**Flavobacterium ummariense** sp. nov., isolated from hexachlorocyclohexane-contaminated soil, and emended description of *Flavobacterium ceti* Vela et al. 2007

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A Gram-negative, strictly aerobic, yellow bacterial strain, designated DS-12<sup>T</sup>, was isolated from hexachlorocyclohexane-contaminated soil in Lucknow, Uttar Pradesh, India. Strain DS-12<sup>T</sup> showed the highest 16S rRNA gene sequence similarity with *Flavobacterium ceti* 454-2<sup>T</sup> (94.2 %). Phylogenetic analysis based on 16S rRNA gene sequences showed that strain DS-12<sup>T</sup> belonged to the genus *Flavobacterium*. Strain DS-12<sup>T</sup> produced flexirubin-type pigments. Gliding motility was not observed. The major fatty acids of strain DS-12<sup>T</sup> were iso-C<sub>15 : 0</sub> (48.0 %), summed feature 9 (comprising iso-C<sub>17 : 0</sub> 9<sup>c</sup> and/or C<sub>16 : 0</sub> 10-methyl; 19.3 %), iso-C<sub>17 : 0</sub> 3-OH (8.5 %) and summed feature 3 (comprising one or more of C<sub>16 : 1</sub> 7<sup>c</sup>, C<sub>18 : 1</sub> 9<sup>c</sup> and iso-C<sub>15 : 0</sub> 2-OH; 7.2 %). The only respiratory quinone was menaquinone-6 and the major polyamine was homospermidine. Strain DS-12<sup>T</sup> contained phosphatidymethylhexanolamine, phosphatidyldimethylethanolamine, one unknown phospholipid and one unknown aminolipid. The DNA G+C content was 37.4 mol%. Phylogenetic inference and phenotypic properties indicated that strain DS-12<sup>T</sup> represents a novel species of the genus *Flavobacterium*, for which the name *Flavobacterium ummariense* sp. nov. is proposed. The type strain is DS-12<sup>T</sup> (=CCM 7847<sup>T</sup> = MTCC 10766<sup>T</sup>). An emended description of *Flavobacterium ceti* is also given.

The genus *Flavobacterium*, initially proposed by Bergey et al. (1923) and subsequently emended by Bernardet et al. (1996), belongs to the family *Flavobacteriaceae* in the phylum *Bacteroidetes* (Bernardet & Bowman, 2011). Members of this genus are aerobic, Gram-negative, non-spore-forming rods containing menaquinone-6 (MK-6) as the major respiratory quinone (Bernardet & Bowman, 2006). *Flavobacterium* strains have been isolated from a variety of habitats such as soil (Horn et al., 2005; Yoon et al., 2006), marine environments (Miyashita et al., 2010; Fu et al., 2011), freshwater (Tamaki et al., 2003; Wang et al., 2006), glacier ice (Zhu et al., 2003) and Antarctic habitats (McCammon & Bowman, 2000; Humphry et al., 2001; Van Trappen et al., 2003, 2004, 2005; Yi et al., 2005; Nogi et al., 2005; Yi & Chun, 2006).

We have been studying the diversity of culturable bacteria at a hexachlorocyclohexane (HCH) dumpsite located at Ummari village (27° 00′ 24.0″ N 81° 09′ 03.8″ E) near Lucknow, Uttar Pradesh, India. A number of bacterial strains have already been isolated and characterized from this dump site (Kumar et al., 2008; Singh & Lal, 2009; Verma et al., 2009; Kumari et al., 2009, 2011; Dadhwal et al., 2009; Sharma et al., 2010; Bala et al., 2010; Nigam et al., 2010; Lal et al., 2010; Kaur et al., 2011) including *Flavobacterium lindanitolerans* (Jit et al., 2008). These bacteria either degrade HCH isomers to varying degrees (Singh & Lal, 2009; Dadhwal et al., 2009; Bala et al., 2010; Nigam et al., 2010) or tolerate them at very high concentrations (Jit et al., 2008; Kumar et al., 2008; Verma et al., 2009; Sharma et al., 2010; Lal et al., 2010; Kaur et al., 2011). In order to further investigate the microbial diversity at this dumpsite, soil samples were collected, serially diluted and plated on Luria–Bertani (LB) agar supplemented with 100 U nystatin ml<sup>−1</sup> and 20 mg streptomycin ml<sup>−1</sup> and incubated at 28 °C. Yellow colonies were picked and purified on LB agar. The cultures were preserved in filtered water with 40 % (v/v) glycerol at −80 °C and routinely cultivated on LB agar or in LB broth at 28 °C.

Isolation of genomic DNA, and 16S rRNA gene sequence amplification and analysis were carried out as described by Gupta et al. (2008) using a 3100 Avant Genetic Analyzer Sequencer (Applied Biosystems) in the Department of Zoology, University of Delhi, Delhi, India. A 16S rRNA gene sequence of approximately 1.5 kb was obtained and assembled using Sequencing Analysis 5.11 and Clone

**Abbreviation**: HCH, hexachlorocyclohexane.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DS-12<sup>T</sup> is HQ329187.

A supplementary figure is available with the online version of this paper.
Manager 5. Similarity searches were conducted using the Sequence Match tool of the Ribosomal Database Project (Maidak et al., 2001) (http://rdp.cme.msu.edu/seqmatch/seqmatch_intro.jsp) and BLAST (Altschul et al., 1990) of NCBI (http://www.ncbi.nlm.nih.gov). Strain DS-12T showed the highest 16S rRNA gene sequence similarity with Flavobacterium ceti 454-2T (94.2%), whereas sequence similarities with other members of the genus Flavobacterium were 89.7–92.1%. Strain DS-12T exhibited 87.9% 16S rRNA gene sequence similarity with the type species of the genus, Flavobacterium aquatile ATCC 11947T. For construction of phylogenetic trees, 16S rRNA gene sequences from the genus Flavobacterium were selected from the RDP and NCBI databases. The 16S rRNA gene sequence of Cellulophaga lytica ATCC 23178T was used as an outgroup. The sequences were aligned using CLUSTAL X (Thompson et al., 1997). Phylogenetic analysis was carried out using TREECON (Van de Peer & De Wachter, 1994) and PHYLIP (Felsenstein, 1993). Evolutionary trees were constructed by neighbour-joining (Saitou & Nei, 1987), maximum-parsimony and maximum-likelihood (Takahashi & Nei, 2000). In the neighbour-joining tree (Fig. 1), strain DS-12T and F. ceti 454-2T formed a distinct cluster within the genus Flavobacterium. This topology was confirmed in the maximum-parsimony and maximump-likelihood trees.

For fatty acid analysis, strain DS-12T, F. ceti CCUG 52969T and F. aquatile ATCC 11947T were grown on LB agar at 28°C for 24 h. The physiological age of the three cultures was standardized following the procedure described by MIDI (http://www.microbialid.com/PDF/TechNote_101.pdf). Bacterial cells were collected and fatty acids were subjected to saponification, methylation and extraction using the methods of Miller (1982) and Kuykendall et al. (1988). The mixture of fatty acid methyl esters was separated by GC (Agilent 6890) equipped with a flame-ionization detector and fatty acids were identified using the RRTSA6 database of the Sherlock Microbial Identification System version 6.0 (MIDI). The major fatty acids of strain DS-12T were iso-C15:0 (48.0%), summed feature 9 (comprising iso-C17:1o9c and/or C16:0 10-methyl; 19.3%), iso-C17:0 3-OH (8.5%) and summed feature 3 (comprising one or more of C16:1o7c, C16:1o9c and iso-C15:0 2-OH; 7.2%). The complete fatty acid compositions of strain DS-12T, F. ceti CCUG 52969T and F. aquatile ATCC 11947T are shown in Table 1. The three strains shared very similar fatty acid profiles with only minor differences in the respective proportions of some components.

The respiratory quinones of strain DS-12T and F. ceti CCUG 52969T were extracted as described by Collins et al. (1977) and analysed by TLC (silica gel 60 F254, 20 × 20 cm; Merck) and HPTLC (silica gel 60 RP-18 F254S, 10 × 10 cm; Merck). The quinone composition of F. ceti had already been determined and included in the original species description. Menaquinone 6 (MK-6) was the only respiratory quinone of strain DS-12T, which is in line with all members of the family Flavobacteriaceae. Polar lipid analysis of strain DS-12T, F. ceti CCUG 52969T and F. aquatile ATCC 11947T was carried out by two-dimensional TLC and spraying the plates with specific reagents (Gupta et al., 2009). Strain DS-12T contained phosphatidyldimethylethanolamine, phosphatidylserine, phosphatidylethanolamine, one unknown phospholipid and one unknown aminolipid (Fig. S1 available in IJSEM Online). F. aquatile ATCC 11947T exhibited the same polar lipid profile as strain DS-12T. F. ceti CCUG 52969T exhibited an unknown phospholipid and an unknown aminolipid that were different from those of the other two strains.

The polyamines were extracted from strain DS-12T and F. ceti CCUG 52969T and analysed as described by Busse & Auling (1988) and Busse et al. (1997). The extracted samples were loaded on TLC plates (silica gel 20 × 20 cm, 105553; Merck) and separated using ethylacetate/cyclohexane (2:3) as the running solvent. Homospermidine was the major polyamine of both strains, which is in line with the other members of the genus Flavobacterium for which polyamine composition has been analysed (Bernardet & Bowman, 2011).

The G+C content of the genomic DNA of strain DS-12T was determined by Applied Biosystems 7500 Real-Time PCR, according to Gonzalez & Saiz-Jimenez (2002). The DNA G+C content was 37.4 mol%, which is in the range reported for the genus Flavobacterium (Bernardet & Bowman, 2011).

All phenotypic tests were performed on LB agar or in LB broth at 28°C using F. ceti CCUG 52969T as a reference strain. Gram-staining was performed using a kit (HiMedia). Colony morphology was examined after growth on LB agar at 28°C for 24 h. The presence of flagella was assessed by transmission electron microscopy (Morgagni 269D TEM; Fei) using cells from an exponentially growing culture. After air drying, the cells were negatively stained with 0.5% uranyl acetate and the grids were examined. Gliding motility was tested on a fresh LB broth culture using the hanging drop method and flagellar motility was assessed on motility agar (Farmer, 1999). The presence of flexirubin-type pigments was tested as described by Reichenbach (1992). The ability of strain DS-12T to grow anaerobically was tested using thioglycolate medium. Growth was tested in LB broth adjusted to pH 2–12 with 1.0 M HCl or 1.0 M NaOH or supplemented with 0–5% (w/v) NaCl (at intervals of 1%), as described by Arden Jones et al. (1979). Growth in LB broth was also tested at 4, 25, 28, 37 and 42°C. Tests for indole production and nitrate reduction were performed according to Smibert & Krieg (1994). Oxidase production was tested as described by McCarthy & Cross (1984). Catalase activity was determined by bubble production in a 3% (v/v) hydrogen peroxide solution. Hydrolysis of casein, gelatin, starch, aesculin, xanthine and hypoxanthine was determined as described by Cowan & Steel (1965). Urease activity was tested according to Christensen (1946). Acid production from carbohydrates and utilization of various substrates were assessed according to Gordon et al. (1974). DNase activity was tested using DNase agar (HiMedia) supplemented with 0.005% methyl green. Hydrolysis of
Tweens 20 and 80 was tested according to Arden Jones et al. (1979). Citrate utilization was tested on Simmons’ citrate agar (Simmons, 1926). Susceptibility to antibiotics was determined on Mueller–Hinton agar using the following commercial discs (HiMedia; µg antibiotic per disc): amikacin (30), chloramphenicol (10), ciprofloxacin (5), gentamicin (10), kanamycin (30), nalidixic acid (30), penicillin G (10), polymyxin B (300), rifampicin (30), tetracycline (30), vancomycin (30) and oxytetracycline (30). Degradation of HCH isomers tested (data not shown). The likelihood trees. Stars indicate that the corresponding nodes were also recovered in the maximum-parsimony and maximum-likelihood trees. Cellulophaga lytica ATCC 23178 was used as an outgroup. Bar, 0.05 substitutions per nucleotide position.

**Fig. 1.** Neighbour-joining phylogenetic tree based on nearly complete 16S rRNA gene sequences showing the relationships of strain DS-12T with members of the genus Flavobacterium. Bootstrap values (>700) based on 1000 resamplings are shown at branch nodes. Stars indicate that the corresponding nodes were also recovered in the maximum-parsimony and maximum-likelihood trees. Cellulophaga lytica ATCC 23178 was used as an outgroup. Bar, 0.05 substitutions per nucleotide position.

Phylogenetic inference based on 16S rRNA gene sequences as well as phenotypic data confirm that strain DS-12T represents a novel species of the genus Flavobacterium, for which the name Flavobacterium ummariense sp. nov. is proposed. On the basis of new data obtained in this study, an emended description of *F. ceti* is also given.

**Description of Flavobacterium ummariense sp. nov.**

Flavobacterium ummariense (um.ma.ri.en’se. N.L. neut. adj. ummariense pertaining to Ummari, the HCH-contaminated site from where the type strain was isolated).

Cells are Gram-negative, strictly aerobic, non-flagellated, non-spore-forming rods approximately 1 μm in length and 0.5 μm in diameter and devoid of gliding motility. Colonies on LB agar are yellow, entire, smooth and circular, approximately 1.5 mm in diameter after 24 h of incubation. Good growth occurs on LB, nutrient, trypticase soy and MacConkey agars. Growth occurs at 25 and 37 °C; optimum growth occurs at 28 °C and no growth occurs at
Strains: 1, F. ummariense sp. nov. DS-12T; 2, F. ceti CCUG 52969T; 3, F. aquatile ATCC 11947T. All data were taken from this study. Fatty acids amounting to <1 % of the total in all strains are not shown.

<table>
<thead>
<tr>
<th>Fatty acid (%)</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
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<tbody>
<tr>
<td>C16:0</td>
<td>1.3</td>
<td>1.0</td>
<td>1.2</td>
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<tr>
<td>iso-C15:0</td>
<td>48.0</td>
<td>46.8</td>
<td>47.1</td>
</tr>
<tr>
<td>iso-C16:0</td>
<td>1.9</td>
<td>1.0</td>
<td>1.7</td>
</tr>
<tr>
<td>anteiso-C15:0</td>
<td>1.5</td>
<td>2.3</td>
<td>1.3</td>
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<tr>
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<td>3.4</td>
<td>4.3</td>
<td>3.7</td>
</tr>
<tr>
<td>iso-C17:0 3-OH</td>
<td>8.5</td>
<td>12.5</td>
<td>9.5</td>
</tr>
<tr>
<td>Summed features*</td>
<td>4</td>
<td>3.7</td>
<td>6.8</td>
</tr>
<tr>
<td>Summed features9</td>
<td>19.3</td>
<td>16.5</td>
<td>18.2</td>
</tr>
</tbody>
</table>

*Summed features represent two or three fatty acids that cannot be separated by the Microbial Identification System. Summed feature 3 consisted of one or more of C16:1ω7c, C16:1ω6c and C15:0 2-OH. Summed feature 9 consisted of iso-C17:1ω9c and/or C16:0 10-methyl.

4 or 42 °C. Optimum growth occurs with 0–1 % NaCl; no growth occurs with >1 % NaCl. Growth occurs at pH 6.0–8.0 (optimum pH 7.5); no growth occurs at pH <6.0 DNase-, catalase- and oxidase-positive. Nitrage is not reduced to nitrite. Hydrolyzes urea, but not aesculin, Tween 20 or 80, gelatin, starch, xanthine, hypoxanthine or casein. Indole is not produced and citrate is not utilized.

Utilizes aminobutyric acid, N-acetylglycosamine, serine, β-alanine, lactose, D-mannose, maltose, D-galactose, L-rhamnose, D-xylene and trehalose, but not D-arabinose, D-glucose, D-mannitol, D-ribose, D-sorbitol, inositol, serine, aminobutyric acid, sodium citrate, potassium acetate, β-alanine, N-acetylglycosamine or proline. Utilizes aminobutyric acid, N-acetylglycosamine, serine, β-alanine, lactose, D-mannose, maltose, D-galactose, L-rhamnose, D-xylene and trehalose, but not D-glucose, L-arabinose, D-sorbitol, D-mannitol, inositol, proline, sodium citrate or potassium acetate. Sensitive to (μg per disc) ciprofloxacin (5), nalidixic acid (30), rifampicin (5), tetracycline (30), vancomycin (30), oxotetracycline (30) and chloramphenicol (10), but resistant to amikacin (30), gentamicin (10), kanamycin (30), polymyxin B (300) and penicillin G (10). The major fatty acids are iso-C15:0, summed feature 9 (comprising iso-C17:1ω9c and/or C16:0 10-methyl), iso-C17:0 3-OH and summed feature 3 (comprising one or more of C16:1ω7c, C16:1ω6c and iso-C15:0 2-OH). The only respiratory quinone is MK-6 and the major polyamine is homospermidine. The polar lipids are phosphatidyl-dimethylethanolamine, phosphatidylserine, phosphatidylethanolamine, one unknown phospholipid and one unknown aminolipid. Contains flexirubin-type pigments.

The type strain is DS-12T (=CCM 7847T =MTCC 10766T), isolated from a HCH dumpsite in Lucknow, India. The DNA G+C content of the type strain is 37.4 mol%.

**Table 1.** Cellular fatty acid compositions of strain DS-12T, F. ceti and F. aquatile

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
</tr>
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<tbody>
<tr>
<td>Colony colour</td>
<td>Dark yellow</td>
<td>Light yellow</td>
</tr>
<tr>
<td>Range for growth</td>
<td>NaCl (%)</td>
<td>pH</td>
</tr>
<tr>
<td></td>
<td>0–1</td>
<td>6.0–8.0</td>
</tr>
<tr>
<td></td>
<td>0–5</td>
<td>6.0–10.0</td>
</tr>
</tbody>
</table>

**Table 2.** Differential phenotypic characteristics of strain DS-12T and F. ceti CCUG 52969T

Strains: 1, F. ummariense sp. nov. DS-12T; 2, F. ceti CCUG 52969T. All data were taken from this study. Both strains are positive for the following characteristics: production of flexirubin-type pigments; growth on nutrient, tryptone soy and MacConkey agars; presence of MK-6 as the major respiratory quinone; presence of urease, catalase and oxidase; acid production from lactose, D-galactose, trehalose, D-xylene, L-rhamnose, D-mannose and maltose; and utilization of aminobutyric acid, N-acetylglycosamine, serine, β-alanine, lactose, D-galactose, trehalose, D-xylene, L-rhamnose, D-mannose and maltose. Both strains are negative for the following characteristics: gliding motility; hydrolysis of xanthine, hypoxanthine, Tween 20 and 80 and aesculin; indole production; nitrate reduction; and utilization of D-glucose, D-mannitol, inositol, L-arabinose and sodium citrate. Opposite results were reported by Vela et al. (2007) for F. ceti 454-2T for the following characteristics: urease; acid production from L-rhamnose; and utilization of N-acetylglycosamine, D-mannose and maltose.

**Emended description of* Flavobacterium ceti***

*Flavobacterium ceti* (ce’ti. L. n. cetus whale; L. gen. n. ceti of a whale).

The description is as given by Vela et al. (2007) with the following additions and modifications. Gliding motility is not observed. Positive for urease. Produces acid from lactose, D-galactose, trehalose, D-xylene, L-rhamnose, D-mannose and maltose. Utilizes aminobutyric acid, N-acetylglycosamine, serine, β-alanine, lactose, D-galactose, trehalose, D-xylene, L-rhamnose, D-mannose and maltose, but not inositol. Does not hydrolyse xanthine, hypoxanthine or Tweenes 20 or 80. The major polyamine is homospermidine. The polar lipids are phosphatidyl-dimethylethanolamine, phosphatidylserine, phosphatidylethanolamine, one unknown phospholipid and one unknown aminolipid.

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