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 LM22^T, was isolated from activated sludge of a sequencing batch reactor for the treatment of malachite green effluent. Cells of strain LM22^T were slightly curved to straight rods (0.3–0.5×0.6–1.0 µm) and motile by a single polar flagellum. Strain LM22^T was negative for oxidase and catalase activities and phototrophic growth. An internal membrane system and bacteriochlorophyll *a* 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 LM22^T 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 C_{18:1}ω7c (32.9 %), C_{19:0} cyclo ω8c (18.7 %), C_{16:0} (12.1 %) and C_{16:0} 2-OH (10.5 %). Phylogenetic analysis of 16S rRNA gene sequences showed that *Inquilinus limosus* AU0476^T 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 LM22^T 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 LM22^T (=CGMCC 1.7660^T =JCM 15798^T).

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* from sludge of a dye works (Yoon *et al.*, 2007), *Fodinicurvata fenggangensis* and *F. sediminis* from a salt mine (Wang *et al.*, 2009), *Marispirillum indicum* from a deep-sea environment (Lai *et al.*, 2009a), *Nisaea denitrificans* and *N. nitritireducens* from surface seawater (Urios *et al.*, 2008), *Oceanibaculum indicum* from deep seawater (Lai *et al.*, 2009b), *Pelagibius litoralis* from seawater (Choi *et al.*, 2009), *Thalassobaculum litoreum* from coastal seawater (Zhang *et al.*, 2008) and *Telmatospirillum siberiense* from a mesotrophic fen (Sizova *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, LM22^T, 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 LM22^T was obtained after subcultivation on YP agar [l⁻: 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 LM22^T is FJ455532.

A transmission electron micrograph of a cell of strain LM22^T 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 LM22^T 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 K₂HPO₄, 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 LM22^T were Gram-negative, slightly curved to straight rods, 0.3–0.5 µm wide and 0.6–1.0 µm long, and were 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 5.0–11.0 (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 LM22^T 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 LM22^T 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 LM22^T 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 LM22^T 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 LM22^T formed a deep lineage in the family *Rhodospirillaceae* with *Inquilinus limosus* AU0476^T (Fig. 1). Strain LM22^T exhibited highest 16S rRNA sequence similarity with *I. limosus* AU0476^T (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 LM22^T 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 LM22^T 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 C_{18:1}ω7c (32.9 %), which is a common feature of members of the class *Alphaproteobacteria* (Labrenz *et al.*, 2000). Strain LM22^T had a slightly smaller amount of C_{18:1}ω7c than its closest phylogenetic neighbour, *I. limosus* AU0476^T, and could be distinguished from *I. limosus* AU0476^T by having C_{19:0} cyclo ω8c (18.7 %), C_{16:0} (12.1 %) and C_{16:0} 2-OH (10.5 %) as major fatty acids but C_{18:1} 2-OH (4.5 %) as a minor fatty acid. Furthermore, strain LM22^T could be differentiated from members of other related genera by having larger proportions of C_{19:0} cyclo ω8c, C_{16:0} 2-OH, 11-methyl C_{18:1}ω7c (5.1 %) and C_{14:0} (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 LM22^T was 65.6 mol%.

Although strain LM22^T was shown to be most closely related phylogenetically to *I. limosus* AU0476^T, it differed from *I. limosus* AU0476^T 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 LM22^T and *I. limosus* AU0476^T was too low (90.4 %) to allow its assignment to the genus *Inquilinus*. Strain LM22^T 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 LM22^T is

Table 1. Characteristics that distinguish strain LM22^T from phylogenetically related genera in the family *Rhodospirillaceae*

Taxa: 1, *Dongia mobilis* gen. nov., sp. nov. (strain LM22^T); 2, *Inquilinus* (data from Coenye *et al.*, 2002); 3, *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, *Nisaea* (Urios *et al.*, 2008); 5, *Oceanibaculum* (Lai *et al.*, 2009b; Dong *et al.*, 2010); 6, *Rhodocista* (Favinger *et al.*, 1989; Kawasaki *et al.*, 1992; Imhoff *et al.*, 1998; Zhang *et al.*, 2003); 7, *Skermanella* (Sly & Stackebrandt, 1999); 8, *Thalassobaculum* (Zhang *et al.*, 2008; Urios *et al.*, 2010). +, Positive; w, weakly positive; v, variable; –, negative; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8
Isolation source(s)	Sludge from bioreactor	Cystic fibrosis patients	Soil, roots, fresh water	Seawater	Deep seawater, marine sediment	Fresh water, wastewater	Lake water	Coastal seawater
Colony colour(s)	White	Not pigmented	Pink, white	Cream	Grey	Red, pink	Apricot	Cream–yellow
Cell shape	Curved rods	Rods	Plump vibrioids or rods	Rods	Rods	Vibrioids, spirals	Rods	Pleomorphic rods
Oxidase	–	v	+	–	+	ND	+	+
Catalase	–	+	+	+	v	ND	+	+
Gelatinase	–	v	v	ND	–	ND	–	+
Growth temperature (°C)								
Range	20–40	25–42	4–41	15–44	10–45	25–47	10–37	10–37
Optimum	30–35	ND	20–41	30	25–37	31–45	28	30–35
pH for growth								
Range	6–10	ND	5–8.5	5–9	6–11	5.7–8	ND	5–10
Optimum	7–7.5	ND	5–7.2	6	7–9	6.8–7	ND	8
Maximum NaCl concentration (% w/v)	≤2	<6	<5	≤6	≤9	ND	<5	≤10
Bacteriochlorophyll <i>a</i>	–	ND	–	ND	–	+	–	–
Major quinone	Q-10	ND	Q-10	Q-10	ND	Q-9	Q-10	Q-10
Utilization of:								
L-Arabinose	w	–	v	–	–	ND	+	v
D-Glucose	w	–	v	+	–	–	+	v
L-Rhamnose	–	–	v	–	v	ND	+	–
Sucrose	w	–	v	–	–	–	ND	v
Citrate	–	–	v	–	–	–	+	–
Inositol	–	–	v	–	–	ND	–	–
DNA G + C content (mol%)	65.6	70.3	64–71	60	64.8–67.7	68.3–69.9	67.2	65–68

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic position of strain LM22^T and representatives of some other related taxa. Bootstrap values (>70%) based on 1000 replications are shown at branch nodes. The sequence of *Gluconacetobacter diazotrophicus* PA1 5^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

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_{18:1}ω7c, C_{19:0} cyclo ω8c, C_{16:0} and C_{16:0} 2-OH. The major ubiquinone is Q-10. The DNA G + C content of the type strain of the type species is 65.6 mol%. The type species is *Dongia mobilis*.

Description of *Dongia mobilis* sp. nov.

Dongia mobilis (mo'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). Negative for β-galactosidase, urease and production of indole and H₂S. Hydrolyses Tweens 20 and 80 weakly, but starch, L-tyrosine, casein, arginine, gelatin and aesculin are not hydrolysed. Weak growth on L-arabinose, cellobiose, glucose, lactose, maltose, raffinose, sucrose, D-xylose, erythritol, glycerol and D-glucitol; no growth on sodium acetate, casein, citrate, citric acid, inositol, malic acid, methanol, L-rhamnose, sorbitol and succinic acid. With API ZYM, positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, phosphatase acid and naphthol-AS-BI-phosphohydrolase and weakly positive for lipase (C14), valine arylamidase and cystine arylamidase.

In addition to the major fatty acids listed for the genus, contains the minor fatty acids 11-methyl C_{18:1}ω7c, C_{18:1} 2-OH, C_{16:0} 3-OH, C_{14:0}, summed feature 3 (C_{16:1}ω7c 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), carbenicillin (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 LM22^T (=CGMCC 1.7660^T =JCM 15798^T), isolated from a sequencing batch reactor for treatment of malachite green effluent.

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