.5 %), ‘*Candidatus D. euplotis*’ CAMP4.4 (95.4 %), *D. geojensis* BD-c194^T (94.7 %) and *D. insulae* DS-56^T (94.5 %). Hence, DNA–DNA hybridization studies between strain IPL20^T, *D. soli* GH2-10^T and *D. riboflavina* DSM 7230^T were conducted by using the membrane filter method (Tourova & Antonov, 1987). The experiment was performed as described by Bala *et al.* (2004). DNA–DNA relatedness values of strain IPL20^T with *D. riboflavina* DSM 7230^T and *D. soli* GH2-10^T were 12.1 and 9.8 %, respectively (see Supplementary

Fig. 1. Phylogenetic tree based on nearly complete 16S rRNA gene sequences (aligned positions) showing the relationship between strains IPL15^T and IPL20^T and related strains. Numbers at nodes represent bootstrap values (based on 1000 resamplings). GenBank accession numbers are shown in parentheses. Bar, 2 nt substitutions per 100 nt.

Table S1, available in IJSEM Online). All the values were well below the threshold value of 70 % that is recommended for delineation of genospecies (Stackebrandt & Goebel, 1994), thus confirming that strain IPL20^T represents a novel species in the genus *Devosia*.

Fatty acid methyl ester analysis of strains IPL15^T and IPL20^T was carried out at 28 °C on TSA at IMTECH, Chandigarh, India, using the method of Miller (1982) and Kuykendall *et al.* (1988). The mixture of cellular fatty acids was separated using the Sherlock Microbial Identification system (Microbial ID) as described by Kumar *et al.* (2008). Fatty acid analysis indicated that strains IPL15^T and IPL20^T contained C_{18:0} 3-OH and C_{10:0} 3-OH, features that are characteristic of members of the genus *Devosia* (see Supplementary Table S2, available in IJSEM Online). The predominant fatty acids of strain IPL15^T were iso-C_{15:0} (23.2 %), summed feature 8 (C_{18:1}ω7c/C_{18:1}ω6c; 14 %) 11-methyl C_{18:1}ω7c (11.5 %) and C_{16:0} (9.9 %). Interestingly, iso-C_{15:0} has only been reported in trace amounts in *D. insulae* DS-56^T (Yoon *et al.*, 2007). Other fatty acids of strain IPL15^T were unsaturated, branched and straight-chain fatty acids that have not been observed in any *Devosia* species described so far: anteiso-C_{15:0} (7.2 %), iso-C_{17:1}ω10c (4.9 %), C_{16:1}ω11c (3.5 %), iso-C_{16:0} (2.2 %), C_{16:1}ω7c alcohol (1.7 %) and iso-C_{14:0} (1.2 %). On the other hand, summed feature 8 (C_{18:1}ω7c/C_{18:1}ω6c; 60.7 %), C_{16:0} (17.8 %), C_{18:0} (6.7 %) and 11-methyl C_{18:1}ω7c (5.0 %) were the major fatty acids in strain IPL20^T. A minor amount of anteiso-C_{17:0} was also detected in strain IPL20^T; this fatty acid has not been identified in other species of the genus *Devosia* described so far (see Supplementary Table S2, available in IJSEM Online). The fatty acid profile of strain IPL20^T differed not only from that of strain IPL15^T, but also from that of its closest neighbour *D. soli* GH2-10^T.

Results of chemotaxonomic analysis are given in the species description. Strains IPL15^T and IPL20^T were maintained on LB agar at 28 °C and all biochemical tests were conducted at 28 °C unless stated otherwise. The strains were tested for oxidase activity using Oxidase discs. Catalase activity was determined by bubble production in a 3 % hydrogen peroxide solution (Merck) as described by McCarthy & Cross (1984). Urease activity was tested using Christensen's method (Christensen, 1946). Assimilation of different carbohydrates was detected in basal media (Gordon *et al.*, 1974). DNase activity was tested using HiMedia's DNase agar supplemented with 0.005 % (w/v) methyl green. Lipase production was assessed using 1 % tributyrin and Tween 80. β-Galactosidase activity was observed using HiMedia ONPG discs. The presence of nitrate and nitrite was tested under anaerobic conditions after 24–48 h (Smibert & Krieg, 1994; Prakash *et al.*, 2007a). Production of indole was tested as described by Smibert & Krieg (1994) and hydrolysis of casein, gelatin, starch and aesculin was determined as described by Cowan & Steel (1965). Citrate utilization was tested on Simmons' citrate agar (Simmons, 1926). Susceptibility towards

antibiotics was determined on LB agar using HiMedia readymade susceptibility test discs. Antibiotics tested were as follows (in µg unless stated otherwise): amikacin (30), ampicillin (10), chloramphenicol (30), ciprofloxacin (5), gentamicin (10), kanamycin (30), polymyxin B (300 U), rifamycin (5), tetracycline (30) and vancomycin (30).

Strain IPL15^T grew well on LB agar and NA, but slow growth was observed on TSA, whereas strain IPL20^T grew well on LB and TSA. Although all species of the genus *Devosia* reported so far are positive for catalase activity, strain IPL20^T was found to be catalase-negative. Strain IPL15^T could utilize glucose as sole carbon source, whereas strain IPL20^T could not. Unlike *D. soli* GH2-10^T, strain IPL20^T was positive for assimilation of maltose, mannose, D-mannitol and L-arabinose (Table 1).

Based on the data presented above, it is apparent that strains IPL15^T and IPL20^T represent two novel species of the genus *Devosia*, for which the names *Devosia albolilva* sp. nov. and *Devosia crocina* sp. nov., respectively, are proposed.

Description of *Devosia albolilva* sp. nov.

Devosia albolilva (al.bo.gil'va. L. fem. adj. *albolilva* whitish yellow).

Cells are Gram-negative, aerobic, rod-shaped, non-spore-forming and motile with a single polar flagellum. Colonies are cream in colour and circular with entire margins (0.5–2.0 mm in size) when grown on LB agar plates for 3 days. Grows well on LB agar and NA, but less growth is seen on TSA. Temperature, pH and NaCl concentration ranges for growth are 5–45 °C, 5–9 and 0–3 %, respectively. Assimilates L-arabinose, galactose, glucose, lactose, fructose, D-mannitol, mannose, xylose, maltose and sucrose, but not *myo*-inositol. Cells are positive for oxidase, catalase, urease and β-galactosidase activities and aesculin hydrolysis. Negative for DNase, lipase and nitrate reductase activities. Does not hydrolyse casein, gelatin or starch. Does not utilize citrate or produce indole. Sensitive to ciprofloxacin, gentamicin, chloramphenicol, rifampicin, amikacin, ampicillin, vancomycin and tetracycline; resistant to polymyxin B and kanamycin. Major cellular fatty acids are iso-C_{15:0}, summed feature 8 and 11-methyl C_{18:1}ω7c.

The type strain is IPL15^T (=CCM 7427^T=MTCC 8594^T), isolated from an HCH dump site in India.

Description of *Devosia crocina* sp. nov.

Devosia crocina (cro.ci'na. L. fem. adj. *crocina* saffron-coloured).

Gram-negative, aerobic, oval, non-spore-forming and motile with a single polar flagellum. Colonies are bright orange in colour, circular with entire margins and 0.5–2.0 mm in size when grown on LB agar plates for 3 days. Grows well on LB agar and TSA. Temperature, pH and NaCl concentration ranges for growth are 5–32 °C, 5–9 and 0–3 %, respectively. Assimilates L-arabinose, galactose,

Table 1. Differential phenotypic characteristics of strains IPL15^T and IPL20^T and other *Devosia* species

Strains: 1, IPL15^T (*Devosia albogilva* sp. nov.); 2, IPL20^T (*Devosia crocina* sp. nov.); 3, *D. riboflavina* DSM 7230^T; 4, *D. soli* KACC 11509^T; 5, *D. neptuniae* LMG 21357^T; 6, *D. geojensis* BD-c194^T (Ryu *et al.*, 2008); 7, *D. chinhatensis* IPL18^T; 8, *D. limi* LMG 22951^T (Vanparys *et al.*, 2005; Yoo *et al.*, 2006); 9, *D. subaequoris* HST3-14^T (Lee, 2007); 10, *D. insulae* DSM 17955^T (Yoon *et al.*, 2007). All strains are positive for β -galactosidase and aesculin hydrolysis. All are negative for gelatin hydrolysis, indole production, nitrate reduction, lipase, citrate production and susceptibility to polymyxin B. +, Positive; –, negative; (+), weakly positive; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8	9	10
Colony colour	Creamish	Orange	Cream	Light beige	Pearl white	White	Cream	Light yellow to light brown	Light yellow to light brown	Ivory
Cell morphology	Ovals or rods	Oval	Rods*	Rods*	Rods*	Rods	Rods	Rods	Rods	Ovals or rods
Motility	+	+	+*	ND	+*	+	+	–	+	+
Oxidase	+	+	+	–	+	+	+	+	+	–
Urease	+	+	+	+	+	+	+	–	–	+
Casein hydrolysis	–	–	–	–	+	+	–	–	–	–
Starch hydrolysis	–	–	–	+	–	–	–	–	–	–
Catalase	+	–	+	+	+	+	+	+	+	+
Assimilation of:										
Glucose	+	–	+	–	+	+	+	–	–	–
L-Arabinose	+	+	+	–	+	–	+	–	–	–
D-Mannitol	+	+	+	–	+	+	+	–	–	–
Mannose	+	+	+	–	+	+	+	–	–	–
Maltose	+	+	+	–	+	+	+	–	–	–
Galactose	+	+	+	–	–	ND	+	ND	ND	ND
Sucrose	+	–	–	–	–	ND	+	ND	ND	ND
Lactose	+	+	+	–	+	ND	+	ND	ND	ND
myo-Inositol	–	–	(+)	–	–	ND	+	ND	ND	ND
Xylose	+	+	+	–	(+)	ND	+	ND	ND	ND
Susceptibility to:†										
Gentamicin	S	S	R	R	R	R	S	R	ND	R
Chloramphenicol	S	S	R	S	R	R	S	R	ND	S
Kanamycin	R	R	R	S	R	R	R	R	ND	R
Tetracycline	S	S	S	S	R	S	S	I	ND	S

*Data from Yoo *et al.* (2006).

† I, Intermediate; R, resistant; S, sensitive.

fructose, D-mannitol, mannose, xylose, maltose and lactose, but not sucrose, myo-inositol or glucose. Positive for oxidase, urease and β -galactosidase activities and aesculin hydrolysis, but negative for catalase, DNase, lipase and nitrate reductase activities. Does not hydrolyse casein, gelatin or starch. Does not utilize citrate or produce indole. Sensitive to ciprofloxacin, gentamicin, chloramphenicol, rifampicin, amikacin, ampicillin, vancomycin and tetracycline; resistant to polymyxin B and kanamycin. Major cellular fatty acids are C_{18:1}ω7c/C_{18:1}ω6c and C_{16:0}.

The type strain is IPL20^T (=CCM 7425^T=MTCC 8590^T), isolated from an HCH dump site in India.

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