Gordonia hankookensis sp. nov., isolated from soil

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A Gram-positive, aerobic and non-motile bacterial strain, designated ON-33T, was subjected to a study based on a polyphasic approach to determine its exact taxonomic position. Strain ON-33T grew optimally at pH 7.0–7.5 and 30 °C. A neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showed that strain ON-33T fell within the clade comprising Gordonia species, clustering with Gordonia soli CC-AB07T, with which it exhibited 16S rRNA gene sequence similarity of 98.5%. The chemotaxonomic properties of strain ON-33T were consistent with those shared by members of the genus Gordonia. The peptidoglycan type was based on meso-diaminopimelic acid and the whole-cell sugars were arabinose and galactose. The predominant menaquinone was MK-9(H2). The major polar lipids were phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and phosphatidylinositol mannoside. The major fatty acids were C16:0, iso-C15:0 2-OH and/or C16:1ω7c, 10-methyl C18:0 and C18:1ω9c. The DNA G+C content was 66.9 mol%. Strain ON-33T exhibited a DNA–DNA relatedness value of 13% to G. soli DSM 44995T and could be differentiated from G. soli by differences in phenotypic characteristics. On the basis of the data obtained, strain ON-33T is considered to represent a novel species of the genus Gordonia, for which the name Gordonia hankookensis sp. nov., is proposed. The type strain is ON-33T (=KCTC 19599T=CCUG 57507T).

The genus Gordonia (formerly Gordona), first proposed by Tsukamura (1971), belongs phylogenetically to the family Gordoniaceae of the suborder Corynebacterineae (Stackebrandt et al., 1997). At the time of writing, the genus comprised at least 25 species with validly published names, including the recently described species Gordonia araii and G. effusa (Kageyama et al., 2006), G. defluvii (Soddell et al., 2006), G. soli (Shen et al., 2006), G. shandongensis (Luo et al., 2007), G. malaquae (Yassin et al., 2007) and G. cholesterolivorans (Drzyzga et al., 2009). In this study, we report the taxonomic characterization of a Gordonia-like bacterial strain, ON-33T, isolated from soil in Korea.

Strain ON-33T was isolated by means of the standard dilution-plating technique on trypticase soy agar (TSA; Difco) at 30 °C. G. soli DSM 44995T, which was used as a reference strain for DNA–DNA hybridization and phenotypic characterization, was obtained from the DSMZ. The morphological, physiological and biochemical characteristics of strain ON-33T were investigated using routine cultivation on TSA at 30 °C. Cell morphology was examined by using light microscopy (Nikon E600) with cells from exponentially growing cultures. The Gram reaction was determined using the bioMérieux Gram stain kit, according to the manufacturer’s instructions. Growth at various temperatures (4, 10, 20, 25, 28, 30, 35, 37, 40, 45 and 50 °C) was measured on TSA. The pH range for growth was determined in nutrient broth (Difco) with the pH adjusted to pH 4.0–10.5 (in increments of 0.5 pH units) by the addition of HCl or Na2CO3 prior to sterilization. Growth under anaerobic conditions was determined after incubation in an anaerobic chamber on TSA and on TSA supplemented with potassium nitrate (0.1%, w/v), both of which had been prepared anaerobically in a nitrogen atmosphere. Catalase and oxidase activities and hydrolysis of casein, gelatin, hypoxanthine, starch, Tweens 20, 40, 60 and 80, tyrosine, urea and xanthine were determined as described by Cowan & Steel (1965). Hydrolysis of aesculin and nitrate reduction was studied as described by Lányi (1987). Susceptibility to antibiotics was tested on TSA plates using antibiotic discs containing (µg per disc unless otherwise stated): polymyxin B (100 U), streptomycin (50), penicillin G (20 U), chloramphenicol (100), ampicillin (10), cephalothin (30), gentamicin (30), novobiocin (5), tetracycline (30), kanamycin (30), lincomycin (15), oleandomycin (15), neomycin (30) and carbenicillin (100). Utilization of various

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substrates, enzyme activity and other physiological and biochemical properties were tested by using the API 20E, API 20NE, API 50 CH and API ZYM systems (bioMérieux). Cells were suspended in AUX medium according to the manufacturer’s instructions to inoculate the API 50 CH system.

Cell biomass of strain ON-33T for DNA extraction and for the analyses of peptidoglycan type, polar lipids and isoprenoid quinones was obtained after cultivation in tryptase soy broth (Difco) at 30 °C. Chromosomal DNA was isolated and purified according to the method described by Yoon et al. (1996) with the exception that RNase T1 was used in combination with RNase A to minimize contamination with RNA. The 16S rRNA gene sequence was amplified by PCR using two universal primers as described previously (Yoon et al., 1998). Sequencing of the amplification product and phylogenetic analysis were performed as described by Yoon et al. (2003). The methods of Komagata & Suzuki (1987) were used to determine the isomer type of the diamino acid in the cell-wall peptidoglycan and the whole-cell sugars. Isoprenoid quinones were extracted according to the method of Minnikin et al. (1984) and analysed using reversed-phase HPLC and a YMC ODS-A (250 × 4.6 mm) column. For cellular fatty acid analysis, cell mass of strain ON-33T was harvested from TSA plates after incubation for 7 days at 30 °C. The fatty acids were extracted and fatty acid methyl esters were prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). Polar lipids were extracted according to the procedures described by Minnikin et al. (1984) and identified by two-dimensional TLC followed by spraying with appropriate detection reagents (Minnikin et al., 1984; Komagata & Suzuki, 1987). The DNA G+C content was determined by using the method of Tamaoka & Komagata (1984) with the modification that DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC. DNA–DNA hybridization was performed fluorometrically according to the method of Ezaki et al. (1989) using photobiotin-labelled DNA probes and microloudet wells. Hybridization was performed with five replications for each sample. The highest and lowest values obtained in each sample were excluded and the mean of the remaining three values was quoted as a DNA–DNA relatedness value.

The morphological, cultural, physiological and biochemical characteristics of strain ON-33T are given in the species description and in Table 1. The almost-complete 16S rRNA gene sequence of strain ON-33T that was determined in this study comprised 1476 nucleotides (approximately 96 % of the Escherichia coli 16S rRNA gene sequence). Comparative 16S rRNA gene sequence analysis showed that strain ON-33T is phylogenetically most closely related to the family Gordoniaceae. In the neighbour-joining phylogenetic tree based on 16S rRNA gene sequences (Fig. 1), strain ON-33T fell within the cluster comprising Gordonia species, clustering with G. soli CC-AB07T with a bootstrap resampling value of 83.1 %. The relationship between strain ON-33T and G. soli CC-AB07T was also found in the tree constructed using the maximum-parsimony algorithm. Strain ON-33T exhibited 98.5 % 16S rRNA gene sequence similarity to G. soli CC-AB07T and 95.3–98.0 % similarities to the type strains of other Gordonia species.

Strain ON-33T had meso-2,6-diaminopimelic acid as the diagnostic diamino acid in the cell-wall peptidoglycan. The whole-cell sugars were arabinose and galactose. Strain ON-33T contained MK-9(H2) as the predominant isoprenoid

### Table 1. Differential phenotypic characteristics of strain ON-33T (G. hankookensis sp. nov.) and G. soli

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>G. hankookensis ON-33T</th>
<th>G. soli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony colour</td>
<td>Moderate yellowish pink</td>
<td>Pale orange*</td>
</tr>
<tr>
<td>Oxidase</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aesculin</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Gelatin</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Starch</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Urea</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Assimilation of:†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythritol</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>D-Arabinose</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Ribose</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Adonitol</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Aesculin</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>L-Arabinitol</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Gluconate</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Adipate</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Phenylacetate</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>API ZYM</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Leucine arylamidase</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

*Pale yellow in this study.
†Data for G. soli are from this study.

Data for G. soli were taken from Shen et al. (2006) and this study. Both species are positive for Gram-staining, catalase, hydrolysis of Tween 80, utilization of galactose, glucose, fructose, mannose, inositol, mannitol, sorbitol, sucrose, trehalose, xylitol, turanose and D-arabitol and activities of alkaline phosphatase, esterase (C4), esterase lipase (C8), acid phosphatase, naphthol-AS-BI-phosphohydrolase and α- and β-glucosidases. Both species are negative for motility, hydrolysis of hypoxanthine, xanthine and tyrosine, utilization of glycerol, L-arabinose, D-xylene, L-xylene, methyl β-D-xylloside, sorbose, dulcitol, methyl α-D-mannoside, methyl α-D-glucoside, N-acetylglucosamine, amygdalin, arbutin, salicin, cellobiose, maltose, lactose, melibiose, inulin, melizitose, raffinose, starch, glycogen, gentiobiase, β-l-lyxose, β-tagatose, β-fucose, 2-ketogluconate and 5-ketogluconate and activities of lipase (C14), valine arylamidase, cystine arylamidase, trypsin, β-chymotrypsin, α- and β-galactosidases, β-glucuronidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. +, Positive; –, negative.
Gordonia hankookensis sp. nov.

Gordonia hankookensis (han.ko.o.ken’sis. N.L. fem. adj. hankookensis of Hankook, the Korean name of South Korea from where the type strain was isolated). Actinomycete that forms rod/coccus-like elements. Cells are Gram-positive, aerobic, slightly acid-fast and non-motile. Colonies on TSA are circular to irregular, wrinkled, moderate yellowish pink in colour and 1.5–2.0 mm in diameter after incubation for 7 days at 30°C. Growth occurs at 10–37°C (optimum, 30°C) but not at 4 or 40°C and at pH 4.5–10.0 (optimum, pH 7.0–7.5) but not at pH 4.0 or 10.5. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase are absent. H2S and indole are not produced. Tweens 20, 40 and 60 are hydrolysed, but casein is not. Susceptible to the following antibiotics: ampicillin, carbenicillin, cephalothin, chloramphenicol, gentamicin, kanamycin, neomycin, penicillin G, polymyxin B, streptomycin and tetracycline. Resistant to the following antibiotics: lincomycin, novobiocin and oleandomycin. The peptidoglycan type is based on meso-2,6-diaminopimelic acid as the diagnostic diaminode, according to 1H NMR data (Smith et al., 2007). Other phenotypic characteristics are given in Table 1. The DNA G+C content of the type strain is 66.9 mol% (HPLC).

The type strain, ON-33T (KCTC 19599T = CCUG 57507T), was isolated from soil around a wastewater treatment plant in Taejon, Korea.

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of strain ON-33T and some other related taxa. Bootstrap values (>50%) based on 1000 replications are shown at branch nodes. Filled circles indicate nodes recovered in the trees generated with the maximum-likelihood and maximum-parsimony algorithms. Open circles indicate nodes recovered in the tree generated with the maximum-parsimony algorithm. Rhodococcus rhodochrous DSM 43241T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.
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References


