**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 Kongiu, 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, diphasphatidylglycerol, phosphatidylserine and phosphatidylglycerol, 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

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**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), *Microlunatus* (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* (Kalakoutski et al., 1967), *Terrabacter* (Collins et al., 1989) and *Terracoccus*.

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**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 161T (T = type strain) was isolated from a gold-mine cave in Kongju, Republic of Korea. Strain LM 161T 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 origin 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 161T has been deposited in the Culture Collection Centre of the Institute of Microbiology, Seoul National University (IMSNU) as strain IMSNU 50530T.

METHODS

Micro-organisms and culture conditions. Strain LM 161T 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 8599T, Aeromicrobium fastidiosum IFO 14987T, Nocardioidei albus IFO 13917T, Nocardioidei jenseni KCTC 9134T, Nocardioidei luteus IFO 14491T, Nocardioidei simplex IFO 12069T, Luteococcus japonicus IFO 12422T, Microflavus phosphovorus JCM 9379T, Propionifexerix innocua DSM 8251T and Kineospora aurantia IFO 13890T.

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 Fadden (1980). Hydrolysis of the DNA was determined using Bacto DNAse test agar (Difco). Peptidolytic 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 and energy sources was tested by growth on several commercially available media (Biolab) supplemented with 1% (w/v) of the relevant compound. Hydrolysis of the DNA was determined using Bacto DNAse test agar (Difco). Peptidolytic 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 and energy sources was tested using the ISP medium system as described previously (Schleifer & Kandler, 1972). For the determination of the fatty acid pattern, cells were grown on yeast extract/glucose broth at 30°C for 3 d by alkaline methanolation (Minnikin, 1988) and then analysed by using 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 methanolation (Minnikin, 1988) and then analysed using a Hewlett Packard model 5890A gas chromatograph equipped with a SPB-1 fused silica capillary column (0.25 mm x 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'-AGAGTTTGATCMTGTCAG-3'); positions 8–27 (Escherichia coli numbering) and 1525r (5'-AAGGAGGT-GWTCARC-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 DH5α 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 43019T, X93187; Aeromicrobium erythreum NRRRL B-3381T, M37200; Aeromicrobium fastidiosum NCIMB 12713T, X76862; Apotobium minutum, ATCC 33267T, M59059; Friedmanniella antarctica, DSM 11053T, Z78206; Intrasporangium calvum, IFO 12989T, D85486; Kinesospora aurantiaca, ATCC 28727T, X87110; Luteococcus japonicus DSM 10546T, Z78208; Microbacterium lacticum, DSM 20427T, X77441; Microlunatus phosphovorus, DSM 10555T, Z78207; Nocardia asteroides, DSM 43757T, X80606; Nocardioles albus DSM 43109T, X53211; Nocardioles jensenii DSM 20641T, X53214; Nocardioles simplex DSM 20130T, Z78212; Propionibacterium.freudenreichii DSM 20271T, X53217; Saccharothrix australiensis, ATCC 31947T, X53193; Sporichthya polymorpha DSM 46113T, X72377; Streptomyces griseus subsp. griseus KCTC 9080T, M76388; Streptosporangium roseum, DSM 43021T, X89947; Terracoccus lutus, DSM 44267T, Y11928; and Terrabacter tunescens DSM 20308T, X83812. The sequence of Propioniferax inosus 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 161T has morphological properties similar to features that are characteristic of nocardioform organisms. Strain LM 161T produced true myelia with well-developed, irregularly branched hyphae. The aerial mycelium was white and 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
budding on the hyphae (Fig. 2b) were observed on oatmeal agar. Strain LM 161T 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 5), 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 161T 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, D-raffinose, L-rhamnose, sucrose, D-ribose, D-sorbitol, 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, D,L-tyrosine and sodium hippurate were decomposed but adenine was not decomposed. Strain LM 161T utilized 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-ribose, D-sorbitol, D-xylose, adonitol, 2, 3-butanediol, meso-erythritol, glycerol, meso-inositol, D-mannitol and 1, 2-propanediol.

Utilization of dextran, D-glucosamine, D-sorbitose, dulcitol, D-sorbitol, D-xyitol, cis-aconitate, sodium benzoate, sodium formate, sodium maleate, sodium salicylate, sebacate, potassium sorbate or sodium tartarate was not observed. Strain LM 161T 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% thallous acetate.

Strain LM 161T 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.

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

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Saturated</th>
<th>Unsaturated</th>
<th>Iso</th>
<th>Anteiso</th>
<th>10-Methyl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C₁₄</td>
<td>C₁₅</td>
<td>C₁₆</td>
<td>C₁₇</td>
<td>C₁₈</td>
</tr>
<tr>
<td>Strain LM 161T</td>
<td>19</td>
<td>66</td>
<td>40</td>
<td>1-7</td>
<td></td>
</tr>
<tr>
<td>Aeromicrobium erythreum DSM 8599T</td>
<td>12</td>
<td>25</td>
<td>20</td>
<td>10-9</td>
<td></td>
</tr>
<tr>
<td>Aeromicrobium fastidiosum IFO 14987T</td>
<td>12-9</td>
<td>18</td>
<td>16-1</td>
<td></td>
<td>1.5</td>
</tr>
<tr>
<td>Nocardiodes albus IFO 13917T</td>
<td>6-4</td>
<td>3-7</td>
<td>67</td>
<td></td>
<td>1.0</td>
</tr>
<tr>
<td>Nocardiodes luteus IFO 14491T</td>
<td>4-2</td>
<td>18</td>
<td>4-0</td>
<td></td>
<td>3-6</td>
</tr>
<tr>
<td>Nocardiodes jensenii KCTC 9134T</td>
<td>10-1</td>
<td>3-4</td>
<td>10-2</td>
<td>2-6</td>
<td>19-5</td>
</tr>
<tr>
<td>Nocardiodes simplex IFO 12069T</td>
<td>4-9</td>
<td>28</td>
<td>7-3</td>
<td></td>
<td>1-9</td>
</tr>
<tr>
<td>Luteococcus japonicus IFO 12422T</td>
<td>2-9</td>
<td>4-5</td>
<td>2-8</td>
<td>4-8</td>
<td>7-1</td>
</tr>
<tr>
<td>Microlunatus phosphovorus JCM 9379T</td>
<td>1-2</td>
<td>1-0</td>
<td>3-1</td>
<td></td>
<td>2-0</td>
</tr>
<tr>
<td>Propioniferax innocua DSM 8251T</td>
<td>4-0</td>
<td>3-3</td>
<td>2-1</td>
<td>3-1</td>
<td>4-8</td>
</tr>
</tbody>
</table>

Values less than 1% not shown.
**Chemotaxonomic characteristics**

The amino acids composing the peptidoglycan layer of strain LM 161\(^T\) were L-L-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\(\gamma\), 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 \([MK-9(H_2)]\). The polar lipid fraction contained phosphatidylcholine, diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol, but phosphatidylethanolamine and gulosamine-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-methyltetrapentadecanoic 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 tuberculostearic 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 actinomycete 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 *Kineosporia, K. aurantiaca* ATCC 28727T, 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 161T 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 161T and the cluster comprising the members of the families *Nocardioidaceae* and *Propionibacterineae* 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 161T contains several nucleotide pairs differing from those of members of the families *Nocardioidaceae* and *Propionibacterineae*, 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 161T exhibits mean nucleotide similarity values of 92.7±1.4% with members of the family *Nocardioidaceae* and 90.9±1.9% with members of the family *Propionibacterineae*. The results of our phylogenetic analysis clearly indicated that strain LM 161T represents a distinct lineage in the order *Actinomycetales*.

In addition to 16S rDNA sequence differences, strain LM 161T 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 161T 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 *Kineosporia 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 gycosamine-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 3) 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., 1982a, b; 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 peptido- glycan, as well as in terms of phospholipid composition, it differs from the isolate in having meso-

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

<table>
<thead>
<tr>
<th>Taxon*</th>
<th>Morphology</th>
<th>Amino acid at position 1 of peptide subunits</th>
<th>Major menaquinone†</th>
<th>Polar lipids</th>
<th>Predominant fatty acids*</th>
<th>G + C content (mol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain LM 161T</td>
<td>Hyphae</td>
<td>l-Ala</td>
<td>MK-9(H₄)</td>
<td>DPG, PC, PG, PL</td>
<td>ai-C₁₂₅₀, i-C₁₆₀</td>
<td>71.3</td>
</tr>
<tr>
<td>Nocardioides</td>
<td>Hyphae/rods</td>
<td>l-Ala</td>
<td>MK-8(H₄)</td>
<td>DPG, PG, PG-OH, PL</td>
<td>i-C₁₀₀, C₁₆₀, C₁₈₀, TSBA</td>
<td>66-72</td>
</tr>
<tr>
<td>Aeromicrobium</td>
<td>Rods</td>
<td>l-Ala</td>
<td>MK-9(H₄)</td>
<td>DPG, PG, PE, PL</td>
<td>C₁₆₀, C₁₈₀, TSBA</td>
<td>71–73</td>
</tr>
<tr>
<td>Propioniferax</td>
<td>Rods</td>
<td>l-Ala</td>
<td>MK-9(H₄)</td>
<td>DPG, PG, PG, PL</td>
<td>ai-C₁₂₀, i-C₁₆₀</td>
<td>59–63</td>
</tr>
<tr>
<td>Friedmanniella</td>
<td>Cocci</td>
<td>Gly</td>
<td>MK-9(H₄)</td>
<td>DPG, GL, PG, PL</td>
<td>C₁₆₀, C₁₇₀, C₁₈₀</td>
<td>66–68</td>
</tr>
<tr>
<td>Luteococcus</td>
<td>Cocci</td>
<td>l-Ala</td>
<td>MK-9(H₄)</td>
<td>DPG, GL, PG, PL</td>
<td>ai-C₁₂₀, i-C₁₆₀, i-C₁₈₀</td>
<td>67-9</td>
</tr>
<tr>
<td>Microplanus</td>
<td>Cocci</td>
<td>Gly</td>
<td>MK-9(H₄)</td>
<td>DPG, PG, PL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


† 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-C₁₂₀, 12-methylexadecanoic acid; i-C₁₆₀, 14-methylpentadecanoic acid; TSBA, tuberculostearic acid (10-methyloctadecanoic acid); C₁₈₀, 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. aurantica* 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), *Nocardiosis* (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 peptidoglycan, its type alkyl side chain and the proportion of the fatty acid profile (Tables 1 and 2). 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 phosphatidylglycerol 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 161T.

**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 rhamnose are present as cell wall sugars. The polar lipids are phosphatidylcholine, diphosphatidylglycerol, phosphatidylglycerol and phosphatidylglycerol. The major menaquinone is MK-9(H4). The predominant fatty acids are 12-methylenetetradecanoic 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 the members of the suborder *Propionibacteriaceae*. All signature nucleotides defined for the suborder *Propionibacteriaceae* are also found in strain LM 161T, except for base-pair positions 671–735, at which strain LM 161T 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-cellubiose, 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-xyllose, 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, D,L-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-cellubiose, 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-xyllose, 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% thallous 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 oleano- mycin 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 phosphatidylglycholine. The major menaquinone is MK-9(H\(_2\)). The predominant fatty acids are 12-methyltetradecanoic acid and 14-methylpenta-decanoic 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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REFERENCES


