Nocardiopsis ganjiahuensis sp. nov., isolated from a soil from Ganjiahu, China

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An actinomycete, strain HBUM 20038^T, was isolated from soil from Ganjiahu Natural Reserve in Xinjiang Province, in north-western China, and then characterized using a polyphasic approach. 16S rRNA gene sequence analysis confirmed that strain HBUM 20038^T was a member of the genus *Nocardiopsis*, and the morphological and chemotaxonomic characteristics of the strain were also consistent with those of species of *Nocardiopsis*. DNA–DNA hybridization between the strain and related type strains gave relatedness values far below 70 %. These results, together with physiological characteristics, showed that strain HBUM 20038^T represents a novel species within the genus *Nocardiopsis*, for which the name *Nocardiopsis ganjiahuensis* sp. nov. is proposed. The type strain is HBUM 20038^T (=DSM 45031^T =CGMCC 4.3500^T).

The genus *Nocardiopsis* was first described by Meyer (1976), and its members typically exhibit a branched substrate mycelium that fragments into rod-shaped and coccoid elements and abundant aerial hyphae that frequently form a zigzag morphology. The genus *Nocardiopsis* was subsequently shown to exhibit distinct chemotaxonomic characteristics: cell-wall chemotype III/C (*meso*-diaminopimelic acid and no diagnostic sugars in whole-cell hydrolysates; Lechevalier & Lechevalier, 1970), phospholipid type PIII (phosphatidylcholine and phosphatidylmethylethanolamine as characteristic phospholipids; Lechevalier *et al.*, 1977), menaquinone MK-10 with variable degrees of saturation in the side chain as the predominant isoprenoid quinone (Kroppenstedt, 1992), fatty acid type 3d (containing iso-branched, anteiso-branched and 10-methyl-branched chain fatty acids; Kroppenstedt, 1985) and DNA G+C contents between 64 and 71 mol% (Grund & Kroppenstedt, 1990). At the time of writing, 25 species and subspecies have validly published names (Euzéby, 2007). Most of them were isolated from habitats such as saline or alkaline soil and marine sediments. We isolated a novel strain, HBUM 20038^T, from an alkaline soil in western China. The aim of the study was to identify whether or not the strain represents a novel species within the genus *Nocardiopsis* by using phenotypic, genotypic and phylogenetic approaches.

Strain HBUM 20038^T was isolated on agar plates of DSMZ medium no. 852 (GYE medium, adjusted to pH 10 with sterile saturated sodium carbonate solution after sterilization of the medium). Cultural characteristics of strain HBUM 20038^T were observed by using 3-, 5-, 7- and 14-day-old cultures grown on GYE agar at 28 °C. The morphological properties and colours of aerial and substrate mycelium, as well as of diffusible pigments, were tested as described by Shirling & Gottlieb (1966). Spore-chain morphology and spore-surface ornamentation of 7-day-old cultures grown on GYE agar were observed using an Olympus BH-2 light microscope and a KYKY-AMRAY-100B scanning electron microscope. Strain HBUM 20038^T formed powdery-surfaced colonies. The strain produced abundant grey–white aerial mycelium which divided into rod-shaped, smooth-surfaced and non-motile spores (0.3–0.5 × 1.0–2.0 µm). It formed long, well-developed and branched substrate mycelium (Fig. 1). The substrate mycelium was light-yellowish. No diffusible pigments were produced on GYE agar medium. These morphological characteristics are consistent with those described for *Nocardiopsis* species (Kroppenstedt, 1992).

Biomass used for chemotaxonomic and molecular systematic studies was grown in shake flasks of GYE liquid medium for 4 days at 28 °C. At maximum growth, the cultures were checked for purity and harvested by centrifugation and washed twice with distilled water. The isomeric form of diaminopimelic acid and diagnostic

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of HBUM 20038^T is AY336513.

Fig. 1. Scanning electron micrographs of HBUM 20038^T, showing aerial mycelium divided into rod-shaped spores (a) and branched substrate mycelium (b). Bars, 10 μm.

sugars of whole-cell hydrolysates were determined following standard procedures described by Lechevalier & Lechevalier (1980) and Hasegawa *et al.* (1983). Menaquinones were determined according to the methods of Collins (1985). Analysis of phospholipids was based on the procedure of Komagata & Suzuki (1987). The cellular fatty acid composition was determined as described by Sasser (1990) using the Microbial Identification System (MIDI, Inc.). The chemical properties of the strain are consistent with those of the genus *Nocardiopsis* (Lechevalier & Lechevalier, 1970). Cell walls of strain HBUM 20038^T contained *meso*-diaminopimelic acid and no characteristic sugars were found in the strain. The diagnostic phospholipids contained quantities of diphosphatidylglycerol, phosphatidylcholine and phosphatidylmethylethanolamine (phospholipid type III; Lechevalier *et al.*, 1977). The predominant menaquinones were MK-10(H₂), MK-10(H₄) and MK-10(H₆). The major cellular fatty acids were iso-C_{16:0} (41.03 %), C_{18:1ω9c} (14.73 %) and tuberculostearic acid (10-methyl-C_{18:0}) (9.69 %). These chemical properties offer evidence that the strain should be classified in the genus *Nocardiopsis* (Collins & Jones, 1981; Lechevalier & Lechevalier, 1970; Lechevalier *et al.*, 1977).

The physiological characteristics of the strain was tested using the methods described for the genus *Nocardiopsis* in *Bergey's Manual of Determinative Bacteriology* (Holt *et al.*,

1994) and the methods described by Evtushenko *et al.* (2000). Growth of strain HBUM 20038^T on agar was observed at pH 8.5–13.0, while optimum growth occurred at pH 8.5–9.5, and there was no growth at pH 7.0. The other results are listed in Table 1 or in the species description.

For 16S rRNA gene sequence analysis, determination of G+C content and DNA–DNA hybridization, chromosomal DNA was extracted using the procedure described by Marmur (1961) and Kutchma *et al.* (1998). The 16S rRNA gene was amplified by PCR with the universal primers 27f (*Escherichia coli* positions 8–27; 5'-GAGTTTGATCCTGGCTCAG-3') and 1525r (*E. coli* positions 1525–1545; 5'-AGAAAGGAGGTGTACCAGCC-3') (Lane *et al.*, 1985). The PCR conditions used were an initial denaturation at 95 °C for 10 min, rapid cooling in an ice bath, addition of *Taq* DNA polymerase and instantaneous centrifugation followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 54 °C for 30 s and extension at 72 °C for 90 s with a final extension at 72 °C for 5 min. The resultant PCR products were purified and sequenced by Sangon Biological Engineering Co. Ltd (Shanghai, China). The G+C content was determined by HPLC of deoxyribonucleosides by using the method of Mesbah *et al.* (1989). The initial reassociation-rate method (De Ley *et al.*, 1970) was used for determining the percentage of DNA–DNA hybridization by using a spectrophotometer (model CE9500; Cecil Instruments) equipped with a programmable melting-temperature control unit.

The 16S rRNA gene sequence of the strain was aligned manually with corresponding sequences of representatives of the genus *Nocardiopsis* retrieved from the GenBank database using CLUSTAL_X version 1.64b (Thompson *et al.*, 1997). Phylogenetic trees were inferred by using the neighbour-joining (Saitou & Nei, 1987), Fitch–Margoliash (Fitch & Margoliash, 1967) and maximum-likelihood (Felsenstein, 1981) methods. Evolutionary distance matrices for the neighbour-joining and Fitch–Margoliash methods were generated according to the algorithm of Jukes & Cantor (1969) and the robustness of the tree topology of the neighbour-joining data was evaluated by bootstrap analysis (Felsenstein, 1993) based on 1000 resamplings using the SEQBOOT and CONSENSE options from the PHYLIP suite of programs.

The nearly complete 16S rRNA gene sequence determined for strain HBUM 20038^T (1434 nt) was aligned with corresponding sequences of representative reference strains of *Nocardiopsis*. It was evident from the phylogenetic tree (Fig. 2) that *Nocardiopsis prasina* DSM 43845^T, *N. metallicus* KBS6^T, *N. exhalans* ES10.1^T, *N. alkaliphila* DSM 44657^T, *N. listeri* DSM 40297^T, *N. alba* DSM 43377^T, *N. tropica* VKM Ac-1457^T, *N. umidischolae* 66/93^T, *N. aegyptia* DSM 44442^T, *N. lucentensis* DSM 44048^T and strain HBUM 20038^T formed a phylogenetic clade. Pairwise similarity values of 16S rRNA gene sequences >97 % were found between HBUM 20038^T and related

Table 1. Differential phenotypic characteristics of HBUM 20038^T and closely related *Nocardiopsis* species

Strains: 1, strain HBUM 20038^T (data from this study); 2, *N. prasina* DSM 43845^T; 3, *N. metallicus* DSM 44598^T (Schippers *et al.*, 2002); 4, *N. exhalans* DSM 44407^T (Peltola *et al.*, 2001); 5, *N. alba* DSM 43377^T; 6, *N. aegyptia* DSM 44442^T (Sabry *et al.*, 2004); 7, *N. lucentensis* DSM 44048^T; 8, *N. listeri* DSM 40297^T. Unless indicated, data were taken from Evtushenko *et al.* (2000). +, Positive; –, negative; ND, no data.

Characteristic	1	2	3	4	5	6	7	8
Colour of aerial mycelium	Grey–white	White	White	White	Yellow or colourless	Beige to light yellow	White	Colourless
Utilization of:								
L-Arabinose	+	+	ND	+	–	+	–	+
D-Xylose	+	+	+	+	–	+	–	+
Sucrose	–	+	+	+	+	+	+	–
L-Rhamnose	+	–	+	+	–	+	+	+
Lactose	–	–	ND	–	–	+	–	+
Cellobiose	–	–	+	+	+	ND	+	–
Glycerol	+	+	ND	ND	+	+	+	–
myo-Inositol	+	–	ND	–	–	+	+	–
Growth at/in:								
10 °C	+	–	+	+	–	+	+	+
28 °C	+	ND	+	+	ND	+	ND	ND
42 °C	–	+	–	ND	+	ND	–	–
10 % NaCl	–	+	+	+	–	–	+	–
Nitrate reductase	+	–	ND	ND	–	ND	+	+
Urease	+	–	ND	ND	+	ND	–	–

Fig. 2. Neighbour-joining tree (Saitou & Nei, 1987), based on almost complete 16S rRNA gene sequences, showing the relationship between strain HBUM 20038^T and representatives of the genus *Nocardiopsis*. *Actinomadura madurae* JCM 7436^T was used as the outgroup. Numbers at nodes indicate percentages of bootstrap support, based on a neighbour-joining analysis of 1000 resampled datasets; only values greater than 50% are given. 'f' represents clades recovered by the Fitch–Margoliash method; 'fm' represents clades recovered by both the Fitch–Margoliash and maximum-likelihood methods. Bar, 0.01 substitutions per nucleotide position.

strains (Table 2). The DNA–DNA relatedness of strain HBUM 20038^T to related type strains was 21.2–52.6% (Table 2). These values were far below the value of 70% recommended by Wayne *et al.* (1987) for strains of the same species. The DNA G+C content of strain HBUM 20038^T is 71.1 mol% (determined by HPLC).

In summary, strain HBUM 20038^T can be classified in the genus *Nocardiopsis* based on 16S rRNA gene sequence

Table 2. 16S rRNA gene sequence similarities and DNA–DNA relatedness between HBUM 20038^T and related type strains of the genus *Nocardiopsis*

Strain	16S rRNA gene sequence similarity (%)	DNA–DNA relatedness (%)
HBUM 20038 ^T	(100)	100
<i>N. prasina</i> DSM 43845 ^T	99.29	52.6
<i>N. metallicus</i> DSM 44598 ^T	99.29	49.9
<i>N. exhalans</i> DSM 44407 ^T	99.0	44.3
<i>N. alba</i> DSM 43377 ^T	98.64	37.2
<i>N. aegyptia</i> DSM 44442 ^T	98.64	33.4
<i>N. lucentensis</i> DSM 44048 ^T	98.35	34.8
<i>N. listeri</i> DSM 40297 ^T	98.21	29.1
<i>N. alkaliphila</i> DSM 44657 ^T	97.84	26.9
<i>N. umidischolae</i> DSM 44362 ^T	97.84	28.5
<i>N. dassonvillei</i> subsp. <i>albirubida</i> DSM 40465 ^T	97.4	25.8
<i>N. synnemataformans</i> DSM 44143 ^T	97.4	21.2

similarity and its morphological characteristics, cell-wall chemotype, menaquinone composition, phospholipid type and DNA base composition. The results of physiological tests (Table 1) show that there are clear differences between strain HBUM 20038^T and other species of the genus *Nocardiopsis*. DNA–DNA hybridization is the standard for designation of species (Wayne *et al.*, 1987) and the criterion for membership of the same species is $\geq 70\%$ DNA–DNA relatedness (Wayne *et al.*, 1987). The levels of DNA–DNA relatedness between strain HBUM 20038^T and related type strains are 21.2–52.6% (Table 2), which were $< 70\%$. It is therefore proposed that strain HBUM 20038^T be classified as the type strain of the novel species *Nocardiopsis ganjiahuensis* sp. nov.

Description of *Nocardiopsis ganjiahuensis* sp. nov.

Nocardiopsis ganjiahuensis (gan.jia.hu.en'sis. N.L. fem. adj. *ganjiahuensis* pertaining to Ganjiahu Natural Reserve, Xinjiang, north-west China, where the type strain was isolated).

Aerobic and Gram-positive. The surface of colonies is powdery. The colour of the aerial mycelium is grey–white and the substrate mycelium is light-yellowish. The aerial mycelium divides into rod-shaped, irregular-sized spores ($0.3\text{--}0.5 \times 1.0\text{--}2.0\ \mu\text{m}$). Spores are smooth and non-motile. The substrate mycelium is long, well-developed and branched. No diffusible pigments are produced. Whole-cell hydrolysates contain *meso*-diaminopimelic acid, but no diagnostic sugars. Polar lipids are diphosphatidylglycerol, phosphatidylcholine and phosphatidylmethylethanolamine. The predominant menaquinones are MK-10(H₂) and MK-10(H₄); in addition, there are substantial amounts of MK-10(H₆). Major cellular fatty acids are iso-C_{16:0} (41.03%), C_{18:1 ω 9c} (14.73%) and tuberculostearic acid (10-methyl C_{18:0}) (9.69%). The G+C content of the DNA of the type strain is 71.1 mol%. Grows occurs at pH 8.5–13, while optimum growth occurs at pH 8.5–9.5. No growth at pH 7.0. Grows optimally at 28–32 °C; growth occurs at 10 °C, with no growth at 42 °C. Grows well on medium without NaCl and with 1, 3 and 5% NaCl, but no growth occurs at 10% (w/v) NaCl. L-Arabinose, D-xylose, *myo*-inositol, glycerol and L-rhamnose are utilized as sole carbon sources, but not sucrose, lactose or cellobiose. Positive reactions are found for reduction of nitrate and production of urease. Tween 80 can be degraded, but not Tween 85.

The type strain is strain HBUM 20038^T (=DSM 45031^T =CGMCC 4.3500^T), isolated from soil.

Acknowledgements

The work was supported by the National Natural Science Foundation of China (3027002), the Foundation of the National Basic Research Program of China (2006CB100206) and the Foundation of the National Program for Basic S and T Platform Construction (2005DKA21201-10).

References

- Collins, M. D. (1985). Analysis of isoprenoid quinones. *Methods Microbiol* **18**, 329–366.
- Collins, M. D. & Jones, D. (1981). Distribution of isoprenoid quinone structural types in bacteria and their taxonomic implication. *Microbiol Mol Biol Rev* **45**, 316–354.
- De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.
- Euzéby, J. P. (2007). *Nocardiopsis* (Brocq-Rousseau 1904) Meyer 1976, genus. In *List of Prokaryotic Names with Standing in Nomenclature*. Accessed February 2007. <http://www.bacterio.cict.fr/n/nocardiopsis.html>
- Evtushenko, L. I., Taran, V. V., Akimov, V. N., Kroppenstedt, R. M., Tiedje, J. M. & Stackebrandt, E. (2000). *Nocardiopsis tropica* sp. nov., *Nocardiopsis trehalosi* sp. nov., nom. rev. and *Nocardiopsis dassonvillei* subsp. *albirubida* subsp. nov., comb. nov. *Int J Syst Evol Microbiol* **50**, 73–81.
- Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.
- Felsenstein, J. (1993). PHYLIP (phylogeny inference package), version 3.5c. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.
- Fitch, W. M. & Margoliash, E. (1967). Construction of phylogenetic trees. *Science* **155**, 279–284.
- Grund, E. & Kroppenstedt, R. M. (1990). Chemotaxonomy and numerical taxonomy of the genus *Nocardiopsis* Meyer 1976. *Int J Syst Bacteriol* **40**, 5–11.
- Hasegawa, T., Takizawa, M. & Tanida, S. (1983). A rapid analysis for chemical grouping of aerobic actinomycetes. *J Gen Appl Microbiol* **29**, 319–322.
- Holt, J. G., Krieg, N. R. & Sneath, P. H. A. (editors) (1994). *Bergey's Manual of Determinative Bacteriology*, 9th edn. Baltimore: Williams & Wilkins.
- Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, vol. 3, pp. 21–132. Edited by H. H. Munro. New York: Academic Press.
- Komagata, K. & Suzuki, K. (1987). Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–207.
- Kroppenstedt, R. M. (1985). Fatty acid and menaquinone analysis of actinomycetes and related organisms. In *Chemical Methods in Bacterial Systematics* (Society for Applied Bacteriology Technical Series vol. 20), pp. 173–199. Edited by M. Goodfellow & D. E. Minnikin. New York: Academic Press.
- Kroppenstedt, R. M. (1992). The genus *Nocardiopsis*. In *The Prokaryotes*, 2nd edn, pp. 1139–1156. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K. H. Schleifer. New York: Springer.
- Kutchma, A. J., Roberts, M. A., Knaebel, D. B. & Crawford, D. L. (1998). Small-scale isolation of genomic DNA from *Streptomyces* mycelia or spores. *Biotechniques* **24**, 452–456.
- Lane, D. J., Pace, B., Olsen, G. J., Stahl, D. A., Sogin, M. L. & Pace, N. R. (1985). Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. *Proc Natl Acad Sci U S A* **82**, 6955–6959.
- Lechevalier, M. P. & Lechevalier, H. A. (1970). Chemical composition as a criterion in the classification of aerobic actinomycetes. *Int J Syst Bacteriol* **20**, 435–443.
- Lechevalier, H. A. & Lechevalier, M. P. (1980). The chemotaxonomy of actinomycetes. In *Actinomycete Taxonomy*, pp. 227–291. Special

Publication No. 6. Edited by A. Dietz & D. Thayer. Arlington, VA: Society for Industrial Microbiology.

Lechevalier, M. P., De Bièvre, C. & Lechevalier, H. A. (1977). Chemotaxonomy of aerobic actinomycetes: phospholipid composition. *Biochem Syst Ecol* **5**, 249–260.

Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.

Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.

Meyer, J. (1976). *Nocardiopsis*, a new genus of the order Actinomycetales. *Int J Syst Bacteriol* **26**, 487–493.

Peltola, J. S. P., Andersson, M. A., Kämpfer, P., Auling, G., Kroppenstedt, R. M., Busse, H.-J., Salkinoja-Salonen, M. S. & Rainey, F. A. (2001). Isolation of toxigenic *Nocardiopsis* strains from indoor environments and description of two new *Nocardiopsis* species, *N. exhalans* sp. nov. and *N. umidischolae* sp. nov. *Appl Environ Microbiol* **67**, 4293–4304.

Sabry, S. A., Ghanem, N. B., Abu-Ella, G. A., Schumann, P., Stackebrandt, E. & Kroppenstedt, R. M. (2004). *Nocardiopsis aegyptia*

sp. nov., isolated from marine sediment. *Int J Syst Evol Microbiol* **54**, 453–456.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Sasser, M. (1990). Identification of bacteria by gas chromatography of cellular fatty acids. *USFCC Newsl* **20**, 16.

Schippers, A., Bosecker, K., Willscher, S., Spröer, C., Schumann, P. & Kroppenstedt, R. M. (2002). *Nocardiopsis metallicus* sp. nov., a metal-leaching actinomycete isolated from an alkaline slag dump. *Int J Syst Evol Microbiol* **52**, 2291–2295.

Shirling, E. B. & Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* **16**, 313–340.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.

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.