Luteococcus japonicus gen. nov., sp. nov., a New Gram-Positive Coccus with LL-Diaminopimelic Acid in the Cell Wall

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A new gram-positive, nonmotile coccus is described. Strains IFO 12422^T (T = type strain) and IFO 15385 in the Institute for Fermentation, Osaka, culture collection, which were isolated from soil and water, respectively, have the following chemotaxonomic characteristics: menaquinone MK-9(H₄); G+C content of DNA of 67 mol%; and LL-diaminopimelic acid, alanine, glycine, and glutamic acid in a molar ratio of ca. 1:2:1:1 (type A3γ). Mycolic acids are not present. The taxonomic characteristics of these organisms are different from those of previously described gram-positive, high-G+C-content cocci. The partial 16S rRNA sequence indicated that IFO 12422^T represents a distinct line of descent among gram-positive bacteria with a high G+C content. The name *Luteococcus japonicus* gen. nov., sp. nov. is proposed. The type strain is strain IFO 12422.

Gram-positive cocci "*Micrococcus aurantiacus*" IFO 12422 and *Micrococcus* sp. IFO 15385 (equivalent to CCM 2142) were isolated in Japan from soil on Tokara island and from water for brewing "miyamizu," respectively (21), and have been maintained in the culture collections of the Institute for Fermentation, Osaka, and Czech Collection of Microorganisms. *Micrococcus* species contain lysine in their cell walls, but strains IFO 12422 and IFO 15385 contain LL-diaminopimelic acid (LL-DAP) instead of lysine. Therefore, the taxonomic position of these strains has remained uncertain.

The following five genera of gram-positive cocci with a high DNA guanine-plus-cytosine (G+C) content have been described previously: *Micrococcus* (31), *Deinococcus* (2), *Stomatococcus* (1), *Kineococcus* (38), and *Pelczaria* (25). However, gram-positive cocci with a high G+C content and containing LL-DAP have not been known.

In this paper, we describe the characterization and classification of these gram-positive cocci and we propose that they be included in a new genus, *Luteococcus*, as *Luteococcus japonicus*, gen. nov., sp. nov.

MATERIALS AND METHODS

Bacterial strains and culture conditions. Strains IFO 12422^T and IFO 15385 (equivalent to CCM 2142), which were isolated from soil on Tokara island, Japan, and from water for brewing "miyamizu" in Hyogo prefecture, Japan, respectively (21), were obtained from the Institute for Fermentation, Osaka, culture collection. For chemotaxonomic studies, these strains were grown in shake cultures (nutrient broth; Difco, Detroit, Mich.) at 28°C, and cells were harvested in the stationary phase, washed twice with water, and then freeze-dried.

Morphological and cultural characteristics. Cell morphology was examined by using cells grown on nutrient agar (Difco). Samples for scanning with a model JSM 5400 scanning electron microscope (JEOL, Ltd., Tokyo, Japan) were prepared by dehydrating cells through a graded ethanol series and then in a Hitachi model HCP-2 critical point drying apparatus.

Observation of cells by transmission electron microscopy was performed as follows. The cells were fixed in 2.5%

glutaraldehyde and 1% OsO₄, dehydrated through a graded ethanol series, and then embedded in Epon 812. Thin sections were stained with uranyl acetate and lead citrate and examined under an electron microscope, model JEM-1200EX (JEOL).

Phenotypic characterization. Unless otherwise indicated, all tests were carried out at 28°C. Catalase activity was determined by bubbling in a 3% hydrogen peroxide solution. Oxidase activity was determined by the oxidation of 1% tetramethyl-*p*-phenylenediamine on filter paper. Acid production from carbohydrates was studied in media containing 0.5% peptone, 0.25% NaCl, 0.003% bromocresol purple, and each carbohydrate at a concentration of 0.5% (pH 7.2). Nitrate reduction and hydrolysis of starch, gelatin, casein, and esculin were tested by the methods described by Cowan and Steel (9).

Chemical analyses. Preparation of peptidoglycan and analysis of amino acids, sugar, cellular fatty acids, polar lipids, mycolic acids, isoprenoid quinones, and glycolyl residues were carried out by the method described previously (38).

DNA base composition. DNA was obtained by the method of Saito and Miura (29). The G+C content of the DNA was determined by the method of Mesbah et al. (19) after treatment with P₁ nuclease and alkaline phosphatase and by high-performance liquid chromatography (HPLC) using a model LC-6AD apparatus (Shimadzu) equipped with a Cosmosil 5C₁₈-AR column (4.6 by 150 mm; Nacalai Tesque, Inc., Kyoto, Japan).

DNA homology experiment. DNA-DNA homology was studied by the photobiotin-microplate hybridization method of Ezaki et al. (10).

In vitro amplification of rRNA genes. DNA was used for in vitro amplification of the rRNA gene (rRNA) by the PCR technique (28) in combination with a 16S rRNA gene specific primer pair, 5'-AGTTTGATCCTGGCTC OH-3' (identical to positions 10 to 25 in the *Escherichia coli* numbering system [3]) and 5'-AAGGAGGTGATCCAGCC OH-3' (complementary to positions 1541 to 1525), according to the method described previously (34). The amplification was carried out with a *Taq* polymerase kit (The Perkin-Elmer Co.). The cycling parameters were as follows: 2.5 min of preheating at 95°C, 1 min of denaturation at 94°C, 2.5 min of annealing at 58°C and 2.5 min of extension at 72°C. After 30 cycles there was a final incubation at 72°C for 5 min. The length of the amplified fragment was checked by agarose gel electrophoresis (1%, wt/vol).

Sequence determination and analysis. The amplified DNA

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was purified with Suprec-01 (Takara Co., Ltd.) after electrophoresis on agarose gel (1%, wt/vol). The purified DNA was sequenced with a Sequenase kit for ^{35}S -dATP (Amersham International plc) with the primers 5'-AGTTTGATCCTGGC TC OH-3' (identical to positions 10 to 25), 5'-GTGTTACT CACCCGT OH-3' (complementary to positions 123 to 109), 5'-TACGGGAGGCAGCAG-3' (identical to positions 343 to 357), 5'-CTGCTGCCTCCCGTAG OH-3' (complementary to positions 357 to 342), 5'-GTGCCAGCAGCCGCGG OH-3' (identical to positions 515 to 530), 5'-ACCGCGGCTGCTG GC OH-3' (complementary to positions 531 to 517), 5'-TCT ACGCATTTACC OH-3' (complementary to positions 704 to 690), 5'-GTCAATTCCTTTGAGTTT OH-3' (complementary to positions 924 to 907), 5'-AGGGTTGCGCTCGTTG OH-3' (complementary to positions 1115 to 1100), 5'-CCAT TGTAGCACGTGT OH-3' (complementary to positions 1242 to 1227), 5'-ACGGGCGGTGTGTAC OH-3' (complementary to positions 1406 to 1392), and 5'-GGCTACCTTGTTACGA OH-3' (complementary to positions 1510 to 1495). DNA sequences were aligned by the ODEN system (12). Nucleotide substitution rates (K_{nuc} values) were calculated (14), and the phylogenetic tree was constructed by the neighbor-joining method (30). The topology of the trees was evaluated by bootstrap analysis of the sequence data with Clustal V software (11).

Nucleotide sequence accession numbers. The sequences were aligned with published sequences from DDBJ, GenBank, and EMBL under the following accession numbers: M37200 (*Aeromicrobium erythreum*), M23411 (*Arthrobacter globiformis*), M38242 (*Micrococcus luteus*), X53211 (*Nocardioides albus*), X53189 (*Nocardioides fastidiosa*), X53214 (*Nocardioides jensenii*), X53212 (*Nocardioides luteus*), X53221 (*Propionibacterium acidipropionici*), X53218 (*Propionibacterium acnes*), X53217 (*Propionibacterium freudenreichii*), X53220 (*Propionibacterium thoenii*), X72377 (*Sporichthya polymorpha*), X61478 (*Streptomyces griseus*), X53215 (*Terrabacter tumescens*). The sequence of *Nocardioides simplex* was taken from reference 5, and that of *Propionibacterium innocuum* was taken from reference 24.

The nucleotide sequence data of *L. japonicus* will appear in the DDBJ under the accession number D21245.

RESULTS AND DISCUSSION

Morphological, physiological, and biochemical characteristics. Both strains IFO 12422^T and IFO 15385 are gram-positive cocci. The cells are 0.7 to 1.0 μm in diameter, occur singly as well as in pairs and in tetrads (Fig. 1), and are nonmotile. Rod-shaped or filamentous cells were not observed at any stage of growth or when IFO 12422^T was cultured under different growth conditions. Observation of thin sections of the cells in the early-growth phase revealed a cross-wall septum (Fig. 2). The physiological and the biochemical characteristics of strains IFO 12422^T and IFO 15385 are summarized in Table 1. On most solid media, the strains formed smooth, yellow colonies. Both strains had urease and catalase activities but no oxidase activity. They hardly utilized the organic acids studied; they were facultative anaerobes. The temperature range for growth was 12 to 38°C, and the optimum temperature was 26 to 28°C.

Chemotaxonomic characteristics. The cellular fatty acids of IFO 12422^T and IFO 15385 are shown in Table 2. The major cellular fatty acids of strains IFO 12422^T and IFO 15385 were unsaturated, straight-chain acids $\text{C}_{16:1}$, $\text{C}_{17:1}$, and $\text{C}_{18:1}$. The strains also contained 2-hydroxy-iso-octadecanoic acid (2-OH iso-18:0). The major menaquinone of IFO 12422^T and IFO 15385 was MK-9(H_4) (Table 3). The chemotaxonomic charac-

TABLE 1. Morphological and biochemical characteristics of *L. japonicus* IFO 12422^T and IFO 15385

Characteristic	Result for strain:	
	IFO 12422 ^T	IFO 15385
Color of colony	Yellow	Yellow
Morphology (diam)	Coccus (0.7–1.0 μm)	Coccus (0.7–1.0 μm)
Motility	—	—
Growth on nutrient agar	Moderate	Moderate
Gram staining	+	+
Spore formation	—	—
Acid production from:		
D-Ribose	+	+
D-Xylose	—	—
D-Arabinose	—	—
L-Arabinose	+	+
D-Glucose	+	+
D-Galactose	+	+
D-Mannose	+	+
D-Fructose	+	+
L-Rhamnose	—	—
Sucrose	+	+
Maltose	+	+
Trehalose	+	+
Raffinose	+	+
Glycerol	+	+
Mannitol	+	+
Inositol	+	+
Assimilation of acid:		
Acetic acid	—	—
Lactic acid	+ weak	—
Malic acid	+ weak	—
Succinic acid	—	—
Fumaric acid	—	—
Citric acid	—	—
Formic acid	—	—
Propionic acid	—	—
Oxalic acid	—	—
Hippuric acid	—	—
Growth in the presence of NaCl	$\leq 5.0\%$	$\leq 5.0\%$
Growth temp	12–36°C	12–38°C
Optimum temp for growth	26°C	28°C
Hydrolysis of:		
Gelatin	+ weak	—
Starch	+	+
Esculin	+	+
Tween 20	—	—
Tween 40	—	—
Tween 60	—	—
Tween 80	—	—
Nitrate reduction	—	—
Voges-Proskauer test	—	—
Methyl red test	+	—
Production of indole	—	—
Production of H_2S	—	—
Urease	—	—
Oxidase	+	+
Catalase	+	+
Oxidation-fermentation test	Fermentation	Fermentation
Principal acid metabolic product	Propionic acid	Propionic acid

teristics of both strains are summarized in Table 4. The amino acids in the cell wall were LL-DAP, alanine, glycine, and glutamic acid (molar ratio, ca. 1:2:1:1); this corresponds to the murein type A3 γ of Schleifer and Kandler (32). Among cell wall sugars, arabinose and glucose were present but galactose

FIG. 1. Phase-contrast (A) (magnification, $\times 800$) and scanning electron (B) micrographs of cells of *L. japonicus* IFO 12422¹ grown on yeast extract-malt extract agar.

FIG. 2. Thin-section electron micrographs of *L. japonicus* IFO 12422^T. The cell division occurs alternately in two planes, the first division (A) and the second division (B). Bar = 0.2 μm.

TABLE 2. Cellular fatty acid compositions of the *L. japonicus* IFO 12422^T and IFO 15385

Strain	Fatty acid composition (%)								2-OH iso-Branched (iso-C _{18:0})
	Nonpolar								
	Saturated				Unsaturated				
	C _{15:0}	C _{16:0}	C _{17:0}	C _{18:0}	C _{15:1}	C _{16:1}	C _{17:1}	C _{18:1}	
IFO 12422 ^T	2.6	5.0	3.1	1.8	5.0	35.6	29.6	15.4	tr
IFO 15385	1.1	7.2	1.5	1.5	2.2	59.5	12.5	12.1	tr

was absent. Mycolic acids were not present. The glycan moiety of the murein contained only acetyl residues. The strains had the polar lipid pattern shown in Fig. 3. Diphosphatidylglycerol and phosphatidylglycerol were identified by their chromatographic behavior and staining characteristics. In addition, unknown glycolipids were present.

The DNA-DNA hybridization study revealed that strains IFO 12422^T and IFO 15385 show very high DNA-DNA similarity values (90 to 99%; data not shown), indicating that strains IFO 12422^T and IFO 15385 belong to the same species.

Phylogenetic analysis. The 16S rRNA sequence of IFO 12422^T, comprising a continuous stretch of 1,207 nucleotides, is shown in Fig. 4. The first and the last nucleotides in the sequence correspond to positions 8 and 1476 of the *E. coli* 16S rRNA sequence (3).

The degree of similarity, based on 957 nucleotides, between IFO 12422^T and sequences of representative species belonging to genera with a high G+C content and LL-DAP in the cell wall was not higher than 93.5% (Table 5). Among them, *P. innocuum* was the closest neighbor of IFO 12422^T. *P. innocuum* differs from the other *Propionibacterium* species in the following characteristics: it is facultatively anaerobic, its cell wall contains arabinose but not galactose, and it hydrolyzes starch (24). Therefore, we consider that *P. innocuum* should be transferred to a different genus. Similarity values between IFO 12422^T and species of the genus *Propionibacterium* and species of the genus *Nocardioides* or *Aeromicrobium* are 91.1 to 93.5% and 91.4 to 93.2%, respectively. Thus, the analysis of partial 16S rRNA clearly indicates that IFO 12422^T represents a distinct line of descent among the gram-positive bacteria with a high G+C content.

Five genera of gram-positive cocci with high G+C contents

have been described previously: *Micrococcus* (4), *Deinococcus* (2), *Stomatococcus* (1), *Kineococcus* (38), and *Pelczaria* (25). However, no genera of cocci with LL-DAP in the cell wall and with a high G+C content have been described. The genus *Sarcina* (31) is the only coccus with LL-DAP in the cell wall, and this genus has a low G+C content in its DNA and is a strict anaerobe. Among gram-positive rods with high G+C contents, the genera *Nocardioides* (26), *Aeromicrobium* (20), *Terrabacter* (5), *Propionibacterium* (23), *Sporichthya* (17), and *Streptomyces* (35) are known. Chemotaxonomy has proved very useful in finding characteristics that distinguish these taxa from neighboring taxa. Strains IFO 12422^T and IFO 15385 can be readily distinguished from all of these taxa on the basis of chemical characteristics (Table 4). The chemotaxonomic characteristics of strains IFO 12422^T and IFO 15385 closely resemble the characteristics of species of the genera of *Nocardioides* (16), *Aeromicrobium* (20), *Terrabacter* (5), and *Propionibacterium* (13), but the fatty acid composition and morphological characteristics are different. With respect to menaquinone composition, the major menaquinone of strains IFO 12422^T and IFO 15385, *N. fastidiosa* and *A. erythreum* is MK-9(H₄), but that of *T. tumescens* and species of *Nocardioides* except for *N. fastidiosa* is MK-8(H₄). The only menaquinone composition which has been reported for species of *Propionibacterium* is MK-9(H₄). Thus, the combination of the presence of LL-DAP and a G+C content of 66 to 68 mol% and the presence of MK-9(H₄) readily distinguishes strains IFO 12422^T and IFO 15385 from all previously described gram-positive cocci with high G+C contents (Table 4).

A comparative analysis of 16S rRNA sequences suggests that the genus *Micrococcus* is more closely related to the genus *Arthrobacter* than to the other genera of gram-positive cocci

TABLE 3. Menaquinone compositions of IFO 12422^T, IFO 15385, and related bacteria

Strain or species	Menaquinone composition ^a							
	MK-7(H ₄)	MK-8	MK-8(H ₂)	MK-8(H ₄)	MK-8(H ₆)	MK-9(H ₄)	MK-9(H ₆)	MK-9(H ₈)
IFO 12422 ^T			—	—	—	+++	tr	tr
IFO 15385			—	—	—	+++	tr	tr
<i>Propionibacterium</i> ^b						+++		
<i>Aeromicrobium erythreum</i> ^c	+			+		+++		
<i>Terrabacter tumescens</i> ^d	+			+++	+	tr		
<i>Nocardioides albus</i> ^e	+	—	+	+++	+	tr		
<i>Nocardioides fastidiosa</i> ^f				+		+++	+	
<i>Nocardioides jensenii</i> ^g				+++				
<i>Nocardioides luteus</i> ^e	+	—	+	+++	+	tr		
<i>Nocardioides simplex</i> ^d	+	+	—	+++	+	tr		

^a +, presence; —, absence.

^b Data from reference 13.

^c Originally reported as lacking menaquinone (20).

^d Data from reference 6.

^e Data from reference 7.

^f Data from reference 8.

^g Data from reference 22.

FIG. 3. Two-dimensional thin-layer chromatogram of the polar lipids of *L. japonicus* IFO 12422^T. Chloroform-methanol-water (65:25:4, vol/vol/vol) was used in the first dimension, and chloroform-methanol-acetic acid-water (80:12:18:5, vol/vol/vol/vol) was used in the second dimension. Lipids were visualized by spraying the plates with 10% molybdophosphoric acid in ethanol, before heating them at 150°C for 10 min. Abbreviations: DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PI, phosphatidylinositol.

(20, 33). Comparative analysis also indicates a similar relationship between the genera *Deinococcus* and *Thermus* (36). These genera form a phylum distinct from the phylum of gram-positive bacteria (37). In this study, the coccoid form IFO 12422^T was revealed to be phylogenetically more closely related to rod-shaped *P. innocuum* than to the other genera of gram-positive cocci, rods, and nocardioforms with high G+C content (Fig. 5).

Thus, on the basis of biochemical and chemical criteria and phylogenetic analysis, strains IFO 12422^T and IFO 15385 can be readily distinguished from all previously described gram-positive cocci and in our opinion warrant a new taxon. Therefore, we formally propose that strains IFO 12422^T and IFO

AGAGTTTGGATCCTGGCTCAGGACGAACGCTGGCGGCTGCTTAACACATGCAAGTCGAAC
GGTAAGCGGTGTTGAAACTCCGTTGATGATGGCTCGCGGCTATCAGCTTGTGGT
GAGGTAGTGGCTCACCAGGCTTCGACGGGTAGCGGCTGAGAGGGCGACCGGCCACAC
TGGGACTGAGATACGGCCCTCTACGGGAGGCGAGCTGGGGAATATGCAATGGGCGA
AGACTGATGACGCAACCGCGCTGCGGGATGACGGCTTCGGGTTGTAACCGCTTTCAA
CGCAGACGAAGCGAAAGTGACGGTATGCGGTAGAGAAAGCACCGGCCAACTACGTGCCGAG
GGTGCGAGCGTTGTCGGAATATTGGGCTTAAAGAGCTTGTAGGCGGTTGTTGCGTCA
GAAGTGAAATCTCAGTGCTTAACACTGAGCTGCTTCTGATACGGGCGAGACTAGAGGAAG
TTAGGGGAGACCGGAATTCCTGGTGGACGTGATAGGCTTAGATACCTTGTAGTCCAGCG
CGTAAACGGTGGGTACTAGGTGTTGGTTTACATTCACGCTGATCCGTGCGCATATAACGC
ATTAAGTACCGCTGGGAGTACGGGCGCAAGGCTAAAGCTCAAGGAATTTGACGGGGG
CCCGCACAAGCGGCGGAGCATCGGATTAATTCGATGCAACGCGAAGAACCTTACCTGGG
TTTGACATATGCCGGAACATTGAGAGATGGATGTTCACTTTTGGTCCGGTATACAGGT
GGTGATGCTGTCGTCAGCTCGTGTGATGATGTTGGGTTAAGTCCCGCAACAGCGCA
ACCTTTTCCTTTGTTGGCAGCACGTAATGGTGGGAACTCAAAGGAGACTGCCGTTGTCAA
ATCGGAGGAAGGTGGGATGACGTCAAGTCATCATGCCCTTATGTCCAGGGGCTTCACGC
ATGCTACAATGGCCGCTACAAAGGGCTGCGAACTGTAAGGGTGAGCTAATCCCAAAAAG
CCGGTCTCAGTTCCGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGTAA
TCGAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCTTGTACACACCGCCCGTCAA
GTCACGAGAGTAGGTAACACCGGAAGCCGGTGGCCCAACCGTGAGGAGGAGCTGTGCAAA
GGTGGGA

FIG. 4. Partial 16S rRNA sequence of *L. japonicus* IFO 12422^T. N, undetermined nucleotide.

TABLE 4. Differential characteristics of the genus *Luteococcus*, gram-positive cocci, and related taxa^a

Genus or species	Cell morphology	Aerobic type	Motility	Endospores	G+C content (mol%)	Diamino acid	Murein type	Mycolic acid	Glycol test	Cell wall sugar	Menaquinone system	Cellular fatty acid(s)
<i>Luteococcus</i>	Coccus	f	—	—	66–68	LL-DAP	A3γ	—	Ace	Ara	MK-9(H ₄)	C _{16:1} , C _{17:1} , C _{18:1}
<i>Nocardioideae</i> ^{b,c}	Hyphae/rod	a	+/+	—	66–72	LL-DAP	A3γ	—	Ace	NC	MK-8(H ₄), MK-9(H ₄)	iso-C _{16:0} , 10-Me18, C _{18:1}
<i>Terrabacter</i> ^d	Rod	a	—/+	—	69–72	LL-DAP	A3γ	—	ND	NC	MK-8(H ₄)	iso-C _{15:0}
<i>Aeromicrobium</i> ^e	Rod	a	—	—	71–73	LL-DAP	A3γ	—	ND	NC	MK-9(H ₄)	10-Me18, C _{18:1} , C _{16:0}
<i>Propionibacterium</i> ^f	Rod	an/f	—	—	53–68	LL-DAP	A3γ	—	ND	Gal, Man	MK-9(H ₄)	A, 1(S)
<i>P. pseudonitro</i> ^g	Rod	an	—	—	—	meso-DAP	A1γ	—	ND	—	—	—
<i>Kineococcus</i> ^h	Coccus	a	+	—	75	meso-DAP	A1γ	—	Ace	Ara, Gal	MK-9(H ₂)	antiso-C _{15:0}
<i>Micrococcus</i> ^h	Coccus	a	—/+	—	65–75	L-Lys	A4α/A3α	—	Ace	NC	MK-8(H ₂), MK-9(H ₂)/MK-8, MK-7	antiso-C _{15:0}
<i>Deinococcus</i> ⁱ	Coccus	a	—	—	62–71	L-Orn/L-Lys	A3β/A3α	—	ND	NC	MK-8	C _{16:0} , C _{16:1}
<i>Stomatococcus</i> ^j	Coccus	f	—	—	56–60	L-Lys	A3α	—	ND	NC	ND	antiso-C _{15:0}
<i>Pelczarid</i> ^k	Coccus	a	—	—	59	L-Lys	A3α	ND	ND	ND	ND	iso-C _{16:0}
<i>Streptomyces</i> ^l	Hyphae	a	—	—	69–73	LL-DAP	A3γ	—	Ace	NC	MK-9(H ₆), MK-(H ₈)	2c ^m
<i>Sporichthya</i> ^m	Hyphae	a	+	—	70	LL-DAP	A3γ	—	ND	NC	MK-9(H ₆), MK-(H ₈)	3d ^m

^a Abbreviations: a, aerobic; f, facultative anaerobe; an, obligate anaerobe; meso-DAP, meso-diaminopimelic acid; L-Lys, L-lysine; L-Orn, L-ornithine; Ace, acetyl residues; Gly, glycol residues; Ara, arabinose; Gal, galactose; ND, not determined; NC, not characteristic; 10Me18, 10 methylheptacosanoic acid; iso, iso-branched; anteiso, anteiso-branched; A, anteiso-branched fatty acid; L, iso-branched fatty acid; S, saturated fatty acid. Designations for murein types are based on those of Schleifer and Kandler (32).

^{b–m} Data were taken from references 16 and 8, 5, 20, 13, 38, 31, 2, 1, 25, 18, and 27, respectively.

ⁿ According to Kroppenstedt (15).

TABLE 5. Homology (upper right-hand triangle) and nucleotide substitution rates (K_{nuc}) values (lower left-hand triangle) for a 957-nucleotide region of 16S rRNAs of *L. japonicus* IFO 12422¹ and other taxa

Strain ^a	% Similarity or K_{nuc} values with:																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1 <i>Luteococcus japonicus</i>																			
2 <i>Propionibacterium innocuum</i>	0.0680	93.5	91.5	91.1	91.6	92.2	92.3	92.2	93.2	91.4	92.4	92.5	91.9	90.3	90.9	89.9	90.5	90.6	81.1
3 <i>Propionibacterium freudenreichii</i>	0.0902	0.0788		94.1	94.4	94.5	91.0	91.4	90.7	92.1	91.0	91.5	91.1	90.3	90.3	89.8	91.0	90.7	80.4
4 <i>Propionibacterium acnes</i>	0.0951	0.0883	0.0611		95.7	95.7	90.7	91.1	90.8	91.6	91.0	91.1	90.7	89.3	89.4	89.6	90.2	90.2	80.5
5 <i>Propionibacterium jensenii</i>	0.0889	0.0717	0.0588	0.0442		99.3	91.6	92.1	91.7	91.9	91.7	92.0	92.0	90.4	91.4	89.8	91.5	90.9	80.3
6 <i>Propionibacterium thoenii</i>	0.0830	0.0659	0.0577	0.0442	0.0074		92.1	92.5	92.3	92.3	92.3	92.5	92.5	90.9	91.7	90.3	91.9	91.4	80.8
7 <i>Nocardioides albus</i>	0.0817	0.0679	0.0959	0.0996	0.0888	0.0841		98.6	97.0	97.0	95.6	95.4	92.8	91.2	92.5	91.3	92.6	92.1	81.0
8 <i>Nocardioides luteus</i>	0.0829	0.0713	0.0912	0.0948	0.0840	0.0794	0.0137		96.7	96.6	95.5	95.3	93.1	91.3	92.3	91.5	92.6	92.1	81.2
9 <i>Nocardioides jensenii</i>	0.0713	0.0644	0.0997	0.0986	0.0878	0.0819	0.0310	0.0343		95.9	96.6	96.3	93.6	91.7	92.7	91.9	92.6	92.1	81.5
10 <i>Nocardioides simplex</i>	0.0913	0.0762	0.0843	0.0890	0.0865	0.0818	0.0310	0.0354	0.0420		95.0	95.0	92.9	90.6	91.6	91.9	92.7	92.7	81.7
11 <i>Nocardioides fastidiosa</i>	0.0805	0.0689	0.0960	0.0960	0.0877	0.0818	0.0453	0.0464	0.0353	0.0520		99.1	93.0	91.1	91.6	92.1	92.7	92.6	81.6
12 <i>Aeromicrobium erythreum</i>	0.0793	0.0678	0.0901	0.0949	0.0853	0.0794	0.0475	0.0486	0.0375	0.0520	0.0095		92.9	91.6	92.2	92.4	92.8	92.7	81.3
13 <i>Terrabacter tumescens</i>	0.0865	0.0831	0.0949	0.0998	0.0853	0.0794	0.0759	0.0724	0.0668	0.0748	0.0737	0.0748		92.7	92.9	93.7	94.6	94.5	83.2
14 <i>Streptomyces griseus</i>	0.1046	0.0949	0.1045	0.1155	0.1033	0.0973	0.0936	0.0924	0.0878	0.1009	0.0949	0.0890	0.0775		97.4	93.3	92.8	92.8	83.4
15 <i>Streptomyces lividans</i>	0.0974	0.0891	0.1047	0.1143	0.0914	0.0878	0.0795	0.0818	0.0772	0.0890	0.0889	0.0831	0.0750	0.0266		93.1	93.4	93.4	83.3
16 <i>Sporichthya polymorpha</i>	0.1094	0.0962	0.1107	0.1132	0.1109	0.1047	0.0924	0.0901	0.0866	0.0866	0.0842	0.0807	0.0659	0.0704	0.0727		93.0	93.3	83.6
17 <i>Micrococcus luteus</i>	0.1020	0.0973	0.0959	0.1057	0.0900	0.0865	0.0782	0.0783	0.0784	0.0771	0.0772	0.0761	0.0566	0.0762	0.0692	0.0742		97.3	83.6
18 <i>Arthrobacter globiformis</i>	0.1010	0.0999	0.0995	0.1057	0.0973	0.0913	0.0843	0.0842	0.0843	0.0771	0.0784	0.0774	0.0577	0.0763	0.0693	0.0706	0.0278		83.1
19 <i>Bacillus subtilis</i>	0.2193	0.2181	0.2290	0.2282	0.2311	0.2239	0.2201	0.2173	0.2131	0.2103	0.2117	0.2161	0.1914	0.1891	0.1906	0.1859	0.1861	0.1931	

^a Strains in the horizontal headings are numbered the same as those listed vertically.

FIG. 5. Unrooted phylogenetic tree displaying the position of *L. japonicus* with regard to some other actinomycete taxa. The tree is based on a comparison of 957 bases.

15385 be classified in a new genus, *Luteococcus*, as *Luteococcus japonicus* gen. nov., sp. nov.

Descriptions for the new genus and new species are given below.

Description of *Luteococcus* gen. nov. *Luteococcus* (Lu.te.o.-coc'cus. Lat. adj. *luteus* yellow; Gr. masc. n. *coccus* a grain; M.L. masc. n. *Luteococcus* yellow coccus). Cells are spherical and 0.7 to 1.0 μm in diameter and occur singly, in pairs, or in tetrads. Endospores are not formed. Gram-positive. Colonies are circular and smooth and may be cream colored to yellow. Facultatively anaerobic. Catalase and oxidase positive. Urease negative. The cells do not reduce nitrate to nitrite. Starch is hydrolyzed. Tween 20, 40, 60, and 80 are not hydrolyzed. Acid is produced from glucose and some other sugars. Propionic acid is formed from glucose as a major product. Optimum growth temperature is 26 to 28°C.

Cell wall peptidoglycan contains LL-diaminopimelic acid, alanine, glycine, and glutamic acid (ca. 1:2:1:1). The major menaquinone is MK-9(H₄). Mycolic acid is not present. The major cellular fatty acid is 16:1, and among the minor components a small amount of 2-OH iso-18:0 is also present. Arabinose is present as a diagnostic sugar in the cell wall. As polar lipids, phosphatidylinositol, diphosphatidylglycerol, and phosphatidylglycerol are present.

Type species is *Luteococcus japonicus*.

Description of *Luteococcus japonicus* sp. nov. *Luteococcus japonicus* (ja.po'ni.cus. M.L. masc. adj. *japonicus*, pertaining to Japan, where the organisms were isolated) cells are spherical and 0.7 to 1.0 μm in diameter and occur singly, in pairs, or in tetrads. Endospores are not formed. Gram-positive. Facultatively anaerobic. Colonies are circular and smooth and may be cream colored to yellow. Catalase and oxidase positive. Urease negative. The cells do not reduce nitrate to nitrite. Oxidation-fermentation is fermentative. Acid is produced from D-glucose, D-ribose, D-galactose, D-mannose, D-fructose, sucrose, maltose, trehalose, raffinose, glycerol, mannitol, inositol and L-arabinose but not from D-xylose, D-arabinose, or L-rhamnose. Propionic acid is produced from glucose. Starch is hydrolyzed. Gelatin is weakly or not hydrolyzed. Optimum growth temperature is 26 to 28°C.

Chemical characteristics such as amino acid composition of

cell wall peptidoglycan, menaquinone system, mycolic acids, cellular fatty acids, and polar lipids are the same as those described for the genus. The DNA base composition of the type strain is 67 mol% as determined by HPLC. Distribution: soil, water.

The type strain is IFO 12422.

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