***Saccharothrix violacea* sp. nov., isolated from a gold mine cave, and *Saccharothrix albidocapillata* comb. nov.**

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The generic position of two isolates from soils inside a gold mine cave in Kongju, Korea, was determined by 16S rDNA sequencing and chemotaxonomic characteristics. Phylogenetic analysis indicated that both of the isolates formed a clade with *Lentzea albidocapillata* and members of the genus *Saccharothrix* of the family *Pseudonocardiaceae*. The chemical composition of the isolates and of *Lentzea albidocapillata* was consistent with that of the genus *Saccharothrix*, which is characterized by a type III cell wall (the *meso*-isomer of diaminopimelic acid, and galactose and rhamnose as characteristic whole-cell sugars), MK-9(H₄) as the major menaquinone, and a phospholipid type PII pattern (phosphatidylethanolamine as a diagnostic phospholipid). The combination of morphological features, chemotaxonomic characters and phylogenetic data supported the proposal that *Lentzea albidocapillata*, the only and type strain of the genus *Lentzea*, should be transferred to the genus *Saccharothrix*. On the basis of physiological properties, cellular fatty acid composition and DNA–DNA hybridization data, two new species within the genus *Saccharothrix* are proposed: *Saccharothrix violacea* sp. nov., type strain LM 036^T (= IMSNU 50388^T), and *Saccharothrix albidocapillata* comb. nov., type strain DSM 44073^T (= IMSNU 21253^T).

Keywords: *Saccharothrix violacea* sp. nov., *Saccharothrix albidocapillata* comb. nov., soil bacteria, phylogeny

INTRODUCTION

The genus *Saccharothrix* was described by Labeda *et al.* (1984) for amycolate actinomycetes that are characterized by fragmentation of both the vegetative and aerial mycelia into ovoid elements and the following cell chemistry: a type III cell wall (the *meso*-isomer of diaminopimelic acid, and rhamnose and galactose as whole-cell sugars); absence of mycolic acids; a major menaquinone of MK-9(H₄); and a phospholipid type PII (phosphatidylethanolamine) or PIV (phosphatidylethanolamine and glucosamine-containing phospholipids) pattern (Labeda & Lechevalier, 1989). The genus currently contains 12 described species: *Saccharothrix aerocolonigenes*, *Saccharothrix aus-*

traliensis, *Saccharothrix coeruleofusca*, *Saccharothrix coeruleoviolacea*, *Saccharothrix cryophilis*, *Saccharothrix espanaensis*, *Saccharothrix flava*, *Saccharothrix longispora*, *Saccharothrix mutabilis*, *Saccharothrix syringae*, *Saccharothrix texasensis* and *Saccharothrix waywayandensis* (Labeda & Lechevalier, 1989; Grund & Kroppenstedt, 1989).

Yassin *et al.* (1995) proposed *Lentzea albidocapillata* gen. nov., sp. nov. for a mesophilic actinomycete which forms a well-developed, fragmenting aerial mycelium and a branched substrate mycelium. The genus *Lentzea* has a type III cell wall (*meso*-isomer of diaminopimelic acid and no characteristic sugar), phosphatidylethanolamine as a diagnostic phospholipid (the phospholipid type PII pattern) and a non-hydrogenated menaquinone with nine isoprene units (MK-9) as a major menaquinone. This genus currently contains the only and type species, *Lentzea albidocapillata*, which was isolated from a tissue specimen

Abbreviation: ISP, International Streptomyces Project.

The EMBL accession numbers for the 16S rDNA nucleotide sequences of isolates LM 036^T and LM 044 are AJ242633 and AJ242634, respectively.

taken from a patient suffering from peritoneal carcinomatosis.

The family *Pseudonocardiaceae* Embley *et al.* 1988 was recently emended by Stackebrandt *et al.* (1997), based on the 16S-rDNA-sequence-based phylogenetic clustering and the presence of family-specific signature nucleotides. According to cell wall chemotype, the 13 genera of the family *Pseudonocardiaceae* are divided into two groups as follows: the type IV cell wall genera comprise *Actinokineospora*, *Actinopolyspora*, *Amycolatopsis*, *Kibdelosporangium*, *Pseudonocardia*, *Saccharomonospora* and *Saccharopolyspora* (Warwick *et al.*, 1994), whereas the type III cell wall genera comprise *Actinosynnema* (Hasegawa *et al.*, 1978), *Kutzneria* (Stackebrandt *et al.*, 1994), *Lentzea* (Yassin *et al.*, 1995), *Saccharothrix* (Labeda *et al.*, 1984), *Streptoalloteichus* (Tomita *et al.*, 1987) and *Thermocrisium* (Korn-Wendisch *et al.*, 1995). The type III cell wall genera and the genus *Actinokineospora*, a member of the type IV cell wall genera, form a coherent cluster within the radiation of the family *Pseudonocardiaceae* (Lee *et al.*, 2000b; Yassin *et al.*, 1995).

During the taxonomic study of soil actinomycetes, we obtained two isolates from soils collected inside a gold mine cave in Kongju, Republic of Korea. Both of the isolates formed well-developed aerial mycelia that fragmented into rod-shaped elements. The comparative analysis of 16S rDNA sequences supported the conclusion that our isolates were related to members of the genera *Lentzea* and *Saccharothrix*. In this paper we describe the characterization and classification of the isolates, and we propose the new taxa *Saccharothrix violacea* sp. nov. and *Saccharothrix albidocapitata* comb. nov. The isolates LM 036^T and LM 044 have been deposited in the Culture Collection Center of the Institute of Microbiology, Seoul National University (IMSNU) under the numbers IMSNU 50388^T and IMSNU 50393, respectively.

METHODS

Micro-organisms and culture conditions. Strains LM 036^T and LM 044 were isolated from soils collected inside a gold mine cave in Kongju, Korea, on tap water agar and oligotrophic medium (M5) by using the dilution plating method. The M5 medium contained (per litre of tap water) glucose, 0.1 g; K₂HPO₄, 0.5 g; Na₂HPO₄, 0.7 g; KNO₃, 0.1 g; NaCl, 0.3 g; MgSO₄·7H₂O, 0.1 g; CaCl₂·2H₂O, 0.02 g; FeSO₄·7H₂O, 200 mg; ZnSO₄·7H₂O, 180 µg; MnSO₄·4H₂O, 20 µg; CuSO₄·5H₂O, 90 µg; CoSO₄·7H₂O, 10 µg; H₃BO₃, 200 µg; and (NH₄)₆Mo₇O₂₄·4H₂O, 5 µg. For comparative purposes, *Lentzea albidocapitata* IMSNU 21253^T (= DSM 44073^T) was used as a reference strain.

Morphological and cultural characteristics. The degree of growth, aerial mycelium, pigmentation and other morphological features were investigated on yeast extract/malt extract agar [International *Streptomyces* Project (ISP) medium 2], oatmeal agar (ISP medium 3) and inorganic salts/starch agar (ISP medium 4) as described by Shirling & Gottlieb (1966), and on yeast extract/glucose agar. Melanin pigment production was determined using peptone/yeast

extract/iron agar (ISP medium 6) and tyrosine agar (ISP medium 7). For scanning electron microscopy, agar blocks on which growth had occurred were fixed in 1% osmium tetroxide, dehydrated with an ethanol series and then critical-point-dried under liquid CO₂. Each specimen was sputter-coated with gold and observed with a scanning electron microscope (Stereoscan 260; Cambridge Instruments).

Physiological characteristics. Utilization of carbohydrates and of organic acids as carbon sources was determined as described by Pridham & Gottlieb (1948) and Gordon *et al.* (1974), respectively. Acid production from carbohydrates was determined by a colour change in Bacto OF basal medium (Difco) supplemented with the substrates at a final concentration of 1% (w/v). Decomposition of adenine, guanine, hypoxanthine, tyrosine and xanthine were determined as described by Gordon *et al.* (1974), and arbutin and aesculin decomposition and casein hydrolysis were determined as described by Williams *et al.* (1983). Decomposition of DNA was determined using Bacto DNase test agar (Difco). Nitrate reduction, hydrogen sulfide production, gelatin liquefaction, starch hydrolysis and hippurate hydrolysis were studied as described by Mac Faddin (1980). Urease activity was determined by a colour change from red to pink in Bacto urea broth (Difco). Catalase activity was checked with 3% hydrogen peroxide. Oxidase production was tested by examining the oxidation of 1% tetramethyl-*p*-phenylenediamine. NaCl tolerance studies were performed on nutrient agar containing NaCl at final concentrations of 0, 2, 4, 7 and 10% (w/v). Thallium acetate tolerance was determined on nutrient agar at a final concentration of 0.01% (w/v). To determine sensitivity to lysozyme, a 0.1% (w/v) solution of lysozyme was filter-sterilized and added to nutrient agar at a final concentration of 0.01%. Growth was tested at temperatures ranging from 10 to 42 °C.

Growth in the presence of various antibiotics was tested using the standard disk diffusion technique (National Committee for Clinical Laboratory Standards, 1994). Disks containing the different antibiotics (Susceptibility test discs; Difco) were applied to Mueller–Hinton agar (Difco) plates which had been inoculated with 100 µl mycelial suspension in 20% glycerol with a Dispense-O-Disc dispenser (Difco). The inverted plates were incubated for 7 d at 30 °C.

To determine the production of antibiotic substances, the ability of the isolates to inhibit the growth of reference organisms was examined using an overlay technique (Williams *et al.*, 1983). Reference organisms selected were *Bacillus subtilis* IMSNU 10011, *Micrococcus luteus* IMSNU 20371, *Streptomyces murinus* IMSNU 20248^T, *Staphylococcus aureus* subsp. *aureus* IMSNU 11089, *Escherichia coli* IMSNU 10080, *Enterobacter aerogenes* IMSNU 10256, *Saccharomyces cerevisiae* IMSNU 30102, *Aspergillus niger* IMSNU 31067 and *Candida albicans* IMSNU 30018. Spot-inoculated cells of the isolates grown on nutrient agar for 7 d at 30 °C were overlaid with 5 ml top agar (0.3% Bacto beef extract, 0.5% Bacto peptone, 0.5% Bacto agar) inoculated with the reference organisms. The degree of inhibition was observed after further incubation for 5 d at 30 °C.

Chemotaxonomy. To determine cellular fatty acid compositions, test strains were cultivated in shake flasks containing trypticase soy broth (BBL) at 30 °C for 3 d. Fatty acid methyl esters were extracted from the biomass as described by Minnikin (1988) and were analysed with a model 5890A Hewlett Packard gas chromatograph. The

resultant peaks were identified by comparison with the Bacterial Acid Methyl Esters CP Mix (catalogue no. 1114; Matreya). Menaquinones were extracted by the method of Minnikin *et al.* (1984) and were analysed by using HPLC (Kroppenstedt, 1985) and a Quattro mass spectrometer (Micromass). The isomer of diaminopimelic acid was determined, and whole-cell sugar, mycolic acid and phospholipid analyses were performed, by the methods of Stanek & Roberts (1974), Saddler *et al.* (1991), Minnikin *et al.* (1977, 1980, 1984) and Collins *et al.* (1982) as described previously (Lee *et al.*, 2000a).

Isolation of DNA, determination of G+C content and DNA–DNA hybridization. Genomic DNA was isolated and purified by the large-scale procedure of Hopwood *et al.* (1985). Test strains were grown on yeast extract/glucose broth for 3 d at 30 °C, harvested by centrifugation and washed twice with distilled water. The wet cells were immediately used for DNA isolation or kept at –20 °C until required. The G+C content of purified DNA was determined by HPLC (Mesbah *et al.*, 1989). DNA–DNA hybridization studies were carried out by the methods described by Hopwood *et al.* (1985). Genomic DNA was broken into fragments of 2–4 kb with an ultrasonic processor (Misonix) in ice-cold water. DNA was labelled with [³²P]dATP by the random-priming method (Sambrook *et al.*, 1989). Unincorporated nucleotides were removed by passing the mixture through Sephadex G-50 filters. Heat-denatured reference DNA was blotted onto nylon membrane (Boehringer Mannheim) by using a Bio-Dot apparatus (Bio-Rad) and cross-linked with an UV cross-linker (CL-1000; UVP). Denatured, labelled DNA was added to the hybridization mixture (Hopwood *et al.*, 1985) and incubated at 55 °C for 18 h. The membranes were then washed twice with 2 × SSC/0.01 % SDS for 15 min at 55 °C and once with 2 × SSC at room temperature. The extent of hybridization on each dot was determined by using a scintillation counter (Hewlett Packard). Each hybridization value represents the mean of three replicates.

Phylogenetic analysis. PCR-mediated amplification and cloning of the 16S rDNAs of the isolates LM 036^T (=IMSNU 50388^T) and LM 044 (=IMSNU 50393) were performed as described previously (Lee *et al.*, 2000b). The purified, cloned 16S rDNA sequences were determined using the Cy5 AutoRead Sequencing kit (Pharmacia). The 16S rDNA sequences obtained were compared with those of representatives of the family *Pseudonocardiaceae* and aligned by using the CLUSTAL W program (Thompson *et al.*, 1994). The evolutionary distance matrices were produced by using the method of Jukes & Cantor (1969), and a phylogenetic tree was constructed by the neighbour-joining method (Saitou & Nei, 1987) contained in the PHYLIP package (Felsenstein, 1993). Bootstrap analysis (Felsenstein, 1985) of the neighbour-joining data, using 1000 resamplings, was carried out for evaluating the reliability of tree topology.

Nucleotide sequence accession numbers. The reference sequences used in this study are available from the DDBJ, EMBL and GenBank nucleotide sequence databases under the following accession numbers: *Actinobispora yunnanensis* IMSNU 22019^T, AJ252822; *Actinosynnema mirum* DSM 43827^T, X84447; *Actinokineospira riparia* IFO 14541^T, X76953; *Amycolatopsis orientalis* subsp. *orientalis* DSM 44040^T, X76958; *Kibdelosporangium aridum* subsp. *aridum* ATCC 39323^T, X53191; *Kutzneria viridigrisea* JCM 3282^T, U58530; *Lentzea albidocapillata* DSM 44073^T, X84321; *Pseudonocardia autotrophica* IMSNU 20050^T, AJ252824;

Pseudonocardia saturnea IMSNU 20052^T, AJ252829; *Saccharomonospora azurea* NA128^T, Z38017; *Saccharomonospora glauca* DSM 43769^T, Z38003; *Saccharomonospora viridis* NCIMB 9602^T, Z38007; *Saccharopolyspora gregorii* NCIMB 12823^T, X76962; *Saccharopolyspora hirsuta* subsp. *hirsuta* ATCC 27875^T, X53196; *Saccharopolyspora rectivirgula* ATCC 33515^T, X53194; *Saccharopolyspora taberi* DSM 43856^T, AF002819; *Saccharothrix aerocolonigenes* subsp. *aerocolonigenes* ISP 5034^T, AB020030; *Saccharothrix aerocolonigenes* subsp. *staurosporea* JCM 9734^T, AB024287; *Saccharothrix australiensis* ATCC 31947^T, X53194; *Saccharothrix coerulescens* DSM 43679^T, X76963; *Saccharothrix longispora* DSM 43749^T, X76964; *Saccharothrix mutabilis* subsp. *capreolus* DSM 40225^T, X76965; *Saccharothrix mutabilis* subsp. *mutabilis* DSM 43853^T, X76966; *Saccharothrix waywayandensis* JCM 9114^T, AB020029; *Thermocrispum agreste* DSM 44070^T, X79183; *Thermocrispum municipale* DSM 44069^T, X79184.

The EMBL accession numbers for the 16S rDNA nucleotide sequences of isolates LM 036^T and LM 044 are AJ242633 and AJ242634, respectively.

RESULTS AND DISCUSSION

Morphological and cultural characteristics

Both of the isolates exhibited good growth on all of the media tested and produced well-developed, white aerial mycelia that fragmented into rod-shaped elements (Figs 1 and 2). Other morphological features, such as sporangia, sclerotia and synnemata, were not observed. The isolates produced violet substrate mycelia and reddish-brown soluble pigments, whereas the substrate mycelium of the *Lentzea albidocapillata* reference strain was yellow–yellowish brown. Neither of the isolates produced melanin on either peptone/yeast extract/iron agar (ISP medium 6) or tyrosine agar (ISP medium 7).

Chemotaxonomic characteristics

The diaminopimelic acid isomer and the sugar composition indicated that both the isolates had cell walls of type III (Lechevalier & Lechevalier, 1970) (*meso*-diaminopimelic acid, galactose, mannose and rhamnose). The phospholipid profiles contained phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol [phospholipid type PII pattern of Lechevalier *et al.* (1981)]. No mycolic acids were present. The major menaquinone of the isolates was MK-9(H₄) (>80 % of the total). Small amounts of MK-9(H₂) and MK-9(H₆) were also detected. The genus *Lentzea* has previously been reported to contain menaquinone MK-9 as the principal type (Yassin *et al.*, 1995), whereas the major menaquinone of *L. albidocapillata* IMSNU 21253^T determined in this study was MK-9(H₄) (72 % of total). The predominant fatty acid was iso-branched hexadecanoate (i-C_{16:0}). A significant amount of saturated, unsaturated, anteiso-branched and 10-methyl branched fatty acids were also present. Small amounts of tuberculostearic acid (10Me-C_{18:0}) were present

Fig. 1. Scanning electron micrograph of strain LM 036^T (=IMSNU 50388^T) grown on ISP medium 4 (inorganic salts/starch agar). Bar, 2 µm.

Fig. 2. Scanning electron micrograph of strain LM 044 (=IMSNU 50393) grown on ISP medium 4 (inorganic salts/starch agar). Bar, 2 µm.

only in strain LM 044. The cellular fatty acid compositions of the isolates and *L. albidocapillata* are shown in Table 1.

Physiological characteristics

All of the test strains grew strictly aerobically. Catalase and urease were produced. Nitrate reductase and oxidase were not produced. H₂S was produced. Growth occurred between 20 and 37 °C. All of the test strains utilized L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, D-lactose, maltose, D-mannose, melibiose, L-rhamnose, sucrose, D-trehalose, D-xylose, adonitol, *meso*-inositol, D-mannitol, acetate, fumarate, α-ketoglutarate, malonate, propionate, pyruvate and succinate as carbon sources. Arbutin, DNA, aesculin, hypoxanthine and tyrosine were decomposed. Casein, hippurate and starch were hydrolysed. All test strains showed acid production from L-arabinose, D-lactose

and L-ribose. None of the test strains utilized inulin, D-melezitose, methyl α-D-glucoside, methyl α-D-mannoside, D-raffinose, dulcitol, 2,3-butanediol, *meso*-erythritol, 1,2-propanediol, D-sorbitol, D-xylitol, benzoate, citrate, formate, maleate or tartarate as carbon sources. Decomposition of adenine, guanine or xanthine, and acid production from D-mannose, melibiose, methyl α-D-mannoside or salicin were not observed. All of the strains grew in the presence of 2 % NaCl and of 0.01 % lysozyme.

All of the test strains were susceptible to 15 µg erythromycin, 10 µg gentamicin, 10 µg kanamycin, 30 µg neomycin, 30 µg novobiocin, 5 µg rifampicin, 10 µg streptomycin, 30 µg tetracycline and 10 µg tobramycin, but were resistant to 10 µg ampicillin and 2 µg lincomycin.

Strain LM 044 showed antibiotic activity against Gram-positive bacteria, such as *Bacillus subtilis*,

Table 1. Cellular fatty acid composition (% of total fatty acids) of the new isolates and of the type strain of *L. albidocapillata*

Values of less than 0.5% are not shown.

Fatty acid	Strain LM 036 ^T	Strain LM 044	<i>L. albidocapillata</i> IMSNU 21253 ^T
C _{12:0}	0.6		1.1
i-C _{14:0}	5.6	1.8	3.9
C _{14:0}	0.6		1.9
i-C _{15:0}	7.0	4.6	12.5
ai-C _{15:0}	4.2	2.3	10.4
C _{15:0}			1.8
i-C _{16:1}	2.8		1.9
i-C _{16:0}	42.5	35.1	28.8
C _{16:1}	9.6	14.9	13.5
C _{16:0}	4.2	19.4	9.4
i-C _{17:1}	0.8		0.6
10Me-C _{16:0}	8.0	5.3	1.4
i-C _{17:0}	0.8	2.5	0.6
C _{17:1}	3.6	4.4	5.7
C _{17:0}			1.0
10Me-C _{17:0}	0.5		
i-C _{18:0}	1.0	1.1	
C _{18:1}		4.3	
C _{18:0}	3.5	3.4	2.7
10Me-C _{18:0}		0.7	

Micrococcus luteus, *Streptomyces murinus* and *Staphylococcus aureus*, and fungal organisms, such as *Candida albicans*, *Saccharomyces cerevisiae* and *Aspergillus niger*.

The results of physiological tests differentiating the isolates and the type strain of *L. albidocapillata* are shown in Table 2.

Phylogenetic analysis

The almost complete 16S rDNA sequences, consisting of 97.7–97.8% of the *E. coli* sequence (Brosius *et al.*, 1978), were determined for isolates LM 036^T (=IMSNU 50388^T) and LM 044 (=IMSNU 50393) and compared with those of representatives of the family *Pseudonocardiaceae*. A total of 1189 unambiguous nucleotide positions present in all sequences between 99 and 1470 (*E. coli* numbering) were used in final tree construction. The phylogenetic tree shown in Fig. 3 indicated that the isolates formed a clade with members of the genera *Lentzea* and *Saccharothrix* within the radiation encompassing the cell wall III type genera of the family *Pseudonocardiaceae*. This relationship was supported by a high bootstrap value of 79%. The pairwise comparison of 16S rDNA sequences indicated that the isolates exhibited the highest 16S rDNA similarity values to *L. albidocapillata* (98.9 ± 0.1%), *Saccharothrix waywayandensis* (98.9 ± 0.1%) and *Saccharothrix aerocolonigenes*

subsp. *staurosporea* (98.7 ± 0.1%). The sequence similarity value of the isolates was 99.8%. DNA–DNA hybridization studies revealed that the level of DNA–DNA relatedness between the isolates and the type strain of *L. albidocapillata* ranged from 40 to 46%, whereas the isolates showed a DNA–DNA relatedness of 68% with each other. The G + C content of the DNA of the isolates was 68.9–69.6 mol%.

A phylogenetic analysis of 16S rDNA sequences indicated that both of the isolates are related to the type strain of *L. albidocapillata* and members of the genus *Saccharothrix*, *Saccharothrix waywayandensis* and *Saccharothrix aerocolonigenes* subsp. *staurosporea* (Fig. 3). The new isolates exhibit morphological and chemotaxonomic characteristics that are typical of the genus *Saccharothrix* as follows: a type III cell wall (the *meso*-isomer of diaminopimelic acid, and rhamnose and galactose in whole-cell hydrolysates), a major menaquinone of MK-9(H₄), a phospholipid type PII pattern (phosphatidylethanolamine as a diagnostic phospholipid) and well-developed aerial and substrate mycelia that fragment into rod-shaped elements. The chemical composition of the genus *Lentzea*, except for the major menaquinone and the composition of cell wall sugars, is also coincident with that of the genus *Saccharothrix* (Yassin *et al.*, 1995; Labeda *et al.*, 1984). It has previously been reported that the only and type strain of genus *Lentzea*, *L. albidocapillata*, contained MK-9 as a major menaquinone and had no characteristic sugars (Yassin *et al.*, 1995). However, our analysis showed that *L. albidocapillata* contained MK-9(H₄) as a predominant menaquinone, and had galactose and rhamnose as characteristic whole-cell sugars. The combination of morphological, chemotaxonomic and phylogenetic data support the proposal that *L. albidocapillata* should be transferred to the genus *Saccharothrix*. *L. albidocapillata* shows a level of 16S rDNA sequence similarity of 98.1 ± 0.1% to its phylogenetic neighbours, *S. waywayandensis* and *Saccharothrix aerocolonigenes* subsp. *staurosporea*. *L. albidocapillata* is differentiated from *Saccharothrix waywayandensis* and *Saccharothrix aerocolonigenes* by its pathogenic properties, G + C base composition, utilization of citrate and lactate, ability to liquefy gelatin and acid production from D-mannose, melibiose, D-raffinose and salicin (Labeda, 1986; Labeda & Lyons, 1989).

The new isolates are readily distinguished from the type strain of *L. albidocapillata* on the basis of the production of violet-coloured substrate mycelium, fatty acid profiles (Table 1), physiological properties (Table 2) and low DNA–DNA hybridization values of 43 ± 0.3%. The isolated strains differ from *Saccharothrix waywayandensis* and *Saccharothrix aerocolonigenes* in utilization of citrate and oxalate, and in acid production from inositol, melibiose, rhamnose, salicin, sucrose and xylose (Labeda, 1986; Labeda & Lyons, 1989). Thus, the isolates merit recognition as a new species of the genus *Saccharothrix*. The isolates show a high similarity in

Table 2. Differential characters of the new isolates and of the type strain of *L. albidocapillata*

Character	Strain LM 036 ^T	Strain LM 044	<i>L. albidocapillata</i> IMSNU 21253 ^T
Use of sole carbon sources:			
Salicin	+	+	–
Lactate	+	+	–
Malate	–	–	+
Oxalate	–	–	+
Acid produced from:			
D-Cellobiose	–	–	+
D-Fructose	+	–	+
D-Galactose	+	–	+
D-Glucose	+	–	+
Maltose	–	–	+
L-Rhamnose	–	–	+
Sucrose	–	–	+
D-Trehalose	–	–	+
D-Xylose	–	–	+
Adonitol	–	–	+
meso-Inositol	–	–	+
D-Mannitol	–	–	+
Hydrolysis of:			
Gelatin	+	+	–
Growth on:			
NaCl (4%)	+	–	+
Thallium acetate (0.01%)	–	–	+
Resistance to:			
Vancomycin (30 µg)	+	+	–
Antibiotic activity against:			
<i>Bacillus subtilis</i>	–	+	–
<i>Micrococcus luteus</i>	–	+	–
<i>Streptomyces murinus</i>	–	+	–
<i>Staphylococcus aureus</i>	–	+	–
<i>Aspergillus niger</i>	–	+	–
<i>Candida albicans</i>	–	+	–
<i>Saccharomyces cerevisiae</i>	–	+	–

genotypic characters, whereas they exhibit a difference in ability to produce an antibiotic, in amount of i-C_{14:0}, i-C_{16:1}, C_{16:1}, C_{16:0} and C_{18:1} fatty acids, in acid production from D-fructose, D-galactose and D-glucose, and in growth with 4% NaCl.

On the basis of cultural and physiological properties, fatty acid profiles and the levels of DNA–DNA relatedness, we propose new species of the genus *Saccharothrix*, *Saccharothrix violacea* sp. nov. and *Saccharothrix albidocapillata* comb. nov. The emended description of *Saccharothrix albidocapillata* below is based on data taken from the original descriptions (Yassin *et al.*, 1995) and our own studies.

Description of *Saccharothrix violacea* sp. nov.

Saccharothrix violacea (vi.o.la.ce'a. L. adj. *violaceus* violet; M.L. adj. *violacea* violet-coloured).

Forms a violet substrate mycelium and a white aerial mycelium. Reddish-brown pigment is produced. Melanin pigment is not produced. Aerial mycelium fragments into rod-shaped elements. Aerobic, catalase- and urease-positive, oxidase-negative. Nitrate is not reduced to nitrite. H₂S is produced. Growth occurs at 10–37 °C. No growth occurs at 42 °C. Utilizes L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, D-lactose, maltose, D-mannose, melibiose, L-rhamnose, salicin, sucrose, D-trehalose, D-xylose, adonitol, meso-inositol, D-mannitol, acetate, fumarate, α-ketoglutarate, lactate, malonate, propionate, pyruvate and succinate as carbon sources. Gelatin liquefaction occurs. Arbutin, DNA, aesculin, hypoxanthine and tyrosine are decomposed. Casein, hippurate and starch are hydrolysed. Acid is produced from L-arabinose, D-fructose, D-galactose, D-glucose, D-lactose and L-ribose. Growth occurs in the presence of 4% NaCl and 0.01% lysozyme. Does not utilize

Fig. 3. Phylogenetic tree showing the relationship of the isolates and related taxa of the family *Pseudonocardiaceae*. Bootstrap values above 40% (based on percentages of 1000 replications) are indicated at the branching points. The scale bar indicates 1 nucleotide substitution per 100 nucleotides.

inulin, D-melezitose, methyl α -D-glucoside, methyl α -D-mannoside, D-raffinose, dulcitol, 2,3-butanediol, *meso*-erythritol, 1,2-propanediol, D-sorbitol, D-xylitol, benzoate, citrate, formate, malate, maleate, oxalate or tartarate as carbon sources. Adenine, guanine and xanthine are not decomposed. No growth occurs in the presence of 0.01% thallium acetate. No acid is produced from D-cellobiose, maltose, D-mannose, melibiose, methyl α -D-mannoside, L-rhamnose, salicin,

sucrose, D-trehalose, D-xylose, adonitol, *meso*-inositol or D-mannitol. Susceptible to erythromycin, gentamicin, kanamycin, neomycin, novobiocin, rifampicin, streptomycin, tetracycline and tobramycin, but resistant to ampicillin, lincomycin and vancomycin. Type III cell wall (*meso*-diaminopimelic acid, galactose, mannose and rhamnose in whole-cell hydrolysates). Phospholipid profiles contain phosphatidylethanolamine, diphosphatidylglycerol, phosphatidyl-

glycerol and phosphatidylinositol. The major menaquinone is MK-9(H₄). Mycolic acids are absent. The predominant fatty acid is i-C_{16:0} (42.5% of total). A considerable amount of C_{16:1} (9.6%), i-C_{15:0} (7.0%) and 10Me-C_{16:0} (8.0%) are also present. The G+C content of the DNA is 69.6 mol% (as determined by HPLC). Isolated from soils inside a gold mine cave in Kongju, Korea. The type strain is strain LM 036^T (=IMSNU 50388^T).

Additional comments. Morphological and cultural characteristics of strain LM 044 (=IMSNU 50393) are similar to those of the type strain of *Saccharothrix violacea*. Differs from the type strain of *Saccharothrix violacea* in acid production from D-fructose, D-galactose and D-glucose, and in growth with 4% NaCl. Shows antimicrobial activity against *Bacillus subtilis*, *Micrococcus luteus*, *Streptomyces murinus*, *Staphylococcus aureus*, *Aspergillus niger*, *Candida albicans* and *Saccharomyces cerevisiae*. The fatty acids are predominated by i-C_{16:0} (35.1%), followed by C_{16:0} (19.4%) and C_{16:1} (14.9%). Small amounts of 10Me-C_{16:0} and 10Me-C_{18:0} acids are also present. The G+C content of the DNA is 68.9 mol% (as determined by HPLC). Isolated from soils inside a gold mine cave in Kongju, Korea.

Emended description of *Saccharothrix albidocapillata* comb. nov. (Yassin et al. 1995)

Saccharothrix albidocapillata (al.bi.do.ca.pil.la' ta. L. adj. *albidus* white; L. adj. *capillatus* hairy; M.L. fem. adj. *albidocapillata* white-haired, referring to the abundant whitish aerial hyphae).

Forms a yellow–yellowish-brown-coloured substrate mycelium and a whitish aerial mycelium. No soluble pigment is produced. Melanin pigment is not produced. Aerial mycelium fragments into rod-shaped elements. Aerobic, Gram-positive, catalase-, phosphatase-, β-glucosidase-, β-galactosidase- and urease-positive. Oxidase-negative. Nitrate is not reduced to nitrite. H₂S is produced. Growth occurs at 20–37 °C. No growth occurs at 42 °C. Utilizes L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, D-lactose, maltose, D-mannose, melibiose, L-rhamnose, sucrose, D-trehalose, D-xylose, adonitol, *meso*-inositol, D-mannitol, acetate, fumarate, gluconate, α-ketoglutarate, malate, malonate, oxalate, propionate, pyruvate, succinate, iso-amylalcohol and paraffin as carbon sources. Arbutin, DNA, aesculin, hypoxanthine and tyrosine are decomposed. Casein, elastin, hippurate and starch are hydrolysed. Growth occurs in the presence of 4% NaCl, 0.01% thallium acetate and 0.01% lysozyme. Acid is produced from L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, D-lactose, maltose, L-rhamnose, L-ribose, sucrose, D-trehalose, D-xylose, adonitol, *meso*-inositol and D-mannitol. Adenine, guanine and xanthine are not decomposed. Keratin and testosterone are not hydrolysed. Does not utilize inulin, D-melezitose, methyl α-D-glucoside, methyl α-D-mannoside, D-

raffinose, salicin, dulcitol, 2,3-butanediol, *meso*-erythritol, 1,2-propanediol, D-sorbitol, D-xylitol, benzoate, citrate, formate, *m*-hydroxybenzoate, *p*-hydroxybenzoate, lactate, maleate, tartarate or adipate as carbon sources. Gelatin liquefaction does not occur. No acid is produced from inulin, D-mannose, D-melezitose, melibiose, methyl α-D-mannoside, D-raffinose or salicin. Susceptible to erythromycin, gentamicin, kanamycin, neomycin, novobiocin, rifampicin, streptomycin, tetracycline, tobramycin and vancomycin, but resistant to ampicillin and lincomycin. Type III cell wall (*meso*-diaminopimelic acid, galactose, mannose and rhamnose in whole-cell hydrolysates). The major menaquinone is MK-9(H₄). The polar lipids contain phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol. The predominant fatty acid is i-C_{16:0} (28.8%), followed by C_{16:1} (13.5%), i-C_{15:0} (12.5%) and ai-C_{15:0} (10.4%). Small amounts of 10Me-C_{16:0} are also present. Mycolic acids are absent. The G+C content of the DNA is 68.6 mol% (as determined by HPLC). Isolated from a tissue specimen taken from an abdominal mass in a patient suffering from peritoneal carcinomatosis. The type strain is strain DSM 44073^T (=IMSNU 21253^T).

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