Leifsonia rubra sp. nov. and *Leifsonia aurea* sp. nov., psychrophiles from a pond in Antarctica

G. S. N. Reddy,¹ J. S. S. Prakash,¹ R. Srinivas,² G. I. Matsumoto³ and S. Shivaji¹

Correspondence

S. Shivaji

shivass@ccmb.res.in

^{1,2}Centre for Cellular and Molecular Biology¹ and Indian Institute of Chemical Technology², Uppal Road, Hyderabad – 500 007, India

³Otsu Women's University, Department of Environment Science, School of Social Information Studies, Karakida 2-7-1, Tama-Shi, Wakazawa, Tokyo 206-0036, Japan

Two unique psychrophilic strains (CMS 76r^T and CMS 81y^T) were isolated from a cyanobacterial mat sample from a pond in Wright Valley, McMurdo, Antarctica. Both isolates were assigned to the genus *Leifsonia*, since they were Gram-positive, curved rods, non-motile, catalase-positive, contained DL-2,4-diaminobutyric acid, menaquinone MK-11, phosphatidylglycerol and diphosphatidylglycerol, had a high content of anteiso- and iso-branched fatty acids and had a DNA G + C content of 64–66 mol%. In addition, both isolates were related to the five reported species of *Leifsonia* at a level of about 95–96 % 16S rDNA sequence similarity and differed from one another by 2.5 %. Strains CMS 76r^T and CMS 81y^T also differed from one another in many other phenotypic characteristics and exhibited only 30 % relatedness at the DNA–DNA level, thus indicating that they represent two different species. Furthermore, these two isolates also showed many distinct differences with respect to the reported species of *Leifsonia* in terms of their phenotypic characteristics, biochemical properties, chemotaxonomic features, sensitivity to various antibiotics and 16S rDNA similarity, clearly indicating that strains CMS 76r^T (=MTCC 4210^T =DSM 15304^T =CIP 107783^T) and CMS 81y^T (=MTCC 4657^T =DSM 15303^T =CIP 107785^T) represent the type strains of two novel species of *Leifsonia*, for which the names *Leifsonia rubra* sp. nov. and *Leifsonia aurea* sp. nov. are proposed.

The genus *Leifsonia* was created by Evtushenko *et al.* (2000) to accommodate Gram-positive, non-spore-forming, irregular rod- or filament-shaped, motile, mesophilic and catalase-positive bacteria containing DL-2,4-diaminobutyric acid in the peptidoglycan, MK-11 as the major menaquinone, phosphatidylglycerol and diphosphatidylglycerol as the major phospholipids, a high content of anteiso- and iso-branched fatty acids and a DNA G + C content of 66–73 mol%. Currently, five species belonging to the genus *Leifsonia* have been described: *Leifsonia poae*, isolated from a nematode gall on *Poa annua* roots (Evtushenko *et al.*, 2000), *Leifsonia aquatica*, isolated from distilled water (Leifson, 1962), *Leifsonia xyli* subsp. *xyli* and *L. xyli* subsp. *cynodontis*, respectively isolated from a *Saccharum* interspecific hybrid and *Cynodon dactylon* (Davis *et al.*, 1984) and *Leifsonia naganoensis* and *Leifsonia shinshuensis*, isolated from soil

(Suzuki *et al.*, 1999). All five species reported so far are mesophilic. In the present study, we report two psychrophilic strains of *Leifsonia* isolated from a cyanobacterial mat sample growing luxuriously in a pond in Wright Valley, McMurdo, Antarctica. Both isolates, CMS 76r^T and CMS 81y^T, were psychrophilic; they could be assigned to the genus *Leifsonia* on the basis of their phenotypic characteristics, chemotaxonomic features and phylogeny based on 16S rRNA gene sequences. Furthermore, strains CMS 76r^T and CMS 81y^T differed from the five reported species of *Leifsonia* with respect to many characteristics and are designated as the type strains of the novel species *Leifsonia rubra* sp. nov. (CMS 76r^T) and *Leifsonia aurea* sp. nov. (CMS 81y^T).

Source of the organisms, media and growth conditions

Seven bacterial cultures (CMS 73y, CMS 74y, CMS 76r^T, CMS 77y, CMS 78y, CMS 79y and CMS 81y^T) were isolated from a cyanobacterial mat sample collected from pond L9 of Wright Valley in McMurdo, Antarctica (Matsumoto, 1993). For this purpose, a fragment of the cyanobacterial mat sample (about 200 mg) was suspended in 1 ml sterile saline (150 mM NaCl) and an aliquot (100 µl) was plated on

Abbreviation: ABM, Antarctic bacterial medium.

The EMBL accession numbers for the 16S rRNA gene sequences of strains CMS 76r^T and CMS 81y^T are AJ438585 and AJ438586.

A UPGMA phenogram including more reference sequences and a table of differences revealed by a comparison of almost-complete 16S rRNA gene sequences of all members of the genus *Leifsonia* are available as supplementary material in IJSEM Online.

Antarctic bacterial medium (ABM) containing 0.5 % (w/v) peptone, 0.2 % (w/v) yeast extract and 1.5 % (w/v) agar (pH 6.9) and incubated at 5 °C (Shivaji *et al.*, 1989). Representative colonies were selected and regrown on ABM plates to obtain pure cultures of the bacteria. The optimum temperature, pH and salt concentration for growth of the cultures were determined using ABM plates.

All seven isolates could grow on either ABM or nutrient agar and colonies were circular, convex, smooth, butyrous and opaque. Furthermore, except for CMS 76r^T (which was red-pigmented), the isolates were yellow-pigmented. These six yellow-pigmented isolates were also identical with respect to their morphology, biochemical and chemotaxonomic characteristics, randomly amplified polymorphic DNA profile and 16S rRNA gene sequence, indicating that they are clonal in origin. Therefore, CMS 81y^T was chosen as a representative strain for all six yellow-pigmented isolates.

Randomly amplified polymorphic DNA analysis was carried out as described previously (Shivaji *et al.*, 2000) using the primers OPA-02 (5'-TGCCGAGCT-3') and OPA-03 (5'-AGTGC GCCAC-3').

Morphology, motility and biochemical characteristics

The morphology of the bacterial cultures during growth and motility were observed by phase-contrast microscopy (1000×). All of the biochemical tests described below were performed by growing the cultures at 18 or 22 °C in the appropriate medium (Hugh & Leifson, 1953; Stanier *et al.*, 1966; Holding & Collee, 1971; Stolp & Gadkari, 1981). For oxidase testing, the method of Kovacs (1956) was used. In this test, stationary-phase cells grown in ABM were used. The ability of the cultures to utilize carbon compounds as sole carbon sources, sensitivity to different antibiotics and the G + C content of the DNA were determined as described previously (Shivaji *et al.*, 1991).

Both strains CMS 76r^T and CMS 81y^T were found to be Gram-positive, curved, rod-shaped bacteria that are non-motile, aerobic and positive for catalase. Other details relating to optimal growth conditions, pigment characteristics,

utilization of carbon compounds as sole carbon sources, ability to oxidize or ferment sugars, sensitivity to antibiotics and other phenotypic characteristics are included below in the species descriptions (see Tables 1 and 2 for diagnostic characteristics). Pigments were extracted from lyophilized bacterial cell pellets by using methanol and their absorption spectra were recorded in a Hitachi 330 spectrophotometer (Jagannadham *et al.*, 1991).

Phylogenetic analysis

The 16S rRNA genes of CMS 76r^T and CMS 81y^T were amplified and sequenced as published elsewhere (Reddy *et al.*, 2000) and aligned with reference sequences that included all the genera of the family using the multiple sequence alignment program CLUSTAL V (Higgins *et al.*, 1992). The program DNADIST, with the Kimura-2 parameter, was used to compute pairwise evolutionary distances for the above aligned sequences (Kimura, 1980). The original sequence dataset was resampled 1000 times using SEQBOOT and subjected to bootstrap analysis. Phylogenetic trees were constructed using parsimony analysis (DNAPARS) and using various distance matrix-based clustering algorithms such as FITCH, KITCH and UPGMA (Felsenstein, 1993). Majority-rule (50 %) consensus trees were also constructed using CONSENSE (Felsenstein, 1993). In all cases, input order of species added to the topology of the tree was randomized with the jumble option of 7 and 10 replications.

The different algorithms gave consistent results. On the basis of the 16S rRNA gene sequence, it was clear that strains CMS 76r^T and CMS 81y^T are closely related to one another (97.5 % similarity) and to the reported species of *Leifsonia* (95–96 %) with an evolutionary distance of 2.5 % between themselves and 4–5 % from the reported species, as calculated by the Kimura-2 parameter of the DNADIST program. In fact, the phylogenetic tree clearly demonstrated that CMS 76r^T and CMS 81y^T are closely related; they formed a robust and separate clade with a bootstrap value of 100 % and clustered with the other five species of *Leifsonia* (Fig. 1) with a bootstrap value of 62 %. Furthermore, the topology of the tree was similar to the one reported previously (Evtushenko *et al.*, 2000).

Fig. 1. UPGMA phenogram showing the phylogenetic relationships between *L. rubra* sp. nov. CMS 76r^T and *L. aurea* sp. nov. CMS 81y^T and other species of the genus *Leifsonia* and related reference microorganisms based on 16S rRNA gene sequence analysis. Bootstrap values are given at the nodes. Branch lengths are not to scale. A version of the phenogram including a wider selection of reference taxa is available as supplementary material in IJSEM Online.

However, although strains CMS 76r^T and CMS 81y^T show 97.5 % relatedness at the 16S rRNA gene level, they probably represent two distinct species, since the overall genome relatedness between the two, based on DNA–DNA hybridization performed using the membrane filter method of Tourova & Antonov (1987) as described previously (Reddy *et al.*, 2002a), was only 30 %.

Chemotaxonomic studies

The methods used for the identification of fatty acids (Sato & Murata, 1988), quinones (Collins *et al.*, 1977; Dunphy *et al.*, 1971), polar lipids (Komagata & Suzuki, 1987), peptidoglycan (Rosenthal & Dziarski, 1994; Schleifer & Kandler, 1972) and cell-wall sugars (Komagata & Suzuki, 1987) and determination of the DNA G + C content were described previously (Reddy *et al.*, 2002b). For fatty acid analysis, strains CMS 76r^T, CMS 81y^T and *L. aquatica* ATCC 14665^T were cultured in CB liquid medium (Zgurskaya *et al.*, 1993) at 25 °C.

The phylogenetic affiliation of strains CMS 76r^T and CMS 81y^T to the genus *Leifsonia* is supported further by their chemotaxonomic properties, such as the presence of glycine, glutamic acid, 2,4-diaminobutyric acid and alanine in the cell-wall peptidoglycan, MK-11 as the major menaquinone, phosphatidylglycerol and diphosphatidylglycerol as the principal phospholipids and anteiso-C_{15:0}, anteiso-C_{17:0} and iso-C_{16:0} as the predominant fatty acids and the DNA G + C content of 64–66 mol%.

Conclusions

Strains CMS 76r^T and CMS 81y^T conform to the description of the genus *Leifsonia* in that they are Gram-positive, curved rods, non-motile and catalase-positive, contain 2,4-diaminobutyric acid in the peptidoglycan, contain menaquinone MK-11, phosphatidylglycerol and diphosphatidylglycerol, have a high content of anteiso- and iso-branched fatty acids and have a DNA G + C content of 64–66 mol%. In addition, CMS 76r^T is closely related to CMS 81y^T with respect to many phenotypic and chemotaxonomic characteristics (Tables 1 and 2). However, it differed from CMS 81y^T in that it was red-pigmented, did not grow at temperatures above 22 °C, gave a negative Voges–Proskauer test, did not utilize arginine or erythritol as sole carbon sources and contained rhamnose as an additional cell-wall sugar (Table 1). Furthermore, CMS 76r^T had higher proportions of C_{15:0} (3.6 %), iso-C_{15:0} (7.8 %) and iso-C_{16:0} (11.5 %) and lower levels of anteiso-C_{17:0} (20.3 %) and iso-C_{17:0} (1.8 %) (Table 2). In addition, there was a difference of 2.5 % at the 16S rDNA sequence level between the two isolates and they were related by only 30 % at the whole-genome level, as evidenced by DNA–DNA relatedness studies. These characteristics clearly establish that CMS 76r^T and CMS 81y^T represent two separate species.

Strain CMS 76r^T is different from all the other reported

species of the genus *Leifsonia* (including CMS 81y^T) in that it is red-pigmented and psychrophilic (with a growth temperature of 0–22 °C). Furthermore, it had higher proportions of C_{15:0} and iso-C_{15:0} compared with CMS 81y^T, *L. aquatica* and *L. poae*, which were analysed following growth in CB liquid medium at 25 °C. Data for *L. aquatica* ATCC 14665^T were acquired in the present study, whereas data for *L. poae* were from Evtushenko *et al.* (2000). The fatty acids C_{14:0}, C_{15:0}, C_{18:0}, C_{15:1} and C_{18:1} were absent from *L. xyli* subsp. *cynodontis*, *L. naganoensis* and *L. shinshuensis* (Suzuki *et al.*, 1999). In addition, CMS 76r^T showed a number of distinct differences from the reported species of *Leifsonia*, as listed in Table 1. Furthermore, at the 16S rRNA gene sequence level, CMS 76r^T differed from *L. aquatica*, *L. poae*, *L. xyli* subsp. *xyli*, *L. xyli* subsp. *cynodontis*, *L. naganoensis* and *L. shinshuensis* by 4.2, 3.8, 4.6, 4.5, 4.1 and 4.9 %, respectively. Thus, on the basis of differences in phenotypic traits (Table 1), distinct fatty acid types (Table 2) and the > 3 % difference at the 16S rRNA gene sequence level (except for CMS 81y^T, for which the value was ~2.5 %), CMS 76r^T has been assigned the status of a novel species, for which the name *L. rubra* sp. nov. is proposed. It is worthwhile to mention that strains with more than a 3 % difference at the 16S rDNA level are unlikely to have a DNA–DNA relatedness of more than 70 % (Stackebrandt & Goebel, 1994).

Strain CMS 81y^T can also be differentiated from CMS 76r^T and *L. aquatica*, *L. poae*, *L. xyli* subsp. *xyli*, *L. xyli* subsp. *cynodontis*, *L. naganoensis* and *L. shinshuensis* on the basis of phenotypic traits and chemotaxonomic properties (Tables 1 and 2). In addition, its 16S rRNA gene sequence differs from those of *L. poae*, *L. aquatica*, *L. xyli* subsp. *xyli*, *L. xyli* subsp. *cynodontis*, *L. naganoensis* and *L. shinshuensis* by 3.9, 4.3, 4.6, 4.2, 4.9 and 4.8 % (but by only 2.5 % from that of CMS 76r^T). Therefore, CMS 81y^T has also been assigned the status of a novel species, for which the name *L. aurea* sp. nov. is proposed.

Despite the fact that strains CMS 76r^T and CMS 81y^T formed a coherent cluster with the clade of *Leifsonia* species, a high level of dissimilarity was observed at the 16S rDNA sequence level. The reason for this is that, when 1407 bp (positions 110–1517 according to *Escherichia coli* numbering) of the 16S rRNA gene sequences of all species of the genus *Leifsonia* were compared, a total of 115 nucleotide changes were observed, spanning the entire region (details available as supplementary material in IJSEM Online). Contributing to this change is an insertion of 14 bp from positions 461 to 474 observed only in CMS 76r^T and CMS 81y^T. In addition, two variable regions were observed, spanning positions 148 to 226 (around 31 variable positions) and positions 613 to 631 (7 variable positions). The significance of these insertions or variable regions is not clear, but the occurrence of the insertion in only the psychrophilic species may prove useful as a signature for these isolates; this awaits clarification in future studies.

Table 1. Characteristics that differentiate strains CMS 76r^T and CMS 81y^T from each other and from reported species of *Leifsonia*

Strains/species: 1, CMS 76r^T; 2, CMS 81y^T; 3, *L. aquatica* (data from Evtushenko *et al.*, 2000); 4, *L. poae* (Evtushenko *et al.*, 2000); 5, *L. xyli* subsp. *xyli* (Davis *et al.*, 1984); 6, *L. xyli* subsp. *cynodontis* (Davis *et al.*, 1984); 7, *L. shinshuensis* (Suzuki *et al.*, 1999); 8, *L. naganoensis* (Suzuki *et al.*, 1999). +, Positive; w+, weakly positive; –, negative; s, sensitive; R, resistant; NA, not available; ND, not determined.

Characteristic	1	2	3	4	5	6	7	8
Colony colour	Red	Yellow	Yellow	Yellow	White	Yellow	White	White
Motility	–	–	+	+	–	–	–	–
Growth temperature (°C)	0–22	0–30	7–37	7–37	7–31	7–35	7–42	7–37
Oxidase, H ₂ S production	–	–	+	–	–	–	NA	NA
Gelatinase, aesculin hydrolysis	–	–	–	+	–	–	NA	NA
Starch hydrolysis	–	–	+	–	–	+	+	+
Methyl red test	–	–	–	+	–	+	NA	NA
Voges–Proskauer test	–	+	–	+	–	+	NA	NA
Major menaquinone(s)	MK-11	MK-11	MK-11, MK-10	MK-11	ND	MK-11, MK-12	MK-11, MK-12	MK-11, MK-10
Cell-wall sugars*	Gal, Glc, Rib, Rha	Gal, Glc, Rib	Gal, Glc, Rib, Rha, Fuc	Gal, Glc, Rib, Rha	Gal, Rha, Fuc	Gal, Rha, Fuc	ND	ND
Production of acid from:								
L-Arabinose	–	–	–	+	–	–	ND	ND
D-Fructose	–	–	+	+	–	–	+	+
D-Galactose, sucrose	–	–	+	+	–	–	–	+
D-Glucose	–	–	+	–	+	+	–	+
D-Maltose	–	–	–	–	+	+	–	+
D-Mannose	–	–	+	+	–	+	–	+
G + C content of DNA (mol%)	66	64	70	NA	66	69	71	71
Utilization of carbon compounds:								
L-Alanine	–	–	–	–	NA	–	+	W+
L-Arabinose	–	–	–	+	NA	–	NA	NA
Adonitol	–	–	+	–	NA	NA	NA	NA
D-Cellobiose, lactose	–	–	+	+	NA	–	–	+
Dextran	–	–	+	+	NA	NA	W+	+
Dulcitol	–	–	+	+	NA	–	NA	NA
meso-Erythritol	–	+	–	–	NA	NA	NA	NA
D-Fructose	+	+	+	+	NA	W+	+	+
D-Galactose	+	+	+	+	NA	–	W+	+
D-Glucose	+	+	+	+	NA	W+	–	+
meso-Inositol	–	–	+	–	NA	–	+	+
D-Maltose	–	–	+	+	NA	–	W+	+
D-Mannitol	–	–	+	+	NA	W+	+	+
D-Mannose	+	+	+	+	NA	W+	W+	+
D-Melibiose	–	–	+	+	NA	–	W+	W+

Table 1. cont.

Characteristic	1	2	3	4	5	6	7	8
Melezitol	—	—	+	—	NA	—	W+	W+
Pyruvate	+	+	+	W+	NA	W+	W+	W+
D-Raffinose	—	—	—	+	NA	NA	NA	NA
D-Rhamnose	—	—	W+	—	NA	—	W+	—
D-Ribose	+	+	—	—	NA	—	W+	W+
Sodium citrate	—	—	NA	ND	—	NA	NA	NA
Sodium acetate	+	+	NA	ND	—	—	NA	NA
Sodium succinate	—	—	NA	ND	NA	—	NA	NA
D-Sorbitol	—	—	W+	—	NA	—	W+	W+
D-Sorbose	+	+	+	—	NA	NA	NA	NA
Sucrose	—	—	+	+	NA	—	+	+
Trehalose	+	+	+	—	NA	—	W+	W+
D-Xylose	—	—	+	+	NA	—	W+	W+
L-Arginine	—	+	NA	NA	NA	—	W+	W+
Sensitivity to antibiotics:								
Penicillin	R	S	R	R	NA	NA	NA	NA
Streptomycin, ampicillin, tetracycline	S	S	R	R	NA	NA	NA	NA
Erythromycin	S	R	NA	NA	NA	NA	NA	NA
Lincomycin	S	R	R	R	NA	NA	NA	NA
Rifampicin	S	S	S	R	NA	NA	NA	NA

*Fuc, Fucose; Gal, galactose; Glc, glucose; Rha, rhamnose; Rib, ribose.

Table 2. Fatty acid composition of strains CMS 76r^T and CMS 81y^T and related species

Strains/species: 1, CMS 76r^T; 2, CMS 81r^T; 3, *L. aquatica* ATCC 14665^T; 4, *L. poae* (data from Evtushenko *et al.*, 2000); 5, *L. xyli* subsp. *cynodontis* (Suzuki *et al.*, 1999); 6, *L. naganoensis* (Suzuki *et al.*, 1999); 7, *L. shinshuensis* (Suzuki *et al.*, 1999). CMS 76r^T, CMS 81y^T and *L. aquatica* ATCC 14665^T were grown in CB liquid medium at 25 °C and analysed for fatty acid methyl esters in the present study. —, Absent.

Fatty acid	1	2	3	4	5	6	7
Saturated fatty acids							
C _{14:0}	1.3	0.2	0.3	—	—	—	—
C _{15:0}	3.6	1.6	0.5	<1.0	—	—	—
C _{16:0}	1.8	1.4	0.5	<1.0	1.6	3.7	<0.5
C _{18:0}	3.7	4.3	—	<1.0	—	—	—
Unsaturated fatty acids							
C _{15:1}	—	—	—	<1.0	—	—	—
C _{18:1}	5.0	4.1	—	<1.0	—	—	—
Branched-chain fatty acids							
iso-C _{14:0}	0.5	0.2	0.02	—	<0.5	<0.5	<0.5
iso-C _{15:0}	7.8	3.3	0.8	1.0	0.5	1.3	0.7
anteiso-C _{15:0}	37.5	43.7	29.8	36.3	34.1	42.1	47.3
iso-C _{16:0}	11.5	5.0	13.2	15.6	10.0	18.5	18.3
iso-C _{17:0}	1.8	6.3	0.5	<1.0	<0.5	<0.5	<0.5
anteiso-C _{17:0}	20.3	28.2	54.4	45.4	52.7	32.8	32.6

Description of *Leifsonia rubra* sp. nov.

Leifsonia rubra (rub'ra. L. fem. adj. *rubra* reddish, referring to the reddish pigment that the bacterium produces).

Colonies are circular, convex, smooth, butyrous, opaque, red-pigmented and 1–2 mm in diameter. Cells are aerobic, Gram-positive, non-motile, curved rods. The pigment is insoluble in water but soluble in methanol and exhibits characteristic peaks at 420, 443, 490 and 520 nm in methanol. Grows between 0 and 22 °C and between pH 6 and 12 and tolerates up to 3 % NaCl. Optimum growth is observed at 15 °C, pH 7 and in the absence of NaCl. Positive for catalase and β -galactosidase and reduces nitrate to nitrite. Negative for oxidase, urease, lipase, gelatinase, phosphatase and indole, gives negative results in methyl red and Voges–Proskauer tests, is negative for H₂S production and does not hydrolyse aesculin or starch. Does not produce acid from L-arabinose, D-fructose, D-galactose, glucosamine, lactose, D-maltose, D-mannose, L-rhamnose, sucrose or D-xylose. Utilizes D-fructose, D-glucose, D-galactose, glucosamine, glycerol, D-mannose, pyruvate, L-rhamnose, D-ribose, sodium acetate, D-sorbose, trehalose, L-glutamic acid and L-phenylalanine as sole carbon sources, and fails to utilize L-arabinose, adonitol, ammonium acetate, D-cellobiose, cellulose, creatinine, dextran, dulcitol, *meso*-erythritol, glycogen, β -hydroxybutyric acid, inositol, inulin, lactose, lactic acid, D-maltose, D-mannitol, D-melibiose, melezitol, myristic acid, polyethylene glycol, potassium hydrogen phthalate, D-raffinose, sodium citrate, sodium succinate, sorbitol, sucrose, D-xylose, L-alanine, L-arginine, L-glycine, L-glutamine, L-lysine, L-methionine, L-serine, L-tyrosine and L-tryptophan. Sensitive to novobiocin, cefaperazone, cefazoline, kanamycin, amoxycillin,

tobramycin, roxithromycin, vancomycin, cephalothime, streptomycin, erythromycin, amikacin, ampicillin, lincomycin, tetracycline and rifampicin and resistant to penicillin, colistin, cefuroxime, nitrofurantoin, ciprofloxacin, cotrimoxazole, norfloxacin and chloramphenicol. The peptidoglycan contains DL-diaminobutyric acid as the diamino acid, along with glycine, glutamic acid and alanine. The cell-wall sugars are galactose, glucose, ribose and rhamnose. The major menaquinone is MK-11. Fatty acid methyl esters are listed in Table 2. The G + C content of the DNA of the type strain is 66 mol%.

The type strain is strain CMS 76r^T (=MTCC 4210^T = DSM 15304^T = CIP 107783^T).

Description of *Leifsonia aurea* sp. nov.

Leifsonia aurea (au're.a. L. fem. adj. *aurea* golden, referring to the yellowish pigment that the bacterium produces).

Colonies are yellowish, circular, convex, smooth, butyrous, opaque, yellow-pigmented and 1–2 mm in diameter. Cells are aerobic, Gram-positive, curved, non-motile rods. The pigment is insoluble in water but soluble in methanol and exhibits four characteristic peaks, at 390, 414, 438 and 468 nm. Grows between 0 and 30 °C and between pH 6 and 11 and tolerates up to 3 % NaCl. Optimum growth occurs at 22 °C, pH 7 and in the absence of salt. Positive for catalase and β -galactosidase, gives a positive result in the Voges–Proskauer test and reduces nitrate to nitrite. Negative for oxidase, urease, lipase, gelatinase, phosphatase and indole, gives a negative result in the methyl red test, is negative for H₂S production and does not hydrolyse aesculin or starch. Does not produce acid from L-arabinose,

D-fructose, D-galactose, D-glucose, lactose, D-maltose, D-mannose, L-rhamnose, sucrose or D-xylose. Does not grow on minimal medium containing L-arabinose, adonitol, ammonium acetate, D-cellobiose, cellulose, creatinine, dextran, dulcitol, glycogen, β -hydroxybutyric acid, inositol, inulin, lactose, lactic acid, D-maltose, D-mannitol, D-melibiose, melezitol, myristic acid, polyethylene glycol, potassium hydrogen phthalate, D-raffinose, sodium citrate, sodium succinate, sorbitol, sucrose, D-xylose, L-alanine, L-glycine, L-glutamine, L-lysine, L-methionine, L-serine, L-tyrosine or L-tryptophan. Can grow on erythritol, D-fructose, D-glucose, D-galactose, glucosamine, glycerol, D-mannose, pyruvate, L-rhamnose, D-ribose, sodium acetate, D-sorbose, trehalose, L-arginine, L-glutamic acid and L-phenylalanine as sole carbon sources. Resistant to colistin, nitrofurantoin, cotrimoxazole, erythromycin, norfloxacin, lincomycin and chloramphenicol and sensitive to novobiocin, cefaperazone, cefazoline, penicillin, kanamycin, cefuroxime, amoxycillin, ciprofloxacin, tobramycin, roxithromycin, vancomycin, cephatoxime, streptomycin, amikacin, ampicillin, tetracycline and rifampicin. The peptidoglycan contains DL-diaminobutyric acid as the diamino acid, along with glycine, glutamic acid and alanine, and the cell-wall sugars are galactose, glucose and ribose. The major menaquinone is MK-11. Fatty acid methyl esters are listed in Table 2. The G+C content of the DNA of the type strain is 64 mol%.

The type strain is strain CMS 81y^T (=MTCC 4657^T=DSM 15303^T=CIP 107785^T).

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