Gordonia hankookensis sp. nov., isolated from soil

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A Gram-positive, aerobic and non-motile bacterial strain, designated ON-33^T, was subjected to a study based on a polyphasic approach to determine its exact taxonomic position. Strain ON-33^T 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-33^T fell within the clade comprising *Gordonia* species, clustering with *Gordonia soli* CC-AB07^T, with which it exhibited 16S rRNA gene sequence similarity of 98.5 %. The chemotaxonomic properties of strain ON-33^T 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 C_{16:0}, iso-C_{15:0} 2-OH and/or C_{16:1}ω7c, 10-methyl C_{18:0} and C_{18:1}ω9c. The DNA G + C content was 66.9 mol%. Strain ON-33^T exhibited a DNA–DNA relatedness value of 13 % to *G. soli* DSM 44995^T and could be differentiated from *G. soli* by differences in phenotypic characteristics. On the basis of the data obtained, strain ON-33^T 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-33^T (=KCTC 19599^T=CCUG 57507^T).

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-33^T, isolated from soil in Korea.

Strain ON-33^T was isolated by means of the standard dilution-plating technique on trypticase soy agar (TSA; Difco) at 30 °C. *G. soli* DSM 44995^T, 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-33^T 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

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain ON-33^T is FJ572038.

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-33^T for DNA extraction and for the analyses of peptidoglycan type, polar lipids and isoprenoid quinones was obtained after cultivation in trypticase 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 Komagata & Suzuki (1987) 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-33^T 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 microdilution 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-33^T are given in the species description and in Table 1. The almost-complete 16S rRNA gene sequence of strain ON-33^T 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-33^T 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-33^T fell within the cluster comprising *Gordonia* species, clustering with *G. soli* CC-AB07^T with a bootstrap

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

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-xylose, L-xylose, methyl β -D-xyloside, sorbose, dulcitol, methyl α -D-mannoside, methyl α -D-glucoside, N-acetylglucosamine, amygdalin, arbutin, salicin, cellobiose, maltose, lactose, melibiose, inulin, melezitose, raffinose, starch, glycogen, gentiobiose, D-lyxose, D-tagatose, D-fucose, L-fucose, 2-ketogluconate and 5-ketogluconate and activities of lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, α - and β -galactosidases, β -glucuronidase, N-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase. +, Positive; –, negative.

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

*Pale yellow in this study.

†Data for *G. soli* are from this study.

resampling value of 83.1 %. The relationship between strain ON-33^T and *G. soli* CC-AB07^T was also found in the tree constructed using the maximum-parsimony algorithm. Strain ON-33^T exhibited 98.5 % 16S rRNA gene sequence similarity to *G. soli* CC-AB07^T and 95.3–98.0 % similarities to the type strains of other *Gordonia* species.

Strain ON-33^T 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-33^T contained MK-9(H₂) as the predominant isoprenoid

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of strain ON-33^T 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 43241^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

quinone. The fatty acid profile of strain ON-33^T contained the following (each constituting >0.5% of the total fatty acids): straight-chain fatty acids C_{16:0} (35.2%), C_{18:0} (4.3%), C_{14:0} (3.0%), C_{17:0} (1.1%) and C_{15:0} (0.7%); summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1}ω7c, 20.5%); 10-methyl C_{18:0} (17.0%); unsaturated fatty acids C_{18:1}ω9c (12.6%), C_{16:1}ω9c (0.8%) and C_{17:1}ω7c (0.5%); hydroxy fatty acid C_{18:0} 3-OH (2.6%); and an unknown fatty acid of equivalent chain-length 14.959 (1.0%). This fatty acid profile was similar to those of *Gordonia* species, although there are differences in the proportions of some fatty acids probably because of differences in cultivation and extraction conditions (Linos *et al.*, 1999; Iida *et al.*, 2005; Shen *et al.*, 2006; Luo *et al.*, 2007). The major polar lipids were phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and phosphatidylinositol mannoside. This polar lipid pattern is the same as that of *G. soli* CC-AB07^T, the closest phylogenetic neighbour to strain ON-33^T. The DNA G+C content of strain ON-33^T was 66.9 mol%. The chemotaxonomic analyses showed that strain ON-33^T exhibited properties that are also observed with *Gordonia* species and were in agreement with the result of the phylogenetic analysis, i.e. strain ON-33^T is a member of the genus *Gordonia*.

Strain ON-33^T exhibited 13% DNA–DNA relatedness to *G. soli* DSM 44995^T, indicating that the two strains are genetically different (Wayne *et al.*, 1987). Strain ON-33^T was distinguishable from *G. soli* by differences in several phenotypic characteristics, including oxidase activity, nitrate reduction and hydrolysis and assimilation of several substrates (Table 1). The phylogenetic and genetic distinctiveness and differential phenotypic properties of strain ON-33^T are sufficient to classify it as a member of a species

that is distinct from the recognized *Gordonia* species (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994), for which the name *Gordonia hankookensis* sp. nov. is proposed.

Description of *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 diamino acid. 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-33^T (=KCTC 19599^T=CCUG 57507^T), was isolated from soil around a wastewater treatment plant in Taejeon, Korea.

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References

- Cowan, S. T. & Steel, K. J. (1965). *Manual for the Identification of Medical Bacteria*. London: Cambridge University Press.
- Drzyzga, O., Navarro Llorens, J. M., Fernández de las Heras, L. F., Fernandez, E. G. & Perera, J. (2009). *Gordonia cholesterolivorans* sp. nov., a cholesterol-degrading actinomycete isolated from sewage sludge. *Int J Syst Evol Microbiol* **59**, 1011–1015.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in micro-dilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.
- Iida, S., Taniguchi, H., Kageyama, A., Yazawa, K., Chibana, H., Murata, S., Nomura, F., Kroppenstedt, R. M. & Mikami, Y. (2005). *Gordonia otitidis* sp. nov., isolated from a patient with external otitis. *Int J Syst Evol Microbiol* **55**, 1871–1876.
- Kageyama, A., Iida, S., Yazawa, K., Kudo, T., Suzuki, S., Koga, T., Saito, H., Inagawa, H., Wada, A. & other authors (2006). *Gordonia araii* sp. nov. and *Gordonia effusa* sp. nov., isolated from patients in Japan. *Int J Syst Evol Microbiol* **56**, 1817–1821.
- Komagata, K. & Suzuki, K. (1987). Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–207.
- Lányi, B. (1987). Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* **19**, 1–67.
- Linos, A., Steinbüchel, A., Spröer, C. & Kroppenstedt, R. M. (1999). *Gordonia polyisoprenivorans* sp. nov., a rubber-degrading actinomycete isolated from an automobile tyre. *Int J Syst Bacteriol* **49**, 1785–1791.
- Luo, H., Gu, Q., Xie, J., Hu, C., Liu, Z. & Huang, Y. (2007). *Gordonia shandongensis* sp. nov., isolated from soil in China. *Int J Syst Evol Microbiol* **57**, 605–608.
- Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A. & Parlett, J. H. (1984). An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* **2**, 233–241.
- Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.
- Shen, F.-T., Goodfellow, M., Jones, A. L., Chen, Y.-P., Arun, A. B., Lai, W.-A., Rekha, P. D. & Young, C.-C. (2006). *Gordonia soli* sp. nov., a novel actinomycete isolated from soil. *Int J Syst Evol Microbiol* **56**, 2597–2601.
- Soddell, J. A., Stainsby, F. M., Eales, K. L., Seviour, R. J. & Goodfellow, M. (2006). *Gordonia defluvii* sp. nov., an actinomycete isolated from activated sludge foam. *Int J Syst Evol Microbiol* **56**, 2265–2269.
- Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.
- Stackebrandt, E., Rainey, F. A. & Ward-Rainey, N. L. (1997). Proposal for a new hierarchic classification system, *Actinobacteria* classis nov. *Int J Syst Bacteriol* **47**, 479–491.
- Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- Tsukamura, M. (1971). Proposal of a new genus, *Gordona*, for slightly acid-fast organisms occurring in sputa of patients with pulmonary disease and in soil. *J Gen Microbiol* **68**, 15–26.
- Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.
- Yassin, A. F., Shen, F.-T., Hupfer, H., Arun, A. B., Lai, W.-A., Rekha, P. D. & Young, C. C. (2007). *Gordonia malaquae* sp. nov., isolated from sludge of a wastewater treatment plant. *Int J Syst Evol Microbiol* **57**, 1065–1068.
- Yoon, J.-H., Kim, H., Kim, S.-B., Kim, H.-J., Kim, W. Y., Lee, S. T., Goodfellow, M. & Park, Y.-H. (1996). Identification of *Saccharomonospora* strains by the use of genomic DNA fragments and rRNA gene probes. *Int J Syst Bacteriol* **46**, 502–505.
- Yoon, J.-H., Lee, S. T. & Park, Y.-H. (1998). Inter- and intraspecific phylogenetic analysis of the genus *Nocardioides* and related taxa based on 16S rRNA gene sequences. *Int J Syst Bacteriol* **48**, 187–194.
- Yoon, J.-H., Kang, K. H. & Park, Y.-H. (2003). *Psychrobacter jeotgali* sp. nov., isolated from jeotgal, a traditional Korean fermented seafood. *Int J Syst Evol Microbiol* **53**, 449–454.