***Hongia* gen. nov., a new genus of the order Actinomycetales**

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An aerobic, nocardioform actinomycete, named LM 161^T, was isolated from a soil sample obtained from a gold mine in Kongju, Republic of Korea. This organism formed well-differentiated aerial and substrate mycelia and produced branched hyphae that fragmented into short or elongated rods. The cell wall contains major amounts of LL-diaminopimelic acid, alanine, glycine, glutamic acid, mannose, glucose, galactose, ribose and acetyl muramic acid. The major phospholipids of this isolate are phosphatidylcholine, diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol, and the major isoprenologue is a tetrahydrogenated menaquinone with nine isoprene units. The whole-cell hydrolysate of strain LM 161^T contains 12-methyltetradecanoic and 14-methylpentadecanoic acids as the predominant fatty acids, but does not contain mycolic acids. The G+C content of the DNA is 71.3 mol%. The phylogenetic position of the test strain was investigated using an almost complete 16S rDNA sequence. The isolate formed the deepest branch in the clade encompassing the members of the suborder *Propionibacterineae* Rainey *et al.* 1997. On the basis of chemical, phenotypic and genealogical data, it is proposed that this isolate be classified within a new genus as *Hongia koreensis* gen. nov., sp. nov. in the order Actinomycetales. The type strain is LM 161^T (= IMSNU 50530^T).

Keywords: *Hongia* gen. nov., actinomycetes, *Propionibacterineae*, phylogeny

INTRODUCTION

Phylogenetic analysis based on 16S rDNA (rDNA) sequence data is currently one of the most effective methods for the delineation of bacteria. The suborder *Propionibacterineae* Rainey *et al.* (Stackebrandt *et al.*, 1997) of the order Actinomycetales (Buchanan Stackebrandt *et al.* (Stackebrandt *et al.*, 1997) encompasses the type family *Propionibacteriaceae* (Delwiche) Rainey *et al.* (Stackebrandt *et al.*, 1997) and the family *Nocardioidaceae* (Nesterenko *et al.*) Rainey *et al.* (Stackebrandt *et al.*, 1997), on the basis of 16S rDNA studies. The family *Nocardioidaceae* contains the genera *Aeromicrobium* (Miller *et al.*, 1991; Tamura & Yokota, 1994) and *Nocardioides* (Collins *et al.*, 1989, 1994; O'Donnell *et al.*, 1982a; Prauser, 1976, 1984). The family *Propionibacteriaceae* contains the genera

Luteococcus (Tamura *et al.*, 1994), *Microthunatus* (Nakamura *et al.*, 1995), *Propioniferax* (Yokota *et al.*, 1994) and *Propionibacterium* (Cummins & Johnson, 1986). The recently described genus *Friedmanniella* (Schumann *et al.*, 1997) was shown to be a new member of the family *Propionibacteriaceae*. All of the members, except for the genus *Propionibacterium* (Cummins & Johnson, 1986), possess cell wall peptidoglycan based on LL-diaminopimelic acid (LL-DAP) as the diagnostic diamino acid and exhibit a range of cell morphology from coccus- or rod-shaped forms to nocardioforms. Recently, the phylogenetic relationship between members of the family *Nocardioidaceae* and related taxa was re-evaluated on the basis of 16S rDNA sequence comparisons (Yoon *et al.*, 1998).

In addition, the following LL-DAP-containing actinomycete genera, which belong to other phylogenetic lineages (Stackebrandt *et al.*, 1997), have been described: *Streptomyces* (Williams *et al.*, 1989), *Sporichthya* (Rainey *et al.*, 1993), *Kineosporia* (Itoh *et al.*, 1989), *Intrasporangium* (Kalakoutskii *et al.*, 1967), *Terrabacter* (Collins *et al.*, 1989) and *Terracoccus*

Abbreviations: LL-DAP, LL-diaminopimelic acid; ISP, International Streptomyces Project.

The EMBL accession number for the 16S rDNA sequence of strain LM 161^T is Y09159.

(Prauser *et al.*, 1997). The members of the suborder *Propionibacterineae* are readily differentiated from each other and from the other LL-DAP-containing genera mentioned above by a combination of morphological, physiological and chemotaxonomic properties (Prauser *et al.*, 1997; Schumann *et al.*, 1997).

During the taxonomic study of soil actinomycetes from natural environments, strain LM 161^T (T = type strain) was isolated from a gold-mine cave in Kongju, Republic of Korea. Strain LM 161^T contained LL-DAP as the diagnostic diamino acid in the peptidoglycan, and the substrate- and aerial mycelia showed a tendency to fragment. In this work, we have determined the taxonomic and phylogenetic position of the isolate by examining its morphological, physiological and chemotaxonomic properties and by analysing its 16S rDNA. Our results indicate that the isolate should be placed in a new species of a novel genus, for which the name *Hongia koreensis* is proposed. Strain LM 161^T has been deposited in the Culture Collection Centre of the Institute of Microbiology, Seoul National University (IMSNU) as strain IMSNU 50530^T.

METHODS

Micro-organisms and culture conditions. Strain LM 161^T was isolated, using the dilution plating method, from soil collected at a gold-mine cave in Kongju, Republic of Korea. The soil suspensions were pretreated at 30 °C for 20 min before plating (Lee, 1996). The medium used for selective isolation was a tap-water agar (pH 7.0) supplemented with 50 g cycloheximide ml⁻¹ and 50 g nystatin ml⁻¹. After the purity had been checked, the organism was subcultured on yeast extract/malt extract agar [medium 2 of the International *Streptomyces* Project (ISP)] (Shirling & Gottlieb, 1966) at 28 °C. The following reference strains were used to compare chemotaxonomic properties: *Aeromicrobium erythreum* DSM 8599^T, *Aeromicrobium fastidiosum* IFO 14987^T, *Nocardioides albus* IFO 13917^T, *Nocardioides jensenii* KCTC 9134^T, *Nocardioides luteus* IFO 14491^T, *Nocardioides simplex* IFO 12069^T, *Luteococcus japonicus* IFO 12422^T, *Microlunatus phosphovorus* JCM 9379^T, *Propioniferax innocua* DSM 8251^T and *Kineosporia aurantiaca* IFO 13890^T.

Morphological and cultural characteristics. Growth and morphology were examined by using the ISP media described by Shirling & Gottlieb (1966) and the media described by Waksman (1961). The formation of melanin was investigated on peptone/yeast extract/iron agar (ISP medium 6) and tyrosine agar (ISP medium 7). For scanning electron microscopy, cultures grown at 28 °C for 14 d on oatmeal agar (ISP medium 3) and inorganic salts/starch agar (ISP medium 4) were prepared by cutting agar blocks from the growth medium. The agar blocks were fixed with 1 % (w/v) osmium tetroxide, were dehydrated through a graded ethanol series and the ethanol was replaced with isoamyl acetate. After critical-point drying, the samples were sputter-coated with gold under a vacuum and observed under a Stereoscan model 260 scanning electron microscope.

Physiological characteristics. All physiological tests were performed at 28 °C (unless otherwise indicated). Oxidase activity was checked by examining the oxidation of 1 % (w/v) tetramethyl-*p*-phenylenediamine on paper discs.

Catalase activity was determined by the production of bubbles after the addition of a drop of 3 % (v/v) hydrogen peroxide solution on to a slide. Growth was tested over a range of temperatures (10–45 °C) and pH values (5–10). Nitrate reduction, urease activity, hydrogen sulfide production, decomposition of hippurate and hydrolysis of arbutin, casein, aesculin, gelatin and starch were tested as described by Mac Faddin (1980). Hydrolysis of the DNA was determined using Bacto DNase test agar (Difco). Pectinolytic activity was examined according to the method of Hankin *et al.* (1971). Acid production from carbohydrates was determined by means of a colour change from green to yellow in Bacto OF basal medium (Difco). The utilization of organic acids as carbon sources and the decomposition of adenine, hypoxanthine, DL-tyrosine and xanthine were examined using previously described methods (Gordon *et al.*, 1974). The utilization of carbohydrates as the sole carbon source was studied according to the method of Pridham & Gottlieb (1948). NaCl-tolerance studies were performed on nutrient agar containing NaCl at final concentrations of 3, 5, 7 and 10 % (w/v). Susceptibility to antibiotics and chemical inhibitors was determined as described previously (Williams *et al.*, 1983). To determine sensitivity to lysozyme, a 0.1 % solution of lysozyme was sterilized by membrane filtration and added to nutrient agar at a final concentration of 0.01 %. Antimicrobial activity was tested for nine target organisms by using the overlay technique of Williams *et al.* (1983).

Cell wall analysis. Purified cell wall peptidoglycan was prepared according to the method of Yamada & Komagata (1972) and its amino acid composition was analysed by two-dimensional, ascending TLC on cellulose plates by using the solvent systems described previously (Schleifer & Kandler, 1972). The molar ratio of amino acids was determined by GC of *N*-heptafluorobutyl amino acid isobutyl esters (O'Donnell *et al.*, 1982b). The isomers of diaminopimelic acid were determined according to the method of Stanek & Roberts (1974). Cell wall sugars as alditol acetates were determined by GC (Saddler *et al.*, 1991). GC analyses were performed with a Hewlett Packard model HP5890A gas chromatograph equipped with a flame-ionization detector and integrator. The acyl type of the cell wall was determined using the colorimetric method of Uchida & Aida (1984).

Lipid analysis. Cellular fatty acid methyl esters were prepared from cells grown on yeast extract/glucose broth at 30 °C for 3 d by alkaline methanolysis (Minnikin, 1988) and were analysed with a Hewlett Packard model 5890A gas chromatograph equipped with a SPB-1 fused silica capillary column (0.25 mm × 30 m; Supelco). The column temperature was programmed according to the manufacturer's instructions. The fatty acid methyl esters were identified by comparison with the Bacterial Acid Methyl Ester CP MIX (catalogue no. 1114; Matreya). Phospholipids and menaquinones were extracted using the integrated method of Minnikin *et al.* (1984). The resultant phospholipids were separated by two-dimensional TLC (Minnikin *et al.*, 1977) and identified by comparison with standards and by spraying with specific reagents (Collins *et al.*, 1982). The purified menaquinones were analysed using HPLC as described by Kroppenstedt (1985). Mycolic acids were determined using TLC (Minnikin *et al.*, 1980).

DNA base composition. Genomic DNA was extracted according to the method described by Hopwood *et al.* (1985). The G+C content of the DNA was determined by using the thermal denaturation method (Marmur & Doty, 1962).

Molecular cloning and sequencing of the 16S rRNA gene (rDNA). The amplification of 16S rDNA was carried out by using a DNA thermal cycler (Perkin-Elmer) in combination with the following two universal primers: 27f (5'-AG-AGTTTGATCMTGGCTCAG OH-3'; positions 8–27 (*Escherichia coli* numbering) and 1525r (5'-AAGGAGGT-GWTCCARCC OH-3'; positions 1541–1525) (Brosius *et al.*, 1978). The temperature programme consisted of 30 cycles of denaturation at 94 °C for 1 min, primer annealing at 55 °C for 1 min and extension at 72 °C for 3 min. For the last cycle, the reaction mixture was further incubated at 72 °C for 10 min. The PCR product was purified with a GeneClean kit (Bio 101) and ligated into vector pGEM-T (Promega) according to the manufacturer's instructions. The ligation product was transformed into competent *E. coli* DH5 α cells. The DNA sequence was determined by using a model ALFexpress DNA sequencer (Pharmacia Biotech) and the Cy5 AutoRead Sequencing kit (Pharmacia).

Phylogenetic analysis. An almost complete 16S rDNA sequence was obtained from partial sequences derived from sequencing reactions by using a DNASIS program. The 16S rDNA sequence determined and previously published sequences of reference actinomycetes were aligned by using the program CLUSTAL W (Thompson *et al.*, 1994). Evolutionary distances were calculated by the method of Jukes & Cantor (1969). A phylogenetic tree was reconstructed by the tree-making algorithms contained in the PHYLIP package (Felsenstein, 1993). The topology of the tree was evaluated by performing bootstrap analysis (Felsenstein, 1985) of the neighbour-joining data, using 1000 resamplings.

Nucleotide sequence accession numbers. The reference sequences, which were used in the phylogenetic analysis, were sequences available from the DDBJ, EMBL and GenBank nucleotide sequence databases under the following accession numbers: *Actinoplanes philippinensis* DSM 43019^T, X93187; *Aeromicrobium erythreum* NRRL B-3381^T, M37200; *Aeromicrobium fastidiosum* NCIMB 12713^T, X76862; *Apotobium minutum*, ATCC 33267^T, M59059; *Friedmanniella antarctica*, DSM 11053^T, Z78206; *Intrasporangium calvum*, IFO 12989^T, D85486; *Kineosporia aurantiaca*, ATCC 28727^T, X87110; *Luteococcus japonicus* DSM 10546^T, Z78208; *Microbacterium lacticum*, DSM 20427^T, X77441; *Microlunatus phosphovorus*, DSM 10555^T, Z78207; *Nocardia asteroides*, DSM 43757^T, X80606; *Nocardioides albus* DSM 43109^T, X53211; *Nocardioides jensenii* DSM 20641^T, X53214; *Nocardioides simplex* DSM 20130^T, Z78212; *Propionibacterium freudenreichii* DSM 20271^T, X53217; *Saccharothrix australiensis*, ATCC 31947^T, X53193; *Sporichthya polymorpha* DSM 46113^T, X72377; *Streptomyces griseus* subsp. *griseus* KCTC 9080^T, M76388; *Streptosporangium roseum*, DSM 43021^T, X89947; *Terracoccus luteus*, DSM 44267^T, Y11928; and *Terrabacter tumescens* DSM 20308^T, X83812. The sequence of *Propioniferax innocua* was obtained from the study of Pitcher & Collins (1991).

RESULTS

Morphological and cultural characteristics

A study of its cultural and morphological characteristics showed that strain LM 161^T has morphological properties similar to features that are characteristic of nocardioform organisms. Strain LM 161^T produced true mycelia with well-developed, irregularly branched hyphae. The aerial mycelium was white and

Fig. 1. Scanning electron micrograph of aerial mycelium of strain LM 161^T grown on inorganic salts/starch agar (ISP medium 4) for 14 d. Bar, 2 μ m.

Fig. 2. Scanning electron micrograph of substrate mycelium of strain LM 161^T grown on oatmeal agar (ISP medium 3) for 14 d. (a) Hyphal swelling. (b) Budding. Bars, 2 μ m.

fragmented into rod-shaped elements (Fig. 1). The substrate mycelium was creamy and also fragmented into short to elongated rod-shaped elements (Fig. 2). Hyphal swelling on the hyphal tips (Fig. 2a) and

Table 1. Cellular fatty acid composition (%) of strain LM 161^T and the type strains of related taxa that belong to the suborder *Propionibacterineae*

Values less than 1 % not shown.

Taxon	Saturated					Unsaturated				Iso				Anteiso		10-Methyl		
	C ₁₄	C ₁₅	C ₁₆	C ₁₇	C ₁₈	C ₁₅	C ₁₆	C ₁₇	C ₁₈	C ₁₄	C ₁₅	C ₁₆	C ₁₇	C ₁₅	C ₁₇	C ₁₆	C ₁₇	C ₁₈
Strain LM 161 ^T		1.9	6.6	4.0	1.7					5.9	12.3	24.0	5.1	24.7	4.9	5.1	4.0	
<i>Aeromicrobium erythreum</i> DSM 8599 ^T	1.2		25.2	2.0	10.9				15.1			1.0						34.5
<i>Aeromicrobium fastidiosum</i> IFO 14987 ^T			12.9	1.8	16.1		1.5		48.2					2.0		8.7	9.1	
<i>Nocardioides albus</i> IFO 13917 ^T			6.4	3.7	6.7	1.0	1.8		2.4	1.6	2.2	20.3	1.7	1.0	3.8	4.8	2.7	34.0
<i>Nocardioides luteus</i> IFO 14491 ^T			4.2	1.8	4.0				3.6		1.0	50.7	1.7	2.4		5.1		13.4
<i>Nocardioides jensenii</i> KCTC 9134 ^T			10.1	3.4	10.2		2.6		19.5		2.8	17.4	3.4	4.8		1.8	6.1	25.9
<i>Nocardioides simplex</i> IFO 12069 ^T			4.9	2.8	7.3		1.9		49.2		1.7	13.4	1.8	6.7				2.3
<i>Luteococcus japonicus</i> IFO 12422 ^T		2.9	4.5	2.8	4.8	7.1	30.4	20.6	24.4									
<i>Microtholunatus phosphovorius</i> JCM 9379 ^T			1.2		1.0	3.1					20.0	11.9	7.5	48.9	7.3			
<i>Propioniferax innocua</i> DSM 8251 ^T		4.0	3.3	2.1			3.1		4.8	3.4	15.8	7.6	2.8	36.2	11.6			

budding on the hyphae (Fig. 2b) were observed on oatmeal agar. Strain LM 161^T grew well on most solid media, as follows: yeast extract/malt extract agar (ISP medium 2), oatmeal agar (ISP medium 3), inorganic salts/starch agar (ISP medium 4), tyrosine agar (ISP medium 7), glucose/asparagine agar, Hickey–Tresner agar, nutrient agar and Bennett agar. Melanin pigments were not produced on any of the media tested.

Physiological characteristics

Strain LM 161^T grew strictly aerobically. The temperature and pH ranges for growth were 10–37 °C and pH 7–10, respectively. Catalase, urease and H₂S were produced. Oxidase and nitrate reductase were not produced. Acid was produced from D-raffinose. No acid was produced from the following compounds: D-arabinose, L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, inulin, D-lactose, maltose, D-mannose, D-melezitose, melibiose, methyl α-D-glucoside, methyl α-D-mannoside, L-rhamnose, L-ribose, salicin, sucrose, D-trehalose, D-xylose, adonitol, 2, 3-butanediol, *meso*-erythritol, glycerol, *meso*-inositol, D-mannitol and 1, 2-propanediol.

Gelatin, casein, starch, arbutin and aesculin were hydrolysed. Pectin and DNA were not hydrolysed. Xanthine, hypoxanthine, DL-tyrosine and sodium hippurate were decomposed but adenine was not decomposed. Strain LM 161^T utilized D-arabinose, L-arabinose, D-cellobiose, D-fructose, D-galactose, inu-

lin, D-lactose, D-lyxose, D-mannose, D-melezitose, melibiose, methyl α-D-glucoside, methyl α-D-mannoside, D-raffinose, L-rhamnose, sucrose, D-trehalose, D-xylose, adonitol, D-mannitol, sodium acetate, *trans*-aconitate, sodium citrate, sodium fumarate, α-ketoglutarate, sodium lactate, sodium malate, sodium malonate, sodium oxalate, sodium propionate, sodium pyruvate and sodium succinate as carbon sources.

Utilization of dextran, D-glucosamine, L-sorbose, dulcitol, D-sorbitol, D-xylitol, *cis*-aconitate, sodium benzoate, sodium formate, sodium maleate, sodium salicylate, sebacate, potassium sorbate or sodium tartarate was not observed. Strain LM 161^T was resistant to lysozyme and grew in the presence of 0.1 % phenol, 0.01 % potassium tellurite, 0.00005 % crystal violet and 0.001 % brilliant green, but not in the presence of 3 % (w/v) sodium chloride, 0.01 % sodium azide, 0.3 % phenylethanol or 0.01 % thallos acetate.

Strain LM 161^T was susceptible to 40 g cephaloridine ml⁻¹ and 20 g neomycin ml⁻¹, but resistant to 4 g tetracycline ml⁻¹, 4 g rifampin ml⁻¹, 20 g streptomycin ml⁻¹, 160 g oleandomycin ml⁻¹ and 4 g vancomycin ml⁻¹. Antimicrobial activity against any of the following organisms was not observed: *Bacillus subtilis*, *Micrococcus luteus*, *Staphylococcus aureus*, *Streptomyces murinus*, *Escherichia coli*, *Enterobacter aerogenes*, *Saccharomyces cerevisiae*, *Candida albicans* and *Aspergillus niger*.

Fig. 3. Phylogenetic tree displaying the position of strain LM 161^T with regard to other related taxa. The neighbour-joining tree was based on 1256 nucleotide positions. The numbers at the branching points are bootstrap values expressed as percentages out of 1000 replications. Bar, 5 inferred nucleotide substitutions per 100 nucleotides.

Chemotaxonomic characteristics

The amino acids composing the peptidoglycan layer of strain LM 161^T were LL-DAP, alanine, glycine and glutamic acid (molar ratio, approx. 1:2:1:1), indicating that the wall chemotype is type I, according to the classification of Lechevalier & Lechevalier (1970), and that the peptidoglycan type is A3 γ , as described by Schleifer & Kandler (1972). The acyl type was acetyl. Mannose, glucose, galactose and ribose were present as cell wall sugars. The major menaquinone was tetrahydrogenated menaquinone with nine isoprene units [MK-9(H₄)]. The polar lipid fraction contained phosphatidylcholine, diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol, but phosphatidylethanolamine and glucosamine-containing phospholipids were absent; this corresponds to the phospholipid type III pattern as defined by Lechevalier *et al.* (1981). The predominant cellular fatty acids of strain LM 161^T were 12-methyltetradecanoic acid (anteiso-C_{15:0} acid) and 14-methylpentadecanoic acid (iso-C_{16:0} acid). Straight-chain saturated and 10-methyl-branched fatty acids were also present in small amounts, but unsaturated fatty acids and tuberculo-stearic acid (10-methyloctadecanoic acid) were not detected. The fatty acid profiles differ somewhat from those of the reference strains. The fatty acid profiles of the reference strains analysed in our study differed

slightly from the profile reported previously (Miller *et al.*, 1991; Nakamura *et al.*, 1995; Schumann *et al.*, 1997; Tamura *et al.*, 1994), but the relative distribution of each chain length was similar. The cellular fatty acid compositions of strain LM 161^T and the type strains of related taxa are shown in Table 1. Mycolic acids were absent. The G + C content of the DNA of strain LM 161^T was 71.3 mol %.

Phylogenetic analysis

The almost complete 16S rDNA sequence of strain LM 161^T, consisting of 1510 nucleotides, was aligned and compared with the sequences of other actinomyces taxa. A total of 1256 nucleotides present in all strains between positions 39 and 1484 (*E. coli* numbering) were used in the analyses. A phylogenetic tree shown in Fig. 3 was reconstructed by the neighbour-joining method (Saitou & Nei, 1987) from evolutionary distances (Jukes & Cantor, 1969). The phylogenetic analysis based on the 16S rDNA sequence comparisons showed that strain LM 161^T represents a distinct line of descent within the radiation of the order Actinomycetales (Fig. 3). It formed a branching point at the root of members of two families Nocardioidaceae and Propionibacteriaceae belonging to the suborder Propionibacterineae Rainey *et al.*

(Stackebrandt *et al.*, 1997). The type and only species of the genus *Kineosporea*, *K. aurantiaca* ATCC 28727^T, which contains *meso*-DAP in the peptidoglycan in addition to LL-DAP and has a phylogenetic position that remains to be determined, was united with *Sporichthya polymorpha* (93.2% sequence similarity) in our analysis.

DISCUSSION

The 16S rDNA sequence analysis placed strain LM 161^T outside the clade corresponding to the suborder *Propionibacterineae* Rainey *et al.* of the order *Actinomycetales* (Buchanan) Stackebrandt *et al.* (1997) (Fig. 3). The relationship between strain LM 161^T and the cluster comprising the members of the families *Nocardioidaceae* and *Propionibacteriaceae* is supported by a high bootstrap value of 88% and by the presence of all but one signature nucleotide previously defined for the suborder *Propionibacterineae* (Stackebrandt *et al.*, 1997). In addition, the secondary structure of the 16S rDNA of strain LM 161^T contains several nucleotide pairs differing from those of members of the families *Nocardioidaceae* and *Propionibacteriaceae*, represented by the 16S rDNA nucleotide pairs at positions 370–391 (C–G), 371–390 (G–C), 378–385 (A–U), 591–648 (C–A), 671–735 (G–C), 776 (A), 1001–1039 (G–C) and 1006–1023 (U–G). Pairwise comparison of the primary 16S rDNA structures shows that strain LM 161^T exhibits mean nucleotide similarity values of $92.7 \pm 1.4\%$ with members of the family *Nocardioidaceae* and $90.9 \pm 1.9\%$ with members of the family *Propionibacteriaceae*. The results of our phylogenetic analysis clearly indicated that strain LM161^T represents a distinct lineage in the order *Actinomycetales*.

In addition to 16S rDNA sequence differences, strain LM 161^T has chemotaxonomic properties that allow it to be clearly differentiated from its phylogenetic neighbours (Fig. 3) and other LL-DAP-containing actinomycete genera. The chemotaxonomic characteristics of LL-DAP-containing actinomycete genera were recently re-examined by Schumann *et al.* (1997) and the majority of the previously reported characteristics were also confirmed. Phospholipid patterns in combination with diamino acids are extremely useful in distinguishing strain LM 161^T from the LL-DAP-containing actinomycete genera and other taxa previously reported in the order *Actinomycetales*. To date, the phospholipid type III pattern (the diagnostic phospholipid is phosphatidylcholine) and the occurrence of LL-DAP have only been reported for the actinomycete *Kineosporea aurantiaca* (Itoh *et al.*, 1989). All of the LL-DAP-containing actinomycete genera, with the exception of *K. aurantiaca*, have phospholipid type I or II patterns according to Lechevalier *et al.* (1981) in that phosphatidylcholine or glucosamine-containing phospholipids were absent from their phospholipid compositions (Prauser *et al.*, 1997; Schumann *et al.*, 1997). The diagnostic phospholipid profiles (of the phospholipid type III pattern; Table 2) and the cellular fatty acid profiles of the isolate (Table 1) are clearly distinguishable from those of actinomycete genera with LL-DAP in their peptidoglycan (Collins *et al.*, 1989; Miller *et al.*, 1991; Nakamura *et al.*, 1995; O'Donnell *et al.*, 1982b; Prauser *et al.*, 1997; Rainey *et al.*, 1993; Schumann *et al.*, 1997; Tamura *et al.*, 1994; Williams *et al.*, 1989). Although *K. aurantiaca* is similar to the isolate in terms of menaquinone types and amino acids in the peptide subunit of the peptidoglycan, as well as in terms of phospholipid composition, it differs from the isolate in having *meso*-

Table 2. Differential characteristics of strain LM 161^T and related taxa that belong to the suborder *Propionibacterineae*

Abbreviations: L-Ala, L-alanine; Gly, glycine; DPG, diphosphatidylglycerol; PC, phosphatidylcholine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PG-OH, phosphatidylglycerol containing 2-hydroxy fatty acids; PL, unknown phospholipid(s); GL, unknown glycolipid(s).

Taxon*	Morphology	Amino acid at position 1 of peptide subunits	Major menaquinone†	Polar lipids	Predominant fatty acids*	G + C content (mol %)
Strain LM 161 ^T	Hyphae	L-Ala	MK-9(H ₄)	DPG, PC, PG, PI	ai-C _{15:0} , i-C _{16:0}	71.3
<i>Nocardioides</i>	Hyphae/rods	L-Ala	MK-8(H ₄)	DPG, PG, PG-OH, PL	i-C _{16:0} , C _{18:1} , TSBA	66.4–72.7
<i>Aeromicrobium</i>	Rods	L-Ala	MK-9(H ₄)	PG, PE	C _{16:0} , C _{18:1} , TSBA	71–73
<i>Propionifera</i>	Rods	L-Ala	MK-9(H ₄)	GL, PE, PG, PL	ai-C _{15:0} , i-C _{15:0}	59–63
<i>Friedmanniella</i>	Cocci	Gly	MK-9(H ₄)	DPG, PG, PI, PL	ai-C _{15:0} , i-C _{15:0}	73
<i>Luteococcus</i>	Cocci	L-Ala	MK-9(H ₄)	DPG, GL, PG, PI,	C _{16:1} , C _{17:1} , C _{18:1}	66–68
<i>Microtholus</i>	Cocci	Gly	MK-9(H ₄)	DPG, PG, PI, PL	ai-C _{15:0} , i-C _{15:0} , i-C _{16:0}	67.9

* Data from Collins *et al.* (1989, 1994), Lechevalier (1989), Miller *et al.* (1991), Nakamura *et al.* (1995), O'Donnell *et al.* (1982a), Schumann *et al.* (1997), Tamura & Yokota (1994), Tamura *et al.* (1994) and Yokota *et al.* (1994).

† The abbreviations for menaquinones and fatty acids are illustrated by the following examples: MK-9(H₄) = menaquinone with two out of nine isoprene units hydrogenated; ai-C_{15:0}, 12-methyltetradecanoic acid; i-C_{16:0}, 14-methylpentadecanoic acid; TSBA, tuberculostearic acid (10-methyloctadecanoic acid); C_{16:1}, hexadecenoic acid.

DAP as an additional diamino acid in the peptidoglycan, in having no iso/anteiso branched fatty acid profiles, in cell morphology and in having spore motility (Itoh *et al.*, 1989). Phylogenetically, *K. aurantica* clusters with the members of the family *Sporichthyaceae* Rainey *et al.*, 1997 of the suborder *Frankineae* Stackebrandt *et al.* (Stackebrandt *et al.*, 1997) (Fig. 3). The chemotaxonomic characteristics of *K. aurantiaca* were also confirmed in our study (data not shown).

The other high G+C DNA bacteria that have been described as having the phospholipid type III pattern are mainly nocardioform (rarely spore-forming actinomycetes) and contain the genera *Actinopolyspora* (Lechevalier, 1989), *Faenia* (Lechevalier, 1989), *Nocardopsis* (Mayer, 1989), *Saccharopolyspora* (Lechevalier, 1989), *Pseudonocardia* (Lechevalier, 1989) and *Catenuloplanes* (Yokota *et al.*, 1993). However, the isolated strain differs from these genera in terms of the diagnostic diamino acid and sugars in the peptidoglycan.

The phylogenetic analysis of 16S rDNA sequence comparisons reveals that the closest (albeit distant) phylogenetic relatives of the isolated strain are members of the families *Nocardioidaceae* and *Propionibacteriaceae* (Fig. 3). Among them, the genera *Aeromicrobium* (Miller *et al.*, 1991; Tamura & Yokota, 1994), *Luteococcus* (Tamura *et al.*, 1994) and *Propioniferax* (Yokota *et al.*, 1994) share, with the isolated strain, the following chemotaxonomic properties: the amino acids of the peptide subunit of the peptidoglycan, the occurrence of glycine in the interpeptide bridge and the menaquinone type MK-9(H₄). However, these genera differ from the isolated strain in terms of the G+C content of the DNA, in the composition of fatty acids and phospholipids and in the cell morphology (Table 2). Morphologically, two species of the genus *Nocardioides*, *Nocardioides albus* (Prauser, 1976) and *Nocardioides luteus* (Prauser, 1984), resemble the isolate in that all of these organisms produce well-developed, fragmented mycelium, but the genus *Nocardioides* is differentiated from our strain in possessing menaquinone type MK-8(H₄), in lacking the diagnostic phospholipid phosphatidylcholine and by the fatty acid profiles (Tables 1 and 2).

Thus, phylogenetic evidence based on 16S rDNA sequence comparisons and chemotaxonomic properties indicate that the isolate can be readily distinguished from the previously described genera of the order *Actinomycetales*. Therefore, the new genus *Hongia* is proposed to accommodate the new isolate. *Hongia koreensis* is the type species. The type strain is strain LM 161^T.

Description of *Hongia* gen. nov.

Hongia [Hong'i.a. M.L. fem. n. *Hongia* named after Soon-Woo Hong (1927–1988), a Korean microbiologist who devoted his life to the study of soil micro-organisms].

The aerial mycelium is white and fragments into rod-shaped elements. The substrate mycelium is creamy and fragments into short or elongated rod-shaped elements. The substrate hyphal swellings and budding are formed on oatmeal agar. Aerobic. Gram-positive. Not acid-fast. Oxidase-negative. Catalase- and urease-positive. Chemo-organotrophic. Non-motile. Grows at 10–37 °C. Does not grow at 45 °C. The cell wall peptidoglycan contains LL-diaminopimelic acid, alanine, glycine and glutamic acid (approx. 1:2:1:1). The acyl type is acetyl. Mannose, glucose, galactose and ribose are present as cell wall sugars. The polar lipids are phosphatidylcholine, diphosphatidylglycerol, phosphatidylglycerol and phosphatidyl-inositol. The major menaquinone is MK-9(H₄). The predominant fatty acids are 12-methyltetradecanoic acid and 14-methylpentadecanoic acid. Straight-chain saturated and 10-methyl fatty acids occur in small amounts. Mycolic acids are absent. The G+C content of the DNA is 71.3 mol%. Phylogenetically, this genus branches at the root of members of the suborder *Propionibacterineae*. All signature nucleotides defined for the suborder *Propionibacterineae* are also found in strain LM 161^T, except for base-pair positions 671–735, at which strain LM 161^T has a G–C pair rather than an A–U pair. The type species is *Hongia koreensis*.

Description of *Hongia koreensis* sp. nov.

Hongia koreensis (ko.re.en'sis. M.L. adj. *koreensis* pertaining to Korea, the location of the soil sample from which the organism was isolated).

The aerial mycelium is white and fragments into rod-shaped elements. The substrate mycelium is creamy and fragments into short or elongated rod-shaped elements. The substrate hyphal swellings and budding are formed on oatmeal agar. Nitrate is not reduced to nitrite. H₂S is produced. Utilizes D-arabinose, L-arabinose, D-cellobiose, D-fructose, D-galactose, inulin, D-lactose, D-lyxose, D-mannose, D-melezitose, melibiose, methyl α-D-glucoside, methyl α-D-mannoside, D-raffinose, L-rhamnose, sucrose, D-trehalose, D-xylose, adonitol, D-mannitol, sodium acetate, *trans*-aconitate, sodium citrate, sodium fumarate, α-ketoglutarate, sodium lactate, sodium malate, sodium malonate, sodium oxalate, sodium propionate, sodium pyruvate and sodium succinate as carbon sources. Gelatin, casein, starch, arbutin and aesculin are hydrolysed. Pectin and DNA are not hydrolysed. Xanthine, hypoxanthine, DL-tyrosine and sodium hippurate are decomposed but adenine is not decomposed. Acid is produced from D-raffinose. No acid is produced from D-arabinose, L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, inulin, D-lactose, maltose, D-mannose, D-melezitose, melibiose, methyl α-D-glucoside, methyl α-D-mannoside, L-rhamnose, L-ribose, salicin, sucrose, D-trehalose, D-xylose, adonitol, 2, 3-butanediol, *meso*-erythritol, glycerol, *meso*-inositol, D-mannitol or 1, 2-propanediol. Tolerant to lysozyme, 0.1% phenol, 0.01% potassium tellurite,

0.00005% crystal violet and 0.001% brilliant green, but not to 3% (w/v) sodium chloride, 0.01% sodium azide, 0.3% phenylethanol or 0.01% thallos acetate. Susceptible to 40 g cephaloridine ml⁻¹ and 20 g neomycin ml⁻¹, but resistant to 4 g tetracycline ml⁻¹, 4 g rifampin ml⁻¹, 20 g streptomycin ml⁻¹, 160 g oleandomycin ml⁻¹ and 4 g vancomycin ml⁻¹. The cell wall peptidoglycan contains LL-diaminopimelic acid, alanine, glycine and glutamic acid (approx. 1:2:1:1). The acyl type is acetyl. Mannose, glucose, galactose and ribose are present as cell wall sugars. The diagnostic polar lipid is phosphatidylcholine. The major menaquinone is MK-9(H₄). The predominant fatty acids are 12-methyltetradecanoic acid and 14-methylpentadecanoic acid. Mycolic acids are absent. The G+C content of the DNA of the type strain is 71.3 mol%. The type strain of *Hongia koreensis* is strain LM 161^T (= IMSNU 50530^T). The only known isolation site is a gold-mine cave in Kongju, Republic of Korea.

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