Yimella lutea gen. nov., sp. nov., a novel actinobacterium of the family *Dermacoccaceae*

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A Gram-stain-positive, coccoid, non-motile, halotolerant actinobacterium, designated YIM 45900^T, was found as a contaminant on an agar plate in the laboratory of Yunnan Institute of Microbiology, China. The peptidoglycan type was A4 α with an L-Lys–L-Ser–D-Asp interpeptide bridge. The cell-wall sugars contained galactose and fucose. The predominant menaquinone was MK-8(H₄). The major fatty acids were iso-C_{15:0}, anteiso-C_{15:0} and anteiso-C_{17:0}. The polar lipids contained diphosphatidylglycerol, phosphatidylinositol, a glucosamine-containing phospholipid and an unknown phospholipid. The DNA G + C content was 65.8 mol%. Phylogenetic analysis based on 16S rRNA gene sequences revealed that the organism falls within the radius of the suborder *Micrococcineae* and its closest phylogenetic neighbours are the genera of the family *Dermacoccaceae*. Strain YIM 45900^T showed 16S rRNA gene sequences similarity values of 93.1–95.9% with members of the genera *Dermacoccus*, *Demetria* and *Kytococcus*. On the basis of the phylogenetic and phenotypic characteristics of the actinobacterium, a novel genus and species, *Yimella lutea* gen. nov., sp. nov., are proposed. The type strain of *Yimella lutea* is YIM 45900^T (=DSM 19828^T =KCTC 19231^T =CCTCC AB 207007^T).

The family *Dermacoccaceae* was first proposed by Stackebrandt & Schumann (2000) and its pattern of 16S rRNA signature nucleotides was emended by Zhi *et al.* (2009). At the time of writing, the family *Dermacoccaceae* comprises the genera *Dermacoccus* (Stackebrandt *et al.*, 1995), *Demetria* (Groth *et al.*, 1997) and *Kytococcus* (Stackebrandt *et al.*, 1995). Most strains have been isolated from human skin. They are Gram-positive, aerobic, non-encapsulated, non-endospore-forming, catalase-positive, non-halophilic, coccoid actinobacteria and the peptidoglycan type is variation A4 α .

Strain YIM 45900^T, a Gram-stain-positive, coccoid, non-motile, halotolerant actinobacterium, was found in the laboratory of Yunnan Institute of Microbiology, China, as a contaminant on a modified ISP medium 5 (Shirling &

Gottlieb, 1966) agar plate (containing l⁻¹ distilled water: 1 g L-asparagine, 10 g glycerol, 5 g yeast extract, 1 g K₂HPO₄, 5 g KNO₃, 50 g NaCl, 15 g agar; final pH 7.5). NaCl was sterilized separately before being added to the medium. Strain YIM 45900^T was maintained on ISP 2 (Shirling & Gottlieb, 1966) agar slants at 4 °C and in 20% (w/v) glycerol suspensions at –20 °C. Biomass for chemical and molecular studies was obtained by cultivation in shaken flasks (about 150 r.p.m.) using ISP 2 broth (pH 7.5) at 28 °C for about 1 week.

Cell morphology was determined on cultures grown for 6, 12, 24, 48 and 72 h on ISP 2 agar medium at 28 °C. Gram staining was carried out by the standard Gram reaction and was confirmed by using KOH lysis (Cerny, 1978). Spore formation was determined by staining with malachite green. Cell motility was confirmed by the development of turbidity throughout a tube containing semisolid medium (Leifson, 1960). Morphological characteristics of the strain were observed by light microscopy (model BH 2; Olympus)

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and by transmission electron microscopy (model H-800; Hitachi). The growth temperature was tested at 4–55 °C at intervals of 2 °C on ISP medium 2. For NaCl tolerance experiments, ISP medium 2 was used as the basal medium and salt concentrations ranging from 0 to 20% (w/v) at intervals of 1% were tested. The pH growth range was investigated between pH 4.0 and 10.0 at intervals of 1 pH unit, using the following buffer systems: pH 4–5 0.1 M citric acid/0.1 M sodium citrate; pH 6–8 0.1 M KH_2PO_4 /0.1 M NaOH; pH 9–10 0.1 M NaHCO_3 /0.1 M Na_2CO_3 . Catalase activity was determined by production of bubbles after the addition of a drop of 3% H_2O_2 . Oxidase activity was observed by oxidation of tetramethyl-*p*-phenylenediamine. Methyl red and Voges–Proskauer tests and tests for melanin production and milk peptonization and hydrolysis of aesculin, casein, cellulose, chitin, dextrin, DNA, gelatin, starch, urea and Tweens 20, 40, 60 and 80 were performed as described by Cowan & Steel (1965). Oxidation of different substrates was tested using GP2 MicroPlates (Biolog). Enzyme activities and acid production from carbohydrates were determined by using the API 20E, API ZYM and API 50CH systems (bioMérieux) according to the manufacturer's instructions. Anaerobic growth was determined using the GasPak Anaerobic System (BBL) according to the manufacturer's instructions. The morphological, cultural and physiological properties of strain YIM 45900^T are given in the genus and species descriptions. The organism can be distinguished from members of the family *Dermacoccaceae* using a range of phenotypic properties (Table 1).

The peptidoglycan structure of the cell wall of strain YIM 45900^T was determined by using established procedures (Schleifer & Kandler, 1972; Schleifer, 1985; MacKenzie, 1987). Sugar analysis of the purified cell wall followed procedures described by Stanek & Roberts (1974). Polar lipids were extracted, examined by two-dimensional TLC and identified using described procedures (Minnikin *et al.*, 1984). Menaquinones were isolated according to Minnikin *et al.* (1984) and separated by HPLC (Kroppenstedt, 1982). For fatty acid analyses, cells of strain YIM 45900^T were cultured on tryptic soy agar (Difco) at 28 °C for 4 days. Cellular fatty acids analysis was performed as described by Sasser (1990) using the Microbial Identification System (MIDI).

The peptidoglycan of strain YIM 45900^T contained Ala, Gly, Ser, Asp, Glu and Lys in a molar ratio of 1.6:1.0:0.7:0.8:1.0:1.2. The peptide Lys–Ser, which is stable under hydrolytic conditions in 4 M HCl at 100 °C for 16 h, was detected. Aspartic acid was revealed by dinitrophenylation to represent the N terminus of the interpeptide bridge. From these data, it was concluded that strain YIM 45900^T contained the peptidoglycan type A4 α with a L-Lys–L-Ser–D-Asp interpeptide bridge and a glycine residue bound to the α -carboxyl group of D-glutamic acid at position 2 of the peptide subunit. The cell-wall sugars were galactose and fucose (with a minor amount of mannose and traces of glucose). The polar lipids consisted

of diphosphatidylglycerol, phosphatidylinositol, glucosamine-containing phospholipid and one unknown phospholipid. The predominant menaquinone was MK-8(H_4) (97.2%); a minor amount of MK-8(H_6) (2.8%) was detected. Strain YIM 45900^T had a cellular fatty acid profile that contained major amounts (>10%) of branched fatty acids, iso- $\text{C}_{15:0}$ (35.2%), anteiso- $\text{C}_{15:0}$ (12.1%) and anteiso- $\text{C}_{17:0}$ (11.1%), and minor amounts of branched, straight-chain, unsaturated and methyl fatty acids, iso- $\text{C}_{13:0}$ (4.2%), anteiso- $\text{C}_{13:0}$ (2.4%), iso- $\text{C}_{14:0}$ (0.5%), $\text{C}_{14:0}$ (3.0%), $\text{C}_{15:0}$ (1.0%), iso- $\text{C}_{16:1}$ H (0.5%), iso- $\text{C}_{16:0}$ (4.8%), $\text{C}_{16:0}$ *cis*-9 (3.3%), $\text{C}_{16:0}$ (5.7%), $\text{C}_{16:0}$ 9-methyl (2.0%), anteiso- $\text{C}_{17:1}$ C (0.8%), iso- $\text{C}_{17:0}$ (5.5%), $\text{C}_{17:1}$ *cis*-9 (1.0%), $\text{C}_{17:0}$ (0.5%), $\text{C}_{18:1}$ *cis*-9 (2.0%) and $\text{C}_{18:1}$ 2-OH (1.6%).

Extraction of genomic DNA and PCR amplification of the 16S rRNA gene sequence were performed as described by Li *et al.* (2007). Multiple alignments with sequences from the most closely related members of the suborder *Micrococcineae* and calculations of levels of sequence similarity were carried out using the EzTaxon server 2.0 (Chun *et al.*, 2007). Phylogenetic analyses were performed using three tree-making algorithms, the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) methods. A phylogenetic tree was constructed using the neighbour-joining method from K_{nuc} values (Kimura, 1980) using MEGA version 4.0 (Tamura *et al.*, 2007). The topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 resamplings. The genomic DNA of strain YIM 45900^T for the determination of G+C content was prepared according to the method of Marmur (1961). The G+C content of the DNA was determined by reversed-phase HPLC of nucleosides according to Mesbah *et al.* (1989).

An almost-complete 16S rRNA gene sequence (1446 bp) was determined for strain YIM 45900^T. Comparative 16S rRNA gene sequence analysis showed that strain YIM 45900^T fell within the radius of the suborder *Micrococcineae* (Fig. 1). Its closest phylogenetic neighbours were members of the genera of the family *Dermacoccaceae*. Strain YIM 45900^T showed 16S rRNA gene sequence similarities of 95.9, 95.9, 95.7, 95.6, 94.0, 93.5 and 93.1% to the type strains of *Dermacoccus profundus*, *Dermacoccus barathri*, *Dermacoccus abyssi*, *Dermacoccus nishinomiyaensis*, *Demetria terrigena*, *Kytococcus sedentarius* and *Kytococcus schroeteri*, respectively. This relationship was supported by all three tree-making methods (data not shown). The G+C content of the DNA was 65.8 mol%.

Strain YIM 45900^T was similar to members of the genera of the family *Dermacoccaceae* (*Dermacoccus*, *Kytococcus* and *Demetria*), which have lysine as the diagnostic diamino acid, and different from members of the genera of the family *Dermabacteraceae* (*Dermabacter* and *Brachybacterium*) and *Dermatophilaceae* (*Dermatophilus* and *Kineosphaera*), which have meso-A₂pm as the diagnostic

Table 1. Differential phenotypic and chemotaxonomic characteristics of the genus *Yimella* gen. nov. and related genera of the family *Dermacoccaceae*

Strains: 1, *Yimella lutea* gen. nov., sp. nov. YIM 45900^T (data from this study); 2, *Demetria terragena* HKI 0089^T (Groth *et al.*, 1997); 3, *Dermacoccus nishinomiyaensis* DSM 20448^T (Stackebrandt *et al.*, 1995); 4, *Kytococcus sedentarius* DSM 20547^T (Stackebrandt *et al.*, 1995). +, Positive; v, variable; –, negative; ND, no data available.

Characteristic	1	2	3	4
Cell/colony characteristics				
Cell size (µm)	0.7–0.9 × 1.3–1.8	0.8 × 1.2 or 0.8 × 3.0	0.9 × 1.6	0.8 × 1.1
Cell morphology	Coccoid	Coccoid or rods	Coccoid	Coccoid
Colony pigmentation	Orange	White to pale yellow	Bright orange	Cream–white or deep yellow
Water-soluble pigment	–	ND	Orange	Brownish
Conditions for growth				
NaCl range (% w/v)	0–8	0–12	0–7	0–10
pH range	5.0–9.0	ND	5.4–6.9	ND
Facultatively anaerobic	+	–	–	–
Methyl red test	+	–	–	ND
Oxidase test	–	–	+	–
Nitrate reduction	+	–	v	v
Hydrolysis of:				
Aesculin	+	–	–	–
Gelatin	+	–	+	+
Starch	–	+	v	–
Tween 80	+	+	v	–
Urea	–	–	+	–
Acid production from:				
D-Fructose	+	+	ND	–
D-Galactose	–	+	ND	–
D-Glucose	+	+	ND	–
Maltose	+	–	ND	–
Trehalose	+	–	ND	ND
Sucrose	+	–	ND	–
D-Xylose	–	–	ND	–
Cell-wall sugar(s)	Galactose, fucose	Galactose	Galactose	ND
Interpeptide bridge (A4z)	L-Lys–L-Ser–D-Asp	L-Lys–L-Ser–D-Asp	L-Lys–L-Ser _{1–2} –D-Glu or L-Lys–L-Ser _{1–2} –L-Ala–D-Glu	L-Lys–Glu ₂
Predominant menaquinone(s)	MK-8(H ₄)	MK-8(H ₄)	MK-8(H ₂)	MK-8, MK-9, MK-10
Polar lipids*	DPG, PI, GlcN-PL, PL	DPG, PG, PE, PI, PL	DPG, PG, PI	DPG, PG, PI
Major fatty acids (>10 %)	i-C _{15:0} , ai-C _{15:0} , ai-C _{17:0}	C _{18:1} , C _{17:0} , C _{18:0}	i-C _{17:0} , ai-C _{17:0} , i-C _{17:1} , i-C _{15:0}	ai-C _{17:0} , C _{17:0} , C _{15:0} , i-C _{17:1}
DNA G + C content (mol%)	65.8	66	66–71†	68–69†

*DPG, Diphosphatidylglycerol; GlcN-PL, glucosamine-containing phospholipid; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PL, unknown phospholipid.

†Range of values for several strains, including the type strain.

diamino acid. In the phylogenetic tree based on the neighbour-joining algorithm, all of the type strains of the family *Dermacoccaceae* together with strain YIM 45900^T clustered in a distinct clade that was strongly supported by a bootstrap value of 60 %. All of the above data confirmed that the isolate should be assigned to the family *Dermacoccaceae*. However, strain YIM 45900^T formed a monophyletic branch within the radiation occupied by the three genera of the family *Dermacoccaceae*. Moreover, strain YIM 45900^T could be differentiated from the genera *Dermacoccus* and *Kytococcus* in some chemotaxonomic

properties (Table 1), i.e. strain YIM 45900^T had L-Lys–L-Ser–D-Asp as the interpeptide bridge of the peptidoglycan and MK-8(H₄) as the predominant menaquinone, whereas the members of genus *Dermacoccus* show L-Lys–L-Ser_{1–2}–D-Glu or L-Lys–L-Ser_{1–2}–L-Ala–D-Glu as the interpeptide bridge and MK-8(H₂) as the predominant menaquinone and the genus *Kytococcus* contains L-Lys–Glu₂ as the interpeptide bridge and MK-8, MK-9 and MK-10 as the predominant menaquinones. Although strain YIM 45900^T was similar to the genus *Demetria* in containing L-Lys–L-Ser–D-Asp as the interpeptide bridge and MK-8(H₄) as the

Fig. 1. Phylogenetic dendrogram obtained by distance-matrix analysis of 16S rRNA gene sequences, showing the position of strain YIM 45900^T and its phylogenetic neighbours. Bootstrap values ($\geq 50\%$) based on 1000 resamplings are shown at branch nodes. The sequence of *Bifidobacterium gallicum* JCM 8224^T was used as an outgroup. Bar, 1% sequence divergence.

predominant menaquinone, it could be differentiated from the genus *Demetria* by the occurrence of a glycine residue bound to the α -carboxyl group of D-glutamic acid of the peptide subunit and by the polar lipid pattern, as strain YIM 45900^T contained a glucosamine-containing phospholipid but no phosphatidylethanolamine. In particular, strain YIM 45900^T was quite different from the genus *Demetria* in its fatty acid profile, i.e. strain YIM 45900^T contained major amounts of branched fatty acids (iso-C_{15:0}, anteiso-C_{15:0} and anteiso-C_{17:0}) whereas the genus *Demetria* contained major amounts of straight and unsaturated fatty acids (C_{17:0}, C_{18:0} and C_{18:1}). Therefore, on the basis of chemotaxonomic and phylogenetic differentiation of the isolate from its closest neighbours in the family Dermacoccaceae, we propose that strain YIM 45900^T represents a novel species in a new genus, *Yimella lutea* gen. nov., sp. nov.

Description of the genus *Yimella* gen. nov.

Yimella (Yi.mel'la. L. fem. dim. suff. -ella; N.L. fem. dim. n. *Yimella* arbitrary name formed from the acronym of Yunnan Institute of Microbiology, YIM, where the first taxonomic studies of this taxon were performed).

Cells are Gram-stain-positive, coccoid, non-encapsulated, halotolerant, aerobic and facultatively anaerobic in the presence of KNO₃. They do not form endospores. The peptidoglycan is of the type A4 α with a L-Lys-L-Ser-D-Asp interpeptide bridge and a glycine residue bound to the α -carboxyl group of D-glutamic acid at position 2 of the peptide subunit. The cell-wall sugars contain galactose and fucose. The predominant menaquinone is MK-8(H₄). The polar lipids are diphosphatidylglycerol, phosphatidylinositol, a glucosamine-containing phospholipid and an unknown

phospholipid. The major fatty acids are iso-C_{15:0}, anteiso-C_{15:0} and anteiso-C_{17:0}. The G+C content of the DNA is about 65–66 mol%. The type species is *Yimella lutea*.

Description of *Yimella lutea* sp. nov.

Yimella lutea (lu.te'a. L. fem. adj. *lutea* orange-coloured).

Cells are approximately spherical (0.7–0.9 $\times$ 1.3–1.8 μ m) on ISP 2 medium, non-motile and arranged in irregular clusters. The orange colonies on ISP 2 agar are circular, smooth, opaque and non-pigmented. Temperature, pH and NaCl tolerance ranges for growth are 22–42 °C, pH 5.0–9.0 and 0–8% and optimal growth occurs at 28 °C, pH 6.0–7.0 and 0–1%. Catalase and methyl red tests are positive. Oxidase and Voges-Proskauer tests are negative. Gelatin, cellulose and Tweens 20, 40, 60 and 80 are hydrolysed, but casein, dextrin, starch, DNA, chitin and urea are not hydrolysed. Nitrate reduction and melanin production are positive. Milk peptonization and coagulation, H₂S and indole production, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase are negative. With the API ZYM system, positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, naphthol-AS-BI-phosphohydrolase, β -glucuronidase and α -glucosidase, but negative for lipase (C14), α - and β -galactosidases, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase. With Biolog GP2 MicroPlates, oxidizes Tweens 40 and 80, D-fructose, trehalose, α -D-glucose, D-mannose, sucrose, maltose, D-xylose, acetic acid, D-ribose, propionic acid, pyruvic acid, glycerol, uridine, D-tagatose, turanose, L-arginine, glycine, L-histidine and xanthine. Acid is pro-

duced from aesculin, D-fructose, D-glucose, glycerol, D-mannose, maltose, sucrose, D-tagatose, trehalose, turanose and potassium 5-ketogluconate, but not from N-acetylglucosamine, D-adonitol, amygdalin, DL-arabitol, arbutin, DL-arabinose, cellobiose, dulcitol, erythritol, DL-fucose, D-galactose, gentiobiose, glycogen, inositol, inulin, lactose, D-lyxose, D-mannitol, melezitose, melibiose, D-raffinose, L-rhamnose, D-ribose, salicin, D-sorbitol, L-sorbose, starch, DL-xylose, methyl β -D-xylopyranoside, methyl α -D-mannopyranoside, methyl α -D-glucopyranoside, xylitol, potassium gluconate or potassium 2-ketogluconate. The DNA G+C content of the type strain is 65.8 mol%.

The type strain is YIM 45900^T (=DSM 19828^T =KCTC 19231^T =CCTCC AB 207007^T), isolated from a contaminated agar plate.

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