**Dongia mobilis** gen. nov., sp. nov., a new member of the family *Rhodospirillaceae* isolated from a sequencing batch reactor for treatment of malachite green effluent

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A Gram-negative, strictly aerobic and heterotrophic, non-spore-forming bacterial strain, designated LM22T, was isolated from activated sludge of a sequencing batch reactor for the treatment of malachite green effluent. Cells of strain LM22T were slightly curved to straight rods (0.3–0.5×0.6–1.0 μm) and motile by a single polar flagellum. Strain LM22T was negative for oxidase and catalase activities and phototrophic growth. An internal membrane system and bacteriochlorophyll were absent. Growth occurred at 20–40 °C (optimum 30–35 °C) and pH 6.0–10.0 (optimum pH 7.0–7.5). Strain LM22T did not require NaCl for growth and tolerated up to 2.0 % NaCl (optimum 0.5 %). The major ubiquinone was Q-10. The major fatty acids (>10 % of the total) were C18 : 1ω7c (32.9 %), C19 : 0 cyclo ω8c (18.7 %), C16 : 0 (12.1 %) and C16 : 0 2-OH (10.5 %). Phylogenetic analysis of 16S rRNA gene sequences showed that *Inquilinus limosus* AU0476T was the closest relative (90.4 % 16S rRNA gene sequence similarity). The DNA G+C content was 65.6 mol%. On basis of phenotypic, chemotaxonomic and phylogenetic data, strain LM22T was considered to represent a novel genus and species of the family *Rhodospirillaceae*, for which the name *Dongia mobilis* gen. nov., sp. nov. is proposed. The type strain of *Dongia mobilis* is LM22T (=CGMCC 1.7660T =JCM 15798T).

The family *Rhodospirillaceae*, of subgroup 1 of the class *Alphaproteobacteria*, comprises 22 genera at the time of writing (http://www.bacterio.cict.fr/classifgenerafamilies.html#Rhodospirillaceae). Members of several novel genera of this family have been isolated recently from various environments, such as *Caenispirillum bisanense* environments, such as *Pelagibius litoralis* from seawater (Choi et al., 2008) and *Telmatospirillum siberiense* from a salt mine (Wang et al., 2007). The wide distribution and metabolic diversity (such as heterotrophism or phototrophism) of the family *Rhodospirillaceae* suggest that its members may have an important role in such aquatic and marine environments.

Malachite green is an N-methylated diamino-triphenyl-methane dye that can be toxic to human cells and promotes liver tumour formation in rodents (Alderman, 1985; Srivastava et al., 2004). In an investigation of the culturable microbial diversity in the activated sludge of a sequencing batch reactor for the treatment of malachite green effluent, many bacterial strains were isolated and characterized taxonomically. In this study, we report the isolation and characterization of a novel strain, LM22T, of the family *Rhodospirillaceae*.

A sludge sample was suspended in normal saline by vigorous vortexing and 0.1 ml suspension was spread onto 1/10-diluted trypticase soy agar (TSA; Difco) and incubated at 30 °C for 1 week. Single colonies were picked and a pure culture of strain LM22T was obtained after subcultivation on YP agar [↓]: 3.0 g tryptone (Difco), 3.0 g yeast extract (Difco), 0.5 g MgSO4·7H2O, 0.3 g

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain LM22T is FJ455532.

A transmission electron micrograph of a cell of strain LM22T and fatty acid compositions of members of the family *Rhodospirillaceae* are available as supplementary material with the online version of this paper.
NaCl, 15 g agar; pH 7.0. Strain LM22T was maintained on YP agar and stored in 15 % (w/v) glycerol at −80 °C.

Growth on other bacteriological media was tested using R2A agar [laboratory prepared, containing (per litre distilled water): 0.5 g tryptone (Difco), 0.5 g yeast extract (Difco), 0.5 g Casamino acids, 0.5 g glucose, 0.5 g soluble starch, 0.3 g sodium pyruvate, 0.3 g K3HPO4, 0.05 g MgSO4.7H2O, 15 g agar; adjusted to pH 7.0–7.2 using 1 M NaOH], Luria–Bertani (LB) agar (Oxoid), nutrient agar (laboratory prepared) and TSA. Abundant growth was observed on R2A. No growth was seen on LB agar, NA or TSA. Gram-staining was performed as described by Gerhardt et al. (1994). Cell morphology was observed by phase-contrast microscopy and transmission electron microscopy (H600; Hitachi). Motility was determined according to Dong & Cai (2001), by checking the turbidity throughout a tube containing semisolid YP agar. Flagellation was confirmed by transmission electron microscopy. Cells of strain LM22T were Gram-negative, slightly curved rods, 0.3–0.5 µm wide and 0.6–1.0 µm long, and motile by a single polar flagellum (Supplementary Fig. S1, available in IJSEM Online).

Conditions for growth were tested in YP broth. Growth was tested at pH 5.0–10.0 (at intervals of 0.5 pH units), at 15–45 °C (at intervals of 5 °C) and with 0–2.5 % (w/v) NaCl (at intervals of 0.5 % NaCl). Growth occurred at 20–40 °C (optimum 30–35 °C) and pH 6.0–10.0 (optimum pH 7.0–7.5). Strain LM22T did not require NaCl for growth and tolerated up to 2.0 % NaCl (optimum 0.5 %).

Catalase and oxidase activities, H2S production, hydrolysis of casein, starch and Tweens 20, 40, 60 and 80 were tested using standard methods (Cowan & Steel, 1965; Bruns et al., 2001). Assimilation of carbon sources was determined at 30 °C after 1, 3, 7 and 14 days according to Dong & Cai (2001) using the standard mineral base of Stanier et al. (1966) and, after autoclaving, adding filter-sterilized compounds to a concentration of 0.5 % (w/v). Other physiological tests were performed using the API ZYM and API 20NE systems (bioMérieux), according to the manufacturer's instructions. Susceptibility to antibiotics was determined on YP agar using filter-paper discs (Beijing Pharmaceutical Company). The physiological and biochemical characteristics of strain LM22T are given in the genus and species descriptions and the characteristics that differentiated the isolate from phylogenetically related genera are listed in Table 1.

Phototrophic growth was determined in an Oxoid AnaeroGen system using the medium of Pfennig & Trüper (1974) according to Imhoff & Caumette (2004). Strain LM22T was unable to grow photoautotrophically with 0.1 % (w/v) thiosulfate or 0.1 % (w/v) NaHCO3 or photoheterotrophically with 0.1 % (w/v) methanol or 0.3 % (w/v) yeast extract, tryptone, sodium acetate or sodium pyruvate.

The 16S rRNA gene of strain LM22T was amplified using two bacterial universal primers, 27F and 1492R (Lane, 1991), and sequenced as described previously (Zhang et al., 2003). An almost-complete 16S rRNA gene sequence (1458 nt) was subjected to comparative analysis in GenBank using the BLAST program (Altschul et al., 1990). A multiple alignment with sequences from closely related members was created using the CLUSTAL_X program (Thompson et al., 1997). Ambiguous bases and gaps in the alignment were manually removed and a phylogenetic tree was constructed using the evolutionary distance matrix calculated using the neighbour-joining method in MEGA version 3.1 (Kumar et al., 2004). The 16S rRNA gene sequence phylogenetic tree showed that strain LM22T formed a deep lineage in the family Rhodospirillaceae with Inquilinus limosus AU0476T (Fig. 1). Strain LM22T exhibited highest 16S rRNA sequence similarity with I. limosus AU0476T (90.4 %) and less than 90 % similarity with other members of the family Rhodospirillaceae.

Isoprenoid quinones were extracted and analysed as described by Komagata & Suzuki (1987). Strain LM22T contained Q-10 as the sole respiratory quinone. For fatty acid methyl ester analysis, cell mass was obtained from YP agar after cultivation at 30 °C for 3 days and the fatty acids were extracted, methylated and analysed using the Sherlock Microbial Identification System (MIDI), according to the manufacturer's instructions. The fatty acid profile of strain LM22T is summarized in the species description and is compared with members of the most phylogenetically closely related genera in Supplementary Table S1. The major fatty acid was C18:1ω7c (32.9 %), which is a common feature of members of the class Alphaproteobacteria (Labrenz et al., 2000). Strain LM22T had a slightly smaller amount of C18:1ω7c than its closest phylogenetic neighbour, I. limosus AU0476T, and could be distinguished from I. limosus AU0476T by having C19:0 cyclo ω8c (18.7 %), C16:0 (12.1 %) and C16:0 2-OH (10.5 %) as major fatty acids but C18:1 2-OH (4.5 %) as a minor fatty acid. Furthermore, strain LM22T could be differentiated from members of other related genera by having larger proportions of C19:0 cyclo ω8c, C16:0 2-OH, 11-methyl C18:1ω7c (5.1 %) and C14:0 (4.1 %). The DNA G+C content was determined by the thermal denaturation method (Sly et al., 1986), using DNA from Escherichia coli K-12 as a control. The DNA G+C content of strain LM22T was 65.6 mol%.

Although strain LM22T was shown to be most closely related phylogenetically to I. limosus AU0476T, it differed from I. limosus AU0476T in several characteristics, such as catalase activity, nutrient requirement for growth and DNA G+C content. Furthermore, the 16S rRNA gene sequence similarity between strain LM22T and I. limosus AU0476T was too low (90.4 %) to allow its assignment to the genus Inquilinus. Strain LM22T showed less than 90 % 16S rRNA gene sequence similarity to other members of the family Rhodospirillaceae, and several important phenotypic and chemotaxonomic properties also differentiated the isolate from other taxa. Therefore, on the basis of phenotypic, chemotaxonomic and phylogenetic data, strain LM22T is...

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Table 1. Characteristics that distinguish strain LM22\textsuperscript{T} from phylogenetically related genera in the family \textit{Rhodospirillaceae

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolation source(s)</td>
<td>Sludge from bioreactor</td>
<td>Cystic fibrosis patients</td>
<td>Soil, roots, fresh water</td>
<td>Seawater</td>
<td>Deep seawater, marine sediment</td>
<td>Fresh water, wastewater</td>
<td>Lake water</td>
<td>Coastal seawater</td>
</tr>
<tr>
<td>Colony colour(s)</td>
<td>White</td>
<td>Not pigmented</td>
<td>Pink, white plump vibrioids or rods</td>
<td>Cream</td>
<td>Grey</td>
<td>Red, pink vibrioids, spirals</td>
<td>Apricot</td>
<td>Cream–yellow pleomorphic rods</td>
</tr>
<tr>
<td>Cell shape</td>
<td>Curved rods</td>
<td>Plump vibrioids or rods</td>
<td>Rods</td>
<td>Rods</td>
<td>Vibrioids, spirals</td>
<td>Rods</td>
<td>Pleomorphic rods</td>
<td></td>
</tr>
<tr>
<td>Oxidase</td>
<td>Positive</td>
<td>Weakly positive</td>
<td>Variable</td>
<td>Negative</td>
<td>ND</td>
<td>Positive</td>
<td>Weakly positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Catalase</td>
<td>Positive</td>
<td>Weakly positive</td>
<td>Variable</td>
<td>Negative</td>
<td>ND</td>
<td>Positive</td>
<td>Weakly positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Gelatinase</td>
<td>Positive</td>
<td>Weakly positive</td>
<td>Variable</td>
<td>Negative</td>
<td>ND</td>
<td>Positive</td>
<td>Weakly positive</td>
<td>Positive</td>
</tr>
<tr>
<td>pH for growth</td>
<td>Range</td>
<td>6–10</td>
<td>ND</td>
<td>5–8.5</td>
<td>5–9</td>
<td>6–11</td>
<td>5.7–8</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Optimum</td>
<td>7–7.5</td>
<td>ND</td>
<td>5–7.2</td>
<td>6</td>
<td>7–9</td>
<td>6.8–7</td>
<td>ND</td>
</tr>
<tr>
<td>Maximum NaCl concentration (%), w/v</td>
<td>≤2</td>
<td>&lt;6</td>
<td>&lt;5</td>
<td>≤6</td>
<td>≤9</td>
<td>ND</td>
<td>≤5</td>
<td>≤10</td>
</tr>
<tr>
<td>Bacteriochlorophyll a</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Major quinone</td>
<td>Q-10</td>
<td>ND</td>
<td>Q-10</td>
<td>Q-10</td>
<td>ND</td>
<td>Q-9</td>
<td>Q-10</td>
<td>Q-10</td>
</tr>
<tr>
<td>Utilization of:</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>L-Arabinose</td>
<td>W</td>
<td>–</td>
<td>V</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>V</td>
</tr>
<tr>
<td>D-Glucose</td>
<td>W</td>
<td>–</td>
<td>V</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>V</td>
</tr>
<tr>
<td>L-Rhamnose</td>
<td>–</td>
<td>–</td>
<td>V</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Sucrose</td>
<td>W</td>
<td>–</td>
<td>V</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Citrate</td>
<td>–</td>
<td>–</td>
<td>V</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Inositol</td>
<td>–</td>
<td>–</td>
<td>V</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>DNA G + C content (mol%)</td>
<td>65.6</td>
<td>70.3</td>
<td>64–71</td>
<td>60</td>
<td>64.8–67.7</td>
<td>68.3–69.9</td>
<td>67.2</td>
<td>65–68</td>
</tr>
</tbody>
</table>

Taxa: 1, \textit{Dongia} mobilis gen. nov., sp. nov. (strain LM22\textsuperscript{T}); 2, \textit{Inquilinus} (data from Coenye et al., 2002); 3, \textit{Azospirillum} (Tarrand et al., 1978; Reinhold et al., 1987; Khammas et al., 1989; Sly & Stackebrandt, 1999; Eckert et al., 2001; Xie & Yokota, 2005; Peng et al., 2006; Mehnaz et al., 2007a, b; Young et al., 2008; Lin et al., 2009); 4, \textit{Nisaea} (Urios et al., 2008); 5, \textit{Oceanibaculum} (Lai et al., 2009b; Dong et al., 2010); 6, \textit{Rhodocista} (Favinger et al., 1989; Kawasaki et al., 1992; Imhoff et al., 1998; Zhang et al., 2003); 7, \textit{Skermanella} (Sly & Stackebrandt, 1999); 8, \textit{Thalassobaculum} (Zhang et al., 2008; Urios et al., 2010). +, Positive; w, weakly positive; v, variable; –, negative; ND, no data available.
considered to represent a novel genus and species of the family Rhodospirillaceae, for which we propose the name *Dongia mobilis* gen. nov., sp. nov.

**Description of Dongia gen. nov.**

*Dongia* (Don’gi.a. N.L. fem. n. *Dongia* after Professor Xiu-Zhu Dong, a bacteriologist and bacterial taxonomist in China).

Cells are Gram-negative, non-spore-forming, motile, slightly curved to straight rods. Strictly aerobic and heterotrophic. Never phototrophic. Internal membrane system and bacteriochlorophyll *a* are absent. Negative for oxidase and catalase. Reduces nitrate to nitrite. Major fatty acids are *C*₁₈:₁ω₇*č*, *C*₁₉:₀ cyclo ω8*č*, *C*₁₆:₀ and *C*₁₆:₀ 2-0H. The major ubiquinone is Q-10. The DNA G+C content of the type strain is 65.6 mol%. The type species is *Dongia mobilis*.

**Description of Dongia mobilis** sp. nov.

*Dongia mobilis* (mö’bi.lis. L. fem. adj. *mobilis* motile, pertaining to the motility of the type strain).

Exhibits the following properties in addition to those given in the genus description. Cells are 0.3–0.5 μm wide and 0.6–1.0 μm long. Colonies on YP agar are white, transparent, smooth, circular, convex and 0.5–1 mm in diameter after incubation at 30 °C for 3 days. Growth occurs at 20–40 °C (optimum 30–35 °C) and pH 6.0–10.0 (optimum pH 7.0–7.5). NaCl is not required for growth and the type strain can tolerate up to 2.0 % NaCl (optimum 0.5 % NaCl). The DNA G+C content of the type strain is 65.6 mol%.

In addition to the major fatty acids listed for the genus, contains the minor fatty acids 11-methyl *C*₁₈:₁ω7*č*, *C*₁₈:₁ 2-OH, *C*₁₆:₀ 3-OH, *C*₁₄:₀, summed feature 3 (*C*₁₆:₁ω7*č* and/or iso-*C*₁₅:₀ 2-OH) and *C*₁₄:₀ 2-OH. Sensitive to (µg per disc unless otherwise stated) vancomycin (30), gentamicin (10), carbencillin (100), polymyxin B (300 U), streptomycin (10), kanamycin (30), ampicillin (10), neomycin (30), chloramphenicol (30) and penicillin (10 U) and weakly sensitive to tetracycline (30), erythromycin (15), novobiocin (5) and rifampicin (5). The DNA G+C content of the type strain is 65.6 mol%.

The type strain is LM22ᵀ (=CGMCC 1.7660ᵀ =JCM 15798ᵀ), isolated from a sequencing batch reactor for treatment of malachite green effluent.

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