Microbacterium radiodurans sp. nov., a UV radiation-resistant bacterium isolated from soil

Wei Zhang,^{1†} Hong-hui Zhu,^{2†} Menglong Yuan,^{1,3†} Qing Yao,⁴ Ran Tang,¹ Min Lin,¹ Song-zhen Yang,² Zhi-kun Li¹ and Ming Chen¹

Correspondence

Hong-hui Zhu
zhuhonghui66@163.net

¹Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, 12 Zhongguancun Nandajie, Beijing 100081, PR China

²Guangdong Provincial Microbial Culture Collection and Application Key Laboratory, Guangdong Institute of Microbiology, Guangzhou, Guangdong 510070, PR China

³College of Life Science, Beijing Normal University, Beijing 100875, PR China

⁴South China Agricultural University, Guangzhou, Guangdong 510642, PR China

Strain GIMN 1.002^T, a UV radiation-tolerant bacterium, was isolated from the upper sand layers of the Gobi desert, Xinjiang, China and characterized in order to determine its taxonomic position. Cells were Gram-reaction-positive, heterotrophic, strictly aerobic, short rods. 16S rRNA gene sequence analysis revealed that strain GIMN 1.002^T belonged to the genus *Microbacterium* and was closely related to *Microbacterium arborescens* DSM 20754^T (98.8% 16S rRNA gene sequence similarity) and *Microbacterium imperiale* DSM 20530^T (98.7%). However, strain GIMN 1.002^T had low DNA–DNA relatedness with *M. arborescens* DSM 20754^T (17.1%) and *M. imperiale* DSM 20530^T (12.89%). Strain GIMN 1.002^T possessed chemotaxonomic markers that were consistent with its classification in the genus *Microbacterium*, i.e. MK-11, MK-12 and MK-10 as major menaquinones and anteiso-C_{15:0} (38.67%), iso-C_{16:0} (18.16%) and iso-C_{15:0} (17.46%) as predominant cellular fatty acids. The DNA G + C content was 67.74 mol%. The cell-wall sugar was rhamnose. On the basis of the data from this study, strain GIMN 1.002^T represents a novel species of the genus *Microbacterium*, for which the name *Microbacterium radiodurans* sp. nov. is proposed. The type strain is GIMN 1.002^T (=CCTCC M208212^T =NRRL B-24799^T).

Orla-Jensen (1919) established the genus *Microbacterium*, class *Actinobacteria*, with only four species. At the time of writing, the genus includes 60 species (<http://www.bacterio.cict.fr/m/microbacterium.html>). Some species of the genera *Brevibacterium* (Collins *et al.*, 1983; Schumann *et al.*, 1999) and *Flavobacterium* (Imai *et al.*, 1984; Takeuchi & Hatano, 1998a) have been reclassified as species of the genus *Microbacterium* and a comparative sequence analysis of the 16S rRNA gene led to the unification of the genera *Microbacterium* and *Aureobacterium* (Takeuchi & Hatano, 1998b; Behrendt *et al.*, 2001). Members of the genus *Microbacterium* have mainly been isolated from environmental habitats. Here, we describe the phenotypic and genotypic properties of a strain belonging to the genus *Microbacterium* that was isolated from the upper sand layers of the Gobi desert,

Xinjiang, China, where bacteria are exposed to prolonged desiccation and cycles of high and low temperatures.

Strain GIMN 1.002^T was isolated on nutrient agar (NA; Difco) after 2–4 days at 30 °C. The Gram reaction was performed by the non-staining method, as described by Buck (1982). Cells for the observation of cell morphology and motility were prepared on NA at 30 °C for 2 days. Cell morphology was observed by light microscopy (Leica DM RAR) and scanning electron microscopy (Hitachi). Motility was assessed using the hanging-drop method (Skerman, 1967). Catalase activity was determined by assessing bubble production with 3% (v/v) H₂O₂ and oxidase activity was determined using 1% (w/v) tetramethyl-*p*-phenylenediamine. Growth at 4, 18, 25, 30, 37 and 42 °C and pH 5.0–10.0 (in increments of 0.5 pH units) was assessed in nutrient broth (NB; Difco) after 5 days. For the pH experiments, the following buffering systems were used (50 mM): acetate buffer (pH 5.0–5.5), phosphate buffer (pH 6.0–8.0) and Tris buffer (pH 8.5–10.0). Salt tolerance was tested in NB supplemented with 1–10% (w/v) NaCl at 28 °C after 5 days. Anaerobic growth was tested at 28 °C for 7 days in 10-ml rubber-sealed

†These authors contributed equally to this work.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain GIMN 1.002^T is GQ329713.

A figure presenting survival curves after exposure to UV light for strain GIMN 1.002^T and its two closest phylogenetic neighbours is available as supplementary material with the online version of this paper.

screw-capped tubes containing 9 ml NB that was covered with liquid paraffin. Hydrogen sulfide production and gelatin hydrolysis were determined by the general characterization methods described by Smibert & Krieg (1981) and Sneath *et al.* (1986), using gelatin agar (Difco) for the gelatin hydrolysis test.

Preparation of cell walls and determination of the cell-wall diamino acids by TLC followed the procedures described by Bousfield *et al.* (1985). The peptidoglycan acyl type was determined according to Uchida & Seino (1997). Polar lipids were extracted according to Minnikin *et al.* (1984) and were identified by two-dimensional TLC followed by spraying with appropriate detection reagents (Minnikin *et al.*, 1984; Komagata & Suzuki, 1987).

The utilization of substrates as sole carbon sources and the metabolic profile of strain GIMN 1.002^T were examined using the GP2 MicroPlate system (Biolog), which includes an array of 96 wells to test the oxidation of 95 carbon sources. Sample preparation and analysis were performed, according to the directions of the manufacturer, in triplicate. Results were obtained after 4 and 24 h using a Microstation (Biolog) and the data were analysed using MICROLOG version 3 (Biolog). Of the 95 carbon sources tested, 23 could be oxidized by strain GIMN 1.002^T: β -cyclodextrin, cellobiose, gentiobiose, α -D-glucose, inositol, maltose, maltotriose, D-mannitol, D-mannose, 3-methyl D-glucose, α -ketoglutaric acid, succinic acid monomethyl ester, pyruvic acid methyl ester, glycyl L-glutamic acid, uridine, uridine 5'-monophosphate, D-xylose, salicin, D-sorbitol, D-fructose, putrescine, trehalose and α -D-glucose 1-phosphate. Oxidation of 43 carbon sources varied between the three replicates: inulin, mannan, N-acetyl-D-glucosamine, N-acetyl- β -D-mannosamine, amygdalin, D-arabitol, arbutin, L-fucose, D-gluconic acid, lactose, melizitol, melibiose, methyl α -D-galactoside, methyl β -D-galactoside, methyl β -D-glucoside, methyl α -D-mannoside, palatinose, D-psicose, stachyose, turanose, lactulose, D-ribose, α -, β - and γ -hydroxybutyric acids, *p*-hydroxyphenylacetic acid, α -ketovaleric acid, pyruvic acid, succinamic acid, succinic acid, D-alanine, L-alanyl glycine, L-asparagine, putrescine, 2,3-butanediol, glycerol, inosine, thymidine, adenosine 5'-monophosphate, thymidine 5'-monophosphate, D-fructose 6-phosphate, D-glucose 6-phosphate and DL- α -glycerol phosphate. The remaining 29 carbon sources tested were not oxidized by strain GIMN 1.002^T.

Genomic DNA from strain GIMN 1.002^T was extracted and purified as described by Moore & Dowhan (1995) and degraded enzymically into nucleosides and the G+C content was determined as described by Mesbah *et al.* (1989). Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v), evaporated under vacuum conditions and re-extracted in n-hexane/water (1:1, v/v). The crude n-hexane/quinone solution was purified using Sep-Pak Vac silica cartridges (Waters) and subsequently analysed by reversed-phase HPLC as described by Hiraishi *et al.* (1996). Cellular fatty acids were determined for cells

prepared on NA at 30 °C for 3 days. The fatty acid methyl esters were prepared according to the protocol of the Sherlock Microbial Identification System (MIDI) and analysed by GC (model 6890; Hewlett Packard) using the Microbial Identification software (Sasser, 1990). The cell-wall sugars were analysed as described by Stanek & Roberts (1974). DNA-DNA hybridization was performed fluorometrically using the method of Ezaki *et al.* (1989), with photobiotin-labelled DNA probes and microdilution wells.

For analysis of the 16S rRNA gene sequence, genomic DNA was extracted using a DNA extraction kit (Solgent). PCR-mediated amplification of the 16S rRNA gene and sequencing of the purified amplification products were performed according to Kim *et al.* (2005). Full sequences of the 16S rRNA gene were compiled using SeqMan software (DNASTAR). The 16S rRNA gene sequences of related taxa were obtained from GenBank. Multiple alignments were performed using CLUSTAL X (Thompson *et al.*, 1997), gaps were edited using the BioEdit program (Hall, 1999) and evolutionary distances were calculated using Kimura's two-parameter model (Kimura, 1983). Phylogenetic trees were constructed with the neighbour-joining method (Saitou & Nei, 1987) using MEGA version 3 (Kumar *et al.*, 2004) and bootstrap values were calculated from 1000 replications (Felsenstein, 1985). The minimum-evolution and maximum-parsimony (Fitch, 1971) methods were also used to construct trees (results not shown).

Cells of strain GIMN 1.002^T were Gram-reaction-positive, aerobic, weakly motile, non-spore-forming, short rods, approximately 0.4–0.6 $\times$ 0.5–0.8 μ m in size after 48 h at 28 °C on NA. Colonies were white, nearly circular, convex with entire margins, shiny and moist. Growth occurred over a wide pH range (6.0–10.0). The physiological and biochemical characteristics, metabolic properties and substrate-utilization results obtained for strain GIMN 1.002^T are presented in Table 1 and the species description.

The cell-wall peptidoglycan contained ornithine as the diagnostic diamino acid. The major menaquinones were MK-11, MK-12 and MK-10. The acyl type of the peptidoglycan was glycolyl. The cellular fatty acids (>1% of the total) were the branched fatty acids anteiso-C_{15:0} (38.67%), iso-C_{16:0} (18.16%), iso-C_{15:0} (17.46%), anteiso-C_{17:0} (7.53%) and iso-C_{14:0} (5.16%) and the straight-chain fatty acid C_{16:0} (2.09%). The major polar lipids detected in strain GIMN 1.002^T were diphosphatidylglycerol, phosphatidylglycerol, an unidentified phospholipid and an unidentified glycolipid. The DNA G+C content of strain GIMN 1.002^T was 67.74 mol%. These characteristics were well matched to the genus description of *Microbacterium*.

The 16S rRNA gene sequence of strain GIMN 1.002^T determined in this study was a continuous stretch of 1430 bp. In the neighbour-joining phylogenetic tree (Fig. 1), strain GIMN 1.002^T clustered within the genus *Microbacterium*. Strain GIMN 1.002^T exhibited the highest levels of 16S rRNA gene sequence similarity with

Table 1. Physiological and biochemical characteristics that serve to differentiate strain GIMN 1.002^T from its closest phylogenetic neighbours

Strains: 1, *Microbacterium radiodurans* sp. nov. GIMN 1.002^T; 2, *M. arborescens* DSM 20754^T (data from Imai *et al.*, 1984); 3, *M. imperiale* DSM 20530^T (Collins *et al.*, 1983); 4, *M. sediminicola* YM10-847^T (Kageyama *et al.*, 2007); 5, *M. ginsengisoli* DSM 18659^T (Park *et al.*, 2008); 6, *M. ulmi* XIL02^T (Rivas *et al.*, 2004). +, Positive; v, variable; w, weak; –, negative; ND, no data available.

Characteristic	1	2	3	4	5	6
Colony colour	White	Dirty orange	Red–orange	Pale yellow	Yellow	White
Motility	w	+	+	–	–	–
Catalase	w	+	+	+	+	w
H ₂ S production	–	+	v	v	ND	–
Gelatin hydrolysis	–	+	–	–	–	–
Utilization of:						
3-Hydroxybutyrate	–	–	–	–	+	–
Acetate	–	+	+	+	+	–
Adipate	–	–	+	+	–	–
Citrate	–	w	–	–	–	–
Phenylacetate	–	–	–	–	–	+
Propionate	–	–	–	–	+	+
Inositol	+	–	–	–	+	–
D-Mannitol	+	+	+	+	+	–
L-Alanine	–	–	–	+	+	–
L-Proline	–	–	–	–	+	–
L-Serine	–	–	–	–	+	–
N-Acetylglucosamine	–	+	w	+	–	–
Valerate	+	–	+	+	+	–
L-Arabinose	–	+	+	+	–	–
D-Fructose	+	+	+	–	+	+
D-Galactose	–	+	+	+	–	+
Mannose	+	–	–	+	+	+
Raffinose	–	+	+	–	+	–
L-Rhamnose	–	+	+	+	+	–
Sucrose	–	+	+	–	ND	–
D-Xylose	+	+	+	+	+	–
Menaquinones*	11, 12, 10	11, 12	11, 12	10, 11	11, 12	12, 13, 11, 14, 10
Major fatty acids (%)						
anteiso-C _{15:0}	38.7	47.7	50.8	40.6	32.8	42.0
iso-C _{15:0}	17.5	–	–	–	–	12.2
iso-C _{16:0}	18.2	21.3	14.0	24.9	19.5	12.5
anteiso-C _{17:0}	7.5	27.3	28.2	31.9	40.4	24.4
Cell-wall components						
Diamino acid†	Orn	Lys	Lys	Orn	Orn	Orn
Sugar(s)*‡	Rha	6dTal, Man, Gal	Rha, Man, Gal	ND	Rha	Gal, Fuc, Xyl, Rha
DNA G + C content (mol%)	67.7	71.0	71.3	67.8	69.4	69.0

*Components listed in decreasing order of abundance.

†Orn, L-Ornithine.

‡6dTal, 6-Deoxytalose; Fuc, fucose; Gal, galactose; Man, mannose; Rha, rhamnose; Xyl, xylose.

Microbacterium arborescens DSM 20754^T (98.8 %) and *Microbacterium imperiale* DSM 20530^T (98.7 %); lower values were found with the next closest phylogenetic neighbours, *Microbacterium sediminicola* YM10-847^T (98.3 %), *Microbacterium ginsengisoli* Gsoil 259^T (97.9 %) and *Microbacterium ulmi* XIL02^T (97.8 %). Levels of DNA–DNA relatedness between strain GIMN 1.002^T and *M. arborescens* DSM 20754^T and *M. imperiale* DSM 20530^T were 17.1 ± 0.2 and 12.89 ± 0.1 %, respectively, which were well below the

70 % cut-off threshold for the delineation of species recommended by Wayne *et al.* (1987). Thus, the 16S rRNA gene sequence analysis confirmed that strain GIMN 1.002^T represents a novel species of the genus *Microbacterium*.

The chemotaxonomic characteristics of strain GIMN 1.002^T were in agreement with the results of the phylogenetic inference and confirmed that the novel bacterium belongs to the genus *Microbacterium*. However, strain GIMN 1.002^T

Fig. 1. Unrooted neighbour-joining tree, constructed from 16S rRNA gene sequences, showing the phylogenetic relationships between strain GIMN 1.002^T and the type strains of other species of the genus *Microbacterium*. Bootstrap values (>50%) based on 1000 replications are given at branch nodes. *Arthrobacter globiformis* DSM 20124^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

clearly differed from its closest phylogenetic neighbours (Table 1); the major fatty acids of the isolate were anteiso-C_{15:0}, iso-C_{16:0}, iso-C_{15:0} and anteiso-C_{17:0}, while those of *M. arborescens* and *M. imperiale* were anteiso-C_{15:0}, anteiso-C_{17:0} and iso-C_{16:0}. The cell-wall diamino acid of GIMN 1.002^T was ornithine, while that of *M. arborescens* and *M. imperiale* was lysine. Only rhamnose was found in the cell wall of strain GIMN 1.002^T, whereas its closest phylogenetic neighbours contained several sugars.

Survival rates after exposure to doses of UV light (254 nm, 200 µW cm⁻², 5 min) were analysed for strain GIMN 1.002^T, *M. arborescens* DSM 20754^T, *M. imperiale* DSM 20530^T and *Escherichia coli* K-12 CGMCC 1.3065. Cells were harvested by centrifugation at 5000 g and washing twice with 100 mM potassium phosphate buffer (pH 7.0). A 20-ml cell suspension was exposed to UV radiation on a 9 cm plate for 5 min. Irradiated cultures were serially diluted and 100 µl aliquots were spread on tryptone/glucose/yeast agar. After incubation at 30 °C for 3–4 days, the total c.f.u. ml⁻¹ was determined. The survival curve of *E. coli* K-12 CGMCC 1.3065 dropped the most sharply, and the three *Microbacterium* strains demonstrated more resistance to UV light, with strain GIMN 1.002^T showing the highest resistance of them all (Supplementary Fig. S1, available in IJSEM Online).

On the basis of the phenotypic, chemotaxonomic and phylogenetic data, strain GIMN 1.002^T represents a novel species of the genus *Microbacterium*, for which the name *Microbacterium radiodurans* sp. nov. is proposed.

Description of *Microbacterium radiodurans* sp. nov.

Microbacterium radiodurans (ra.di.o.du'rans. L. n. *radius* a beam or ray; N.L. pref. *radio-* pertaining to radiation;

L. part. adj. *durans* enduring; N.L. part. adj. *radiodurans* resisting radiation).

Cells are Gram-reaction-positive, heterotrophic, strictly aerobic, weakly motile, short rods, about 0.4–0.6 µm wide and 0.5–0.8 µm long. Colonies grown on NA agar at 28 °C for 2 days are smooth, nearly circular, convex, transparent and white. Growth occurs at 18–30 °C, at pH 6.0–10.0 and with <7% (w/v) NaCl. Weakly positive for catalase and negative for oxidase. Negative for aerobic and anaerobic nitrate reduction. Gelatin is not hydrolysed. With the Biolog GP2 MicroPlate system, utilizes β-cyclodextrin, cellobiose, gentiobiose, α-D-glucose, inositol, maltose, maltotriose, D-mannitol, D-mannose, 3-methyl D-glucose, α-ketoglutaric acid, succinic acid monomethyl ester, pyruvic acid methyl ester, glycyl L-glutamic acid, uridine, uridine 5'-monophosphate, D-xylose, salicin, D-sorbitol, D-fructose, putrescine, trehalose and α-D-glucose 1-phosphate. Does not utilize α-cyclodextrin, dextrin, glycogen, Tween 40 or 80, L-arabinose, D-galacturonic acid, sedoheptulosan, methyl α-D-glucoside, D-tagatose, xylitol, L-glutamic acid, lactamide, D-lactic acid methyl ester, L-alanine, L-lactic acid, D-malic acid, L-alanine, N-acetyl-L-glutamic acid, L-serine, sucrose, raffinose, propionic acid, L-malic acid, L-rhamnose, L-alaninamide, L-pyrogutamic acid, adenosine or 2'-deoxyadenosine. The cell-wall sugar is rhamnose and the diagnostic diamino acid is ornithine. The major menaquinones are MK-11, MK-12 and MK-10. The acyl type of the peptidoglycan is glycolyl. The major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, an unidentified phospholipid and an unidentified glycolipid. The fatty acids are anteiso-C_{15:0}, iso-C_{16:0}, iso-C_{15:0}, anteiso-C_{17:0}, iso-C_{14:0} and C_{16:0}. The DNA G+C content of the type strain is 67.74 mol%.

The type strain, GIMN 1.002^T (=CCTCC M208212^T =NRRL B-24799^T), was isolated from the upper sand layers of the Gobi desert, Xinjiang, China.

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