Rubellimicrobium mesophilum sp. nov., a mesophilic, pigmented bacterium isolated from soil

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A pink- to light-reddish-pigmented, aerobic, pointed, budding, mesophilic strain designated MSL-20^T was isolated from soil from Bigeum island, Korea. The isolate was Gram-negative. Cells were motile rods without flagella. The temperature and pH for growth were 20–37 °C (optimum 28 °C) and pH 7.0–11.0 (optimum pH 7.0 ± 0.2). Q-10 was the predominant respiratory ubiquinone. The major fatty acids were C_{16:0} (37 %), C_{18:1ω7c} (36 %) and 11-methyl C_{18:1ω7c} (12 %). Phylogenetic analysis based on the 16S rRNA gene sequence revealed that *Rubellimicrobium thermophilum* DSM 16684^T was its closest neighbour (95.2 % similarity); the isolate had less than 97.0 % similarity to all species with validly published names.

Chemotaxonomic data were determined for this isolate, including peptidoglycan type, diagnostic sugar composition, fatty acid profiles, ubiquinones and polar lipids; the results supported the affiliation of strain MSL-20^T to the genus *Rubellimicrobium*. The DNA G + C content was 72.3 mol%. On the basis of the phenotypic, chemotaxonomic and phylogenetic data, strain MSL-20^T represents a novel species of the genus *Rubellimicrobium*, for which the name *Rubellimicrobium mesophilum* sp. nov. is proposed. The type strain is MSL-20^T (=KCTC 22012^T =DSM 19309^T).

The genus *Rubellimicrobium* was proposed by Denner *et al.* (2006) for a reddish-coloured, Gram-negative, rod-shaped bacterium that is phylogenetically considered to be a member of the order *Rhodobacterales* of the class *Alphaproteobacteria*. Members of this order are phenotypically, metabolically and ecologically diverse (Garritty *et al.*, 2005) and include photoheterotrophs that can also grow photoautotrophically or chemotrophically under appropriate environmental conditions. The genus *Rubellimicrobium* contains only one species, *Rubellimicrobium thermophilum* (Denner *et al.*, 2006), isolated from coloured slime deposits on paper machines. In this paper, we report a mesophilic species of *Rubellimicrobium* isolated from soil from Bigeum island, Korea.

During a study of the microbial flora of Bigeum island, a red-pigmented strain, MSL-20^T, was isolated and identified from a sediment soil sample. Further study of strain MSL-20^T based on a polyphasic approach including morphological observation and physiological and chemotaxonomic analysis showed that this isolate represents a novel species of the genus *Rubellimicrobium*.

Strain MSL-20^T was isolated on 10-fold-diluted R2A medium at 28 °C and maintained routinely on the same

medium and as a glycerol suspension (20 %, v/v) at –70 °C. Biomass for chemical and molecular systematic studies was obtained by cultivation at 28 °C for 1 week in flasks of R2A broth (Difco) (pH 7.0). Cultural characteristics were determined after 7 days at 28 °C by methods used in the International *Streptomyces* Project (ISP) (Shirling & Gottlieb, 1966). Morphological properties, spores and mycelia were examined by light microscopy (Nikon; HFX-DX) and scanning electron microscopy with JEOL model apparatus Philips SEM 515 (Fig. 1). Morphological observation of 48–72 h cultures of strain MSL-20^T grown on diluted R2A medium revealed that the cells were motile, short rods, 0.4–0.7 × 1.6–3.4 µm. The strain was examined for a range of phenotypic properties using standard procedures (Goodfellow, 1971; Williams *et al.*, 1983). Utilization of sole carbon and nitrogen sources was investigated according to Tsukamura (1966). Tolerance to temperature (10, 20, 28, 37, 45 and 50 °C) and NaCl (0–15 %, at intervals of 0.5 %) was tested using R2A as basal medium.

Phospholipid analysis was carried out as described by Lechevalier *et al.* (1981). Respiratory lipoquinone analysis was performed by HPLC according to the procedure of Altenburger *et al.* (1996). Biomass for quantitative fatty acid analysis was prepared by scraping growth from R2A plates that had been incubated for 4–5 days at 28 °C. The

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain MSL-20^T is EF547368.

Fig. 1. Scanning electron micrograph showing monopolar cell growth of strain MSL-20^T incubated for 7 days on R2A medium at 28 °C. Bar, 2 µm.

fatty acids were extracted, methylated and analysed using the standard MIDI Microbial ID system (Sasser, 1990; Kämpfer & Kroppenstedt, 1996).

Extraction of genomic DNA and amplification of the 16S rRNA gene were done as described by Rainey *et al.* (1996). An almost-complete 16S rRNA gene sequence of strain MSL-20^T, comprising 1429 bp, was obtained and compared with sequences of type strains retrieved from the EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007) and GenBank database. Phylogenetic analysis was performed using the software packages PHYLIP (Felsenstein, 1993) and MEGA version 2.1 (Kumar *et al.*, 2001) after multiple alignment of the data using CLUSTAL_X (Thompson *et al.*, 1997). Distances (using distance options according to the Kimura two-parameter model; Kimura, 1980, 1983) were calculated and clustering was performed with the neighbour-joining method (Saitou & Nei, 1987).

Bootstrap analysis (1000 resamplings) was used to evaluate the tree topology of the neighbour-joining data (Felsenstein, 1985).

Strain MSL-20^T contained ubiquinone Q-10 as the major quinone. Phospholipids detected were diphosphatidylglycerol, phosphatidylcholine and an unidentified aminolipid. The cellular fatty acid composition of strain MSL-15^T is given in the species description. 16S rRNA gene sequence similarities between strain MSL-20^T and type strains of species with validly published names were below 97.0%. Phylogenetic analysis based on its complete 16S rRNA gene sequence showed that the novel isolate was most closely related to *Rubellimicrobium thermophilum* DSM 16684^T. A dendrogram (Fig. 2) confirmed that strain MSL-20^T and *R. thermophilum* DSM 16684^T are phylogenetic neighbours, with 95.2% 16S rRNA gene sequence similarity and a bootstrap value of 99%.

A comparison of the phenotypic properties of strain MSL-20^T and *R. thermophilum* DSM 16684^T revealed marginal differences, though they can be differentiated easily by their physiological properties, particularly their growth temperatures: *R. thermophilum* DSM 16684^T is a thermophile (optimum growth at 45–54 °C), whereas MSL-20^T is mesophilic (growth at 20–37 °C) (Table 1).

In conclusion, the genotypic and phenotypic data show that strain MSL-20^T represents a novel species of the genus *Rubellimicrobium*, for which we propose the name *Rubellimicrobium mesophilum* sp. nov.

Description of *Rubellimicrobium mesophilum* sp. nov.

Rubellimicrobium mesophilum [me.so.phi'lum. Gr. adj. *mesos* middle; Gr. adj. *philos* loving; N.L. neut. adj. *mesophilum* middle (temperature)-loving, mesophilic].

Fig. 2. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of strain MSL-20^T and some related taxa. Bootstrap values (expressed as percentages of 1000 replications) above 50% are shown at branch points. Bar, 0.01 substitutions per nucleotide position.

Table 1. Characteristics that distinguish strain MSL-20^T and *R. thermophilum* DSM 16684^T

Data for *R. thermophilum* DSM 16684^T were taken from Denner *et al.* (2006). Both strains are positive for utilization of D-fructose and negative for utilization of citrate and propionate.

Characteristic	Strain MSL-20 ^T	<i>R. thermophilum</i> DSM 16684 ^T
Optimum growth temperature (°C)	28	45–54
Substrate utilization		
L-Rhamnose	–	+
L-Histidine	+	–
L-Leucine	+	–
Utilization of carbon compounds		
Cellobiose	–	+
D-Mannose	–	+
Salicin	–	+
D-Xylose	–	+
α-Melibiose	–	+
D-Sorbitol	–	+
L-Malate	–	+
D-Ribose	–	+
Major fatty acids	C _{16:0} , C _{18:1ω7c} , 11-methyl C _{18:1ω7c}	C _{19:0} cyclo ω8c, C _{18:0} , C _{16:0}
DNA G + C content (mol%)	72.3	69.4–70.2 (70.0)*
Source of isolation	Soil	Paper mill

*Range of values for four strains. The DNA G + C content of the type strain is given in parentheses.

Cells are Gram-negative, motile, non-flagellated, irregular rods, 0.4–0.7 × 1.6–3.4 µm. Colonies on R2A medium are pink to light reddish. Oxidase and nitrate reduction activities are negative, whereas catalase activity is weakly positive. Positive for starch and Tween 80 hydrolysis and assimilation of cellulose, histidine, leucine and fructose. Tests for leucine arylamidase, naphthol-AS-BI-phosphohydrolase and α-glucosidase are positive (API ZYM; bioMérieux). Growth is observed at 20–37 °C (optimum 28 °C) and at pH 7–11 (optimum pH 7.0 ± 0.2); no growth in the presence of NaCl. The major respiratory quinone is ubiquinone Q-10. The dominant fatty acids are C_{16:0} (36.91 %), C_{18:1ω7c} (36.50 %), 11-methyl C_{18:1ω7c} (12.40 %), C_{18:0} (3.63 %), C_{10:0} (1.27 %), C_{12:0} (1.26 %) and C_{17:0} (1.22 %). The DNA G + C content of the type strain is 72.3 mol%.

The type strain, MSL-20^T (=KCTC 22012^T =DSM 19309^T), was isolated from soil from Bigeum island, Republic of Korea.

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