Hymenobacter xinjiangensis sp. nov., a radiation-resistant bacterium isolated from the desert of Xinjiang, China

Qiuju Zhang,¹ Cong Liu,¹ Yali Tang,¹ Guoling Zhou,¹ Ping Shen,¹ Chengxiang Fang¹ and Akira Yokota²

Correspondence
Chengxiang Fang
cxfang@whu.edu.cn

¹College of Life Sciences, Wuhan University, Wuhan 430072, China

²Institute of Molecular and Cellular Biosciences, University of Tokyo, Tokyo 113-0032, Japan

A Gram-negative, non-motile, rod-shaped and pink-pigmented bacterium, designated strain X2-1g^T, was isolated from a mixture of sand samples collected from the desert of Xinjiang, China, after exposure of the sand to 8 kGy gamma radiation. Phylogenetic analysis based on 16S rRNA gene sequencing indicated that this isolate represents a novel member of the genus *Hymenobacter*, with low sequence similarities (<97 %) to recognized *Hymenobacter* species. Biolog GN2 assays supported this conclusion. Optimum growth was observed at pH 7 and 28 °C. The strain contained MK-7 as the predominant menaquinone and the major fatty acids were iso-C_{15:0} (19.5 %), C_{16:1ω7cl}/iso-C_{15:0} 2-OH (20.2 %), C_{16:1ω5c} (10.6 %), C_{16:0} (6.2 %), anteiso-C_{17:1} B/iso-C_{17:1} I (8.5 %) and C_{18:0} (6.5 %). The DNA G+C content was 54 mol% (*T_m*). On the basis of the polyphasic evidence presented, it is proposed that strain X2-1g^T represents a novel species of the genus *Hymenobacter*, for which the name *Hymenobacter xinjiangensis* sp. nov. is proposed. The type strain is X2-1g^T (=CCTCC AB 206080^T =IAM 15452^T).

Ionizing-radiation resistance has been observed in several members of the domains *Bacteria* and *Archaea*. Among the genera containing ionizing-radiation-resistant organisms, *Deinococcus* and *Rubrobacter* show the highest levels of resistance (Rainey *et al.*, 2005). Species of these genera have been shown to survive exposure to doses greater than 25 kGy. Other ionizing-radiation-resistant bacteria, although they are less resistant and have been shown to survive after exposure to lower levels of radiation, have also been isolated and described; these include some species of the genera *Acinetobacter*, '*Chroococcidiopsis*', *Methylobacterium*, *Kineococcus*, *Kocuria* and *Hymenobacter* (Rainey *et al.*, 2005).

At the time of writing, eight species are included in the genus *Hymenobacter*, namely *Hymenobacter roseosalivarius* (Hirsch *et al.*, 1998), *H. actinosclerus* (Collins *et al.*, 2000), *H. aerophilus* (Buczolits *et al.*, 2002), *H. norwichensis*, *H. ocellatus*, *H. gelipurpurascens*, *H. chitinivorans* (Buczolits *et al.*, 2006) and *H. rigui* (Baik *et al.*, 2006). Only *H. actinosclerus* has been reported to be resistant to ionizing radiation (Collins *et al.*, 2000). During an investigation of radiotolerant species in the desert of Xinjiang, China, we isolated a Gram-negative, non-motile, rod-shaped, pink-pigmented bacterium. Phylogenetic analysis based on 16S rRNA gene sequencing, supported by the results of

polyphasic taxonomic studies, suggested that the newly isolated strain, designated X2-1g^T, represents a novel species of the genus *Hymenobacter*.

For isolation of strain X2-1g^T, sand samples (1.0 g) collected from the desert were exposed to 8 kGy in a ⁶⁰Co source at a dose rate of 10 Gy min⁻¹ and then suspended in 1.0 ml sterile water and homogenized for 5 min in a sterile mortar. The resulting supernatant was spread on 0.1 × trypticase soy agar (TSA) (Chanal *et al.*, 2006), and isolation was achieved after 7 days incubation at 30 °C. The isolate was routinely cultured on the same medium and stored as a glycerol suspension (20 %, w/v) at -80 °C.

Extraction of genomic DNA, PCR amplification of the 16S rRNA gene and sequencing of the purified PCR product were carried out according to the methods of Lin *et al.* (2004). Phylogenetic analysis was performed by using MEGA, version 3.1 (Kumar *et al.*, 2004), after multiple alignment of the data via CLUSTAL_X (Thompson *et al.*, 1997). Distances were obtained using options according to the Kimura two-parameter model (Kimura, 1980) and clustering was performed by using the neighbour-joining method (Saitou & Nei, 1987). Bootstrap values from 1000 replications were used to determine the confidence level of the branches (Felsenstein, 1985).

Analysis of the 16S rRNA gene sequence of strain X2-1g^T resulted in a sequence of 1407 bases. Sequence similarities

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain X2-1g^T is DQ888329.

to recognized members of the genus *Hymenobacter* were below the threshold for demarcating bacterial species, as follows: *H. rigui* WPCB131^T (96.7%), *H. gelipurpurascens* Txg1^T (95.1%), *H. chitinivorans* Txc1^T (95.0%), *H. norwichensis* NS/50^T (94.5%), *H. actinosclerus* CCUG 39621^T (94.3%), *H. roseosalivarius* AA-718^T (93.7%), *H. aerophilus* I/26-Cor1^T (93.4%) and *H. ocellatus* Txo1^T (93.4%). No other recognized bacterial species showed more than 90% 16S rRNA gene sequence similarity to the new isolate. This relationship between strain X2-1g^T and other members of the genus *Hymenobacter* is also evident in the phylogenetic tree. As shown in Fig. 1, strain X2-1g^T clustered with species of the genus *Hymenobacter*, but K_{nuc} values clearly differentiated the novel strain from recognized species. Therefore, 16S rRNA gene sequence similarity data and phylogenetic analysis suggest that strain X2-1g^T represents a novel species in the genus *Hymenobacter*.

Gram behaviour was investigated by the KOH-lysis test (Moaledji, 1986) and by staining as described by Smibert & Krieg (1994). Cell morphology, size and motility were examined by phase-contrast microscopy (Olympus) from PYES cultures (Buczolits *et al.*, 2002) of different growth stages. Growth at different temperatures (4, 28, 37 and 42 °C), pH (4–10) and NaCl concentration (0, 1, 2, 4 and 10%, w/v) was investigated on PYES agar for up to 1 week. Conventional biochemical tests were performed as described by Smibert & Krieg (1994), including tests for oxidase, catalase, nitrate reduction, H₂S production, citrate utilization, indole production and urease. Additional phenotypic characteristics were determined by using the Biolog microbial identification system following the

protocol provided by the manufacturer. Enzyme activities were tested by using the API ZYM kit (bioMérieux) following the manufacturer's instructions. Antimicrobial susceptibility testing and pigment analysis were performed according to the method of Buczolits *et al.* (2002). The following antibiotics were tested: chloramphenicol (30 µg), colistin sulfate (10 µg), erythromycin (15 µg), gentamicin (10 µg), kanamycin (30 µg), penicillin G (10 IU), bacitracin (10 IU), polymyxin B sulfate (300 IU), tetracycline (10 µg) and vancomycin (30 µg). Any sign of growth inhibition was scored as sensitivity to that antibiotic. Resistance to an antimicrobial drug was indicated if no inhibition zone was observed. To determine the survival rate after exposure to gamma radiation, cultures were grown to an OD₆₀₀ of about 0.5, irradiated at the desired dose, diluted serially and plated (de Groot *et al.*, 2005). Percentage survival was determined by comparing against unirradiated cultures.

The G+C content of the genomic DNA was determined according to the thermal denaturation (T_m) method (Marmur & Doty, 1962). The respiratory quinone system was extracted and determined by HPLC (Shimadzu) as described by Xie & Yokota (2003). Cellular fatty acids of strain X2-1g^T were analysed as methyl esters by GC (Hewlett Packard 6890) according to the instructions of the Sherlock Microbial Identification System (MIDI).

The physiological and biochemical characteristics of strain X2-1g^T are listed in the species description below and in Table 1. The phenotypic features that can be used to differentiate strain X2-1g^T from its close relatives within the genus *Hymenobacter* are given in Table 1. The results of phenotypic examination demonstrated that the strain studied has many traits in common with *H. rigui*. However, the habitats occupied by these two taxa are different: *H. rigui* lives in freshwater (Baik *et al.*, 2006), whereas strain X2-1g^T was isolated from desert sand samples. In contrast to *H. rigui*, strain X2-1g^T tolerated high doses of gamma radiation, utilized D-mannitol, failed to assimilate D-glucose and was unable to grow at 1% NaCl or high pH. Furthermore, the genomic G+C content of X2-1g^T (54 mol%) was much lower than that of *H. rigui* (65 mol%). Differences were also observed in the cellular fatty acid compositions of the novel strain and strains of recognized *Hymenobacter* species, such as the presence of C_{18:0} and C_{18:1}ω7c fatty acids and some quantitative differences in certain components (Table 2).

It is therefore evident from its phylogenetic distinctness in combination with differences in cellular fatty acid composition and phenotypic findings that strain X2-1g^T can be differentiated from its nearest neighbours. Thus, the results of the polyphasic analysis presented here demonstrate that the bacterium studied could not be assigned to any of the currently described species of the genus *Hymenobacter*, and support the placement of strain X2-1g^T in a novel species, for which the name *Hymenobacter xinjiangensis* sp. nov. is proposed.

Fig. 1. Neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing the phylogenetic position of strain X2-1g^T. Bootstrap values, expressed as percentages of 1000 replications, are given at branching points. *Flavobacterium aquatile* ATCC 11947^T was used as an outgroup. Bar, 10% sequence divergence.

Table 1. Differential characteristics among *Hymenobacter* type strains

Strains: 1, X2-1g^T; 2, *H. norwichensis* LMG 21876^T; 3, *H. rigui* IMSNU 14116^T; 4, *H. actinosclerus* CCUG 39621^T; 5, *H. aerophilus* LMG 19657^T; 6, *H. roseosalivarius* DSM 11622^T. Data were taken from Hirsch *et al.* (1998), Collins *et al.* (2000), Buczolits *et al.* (2002, 2006), Baik *et al.* (2006) and this study. +, Positive; −, negative; (+), weakly positive; ND, no data available.

Characteristic	1	2	3	4	5	6
Pigmentation	Pink	Brick red	Pinkish red	Red	Red	Reddish
Growth at/in:						
4 °C	+	(+)	+	−	+	+
28 °C	+	+	+	+	+	−
37 °C	(+)	−	+	+	−	−
42 °C	−	−	−	+	−	−
1 % NaCl	−	−	+	ND	+	+
3 % NaCl	−	−	−	ND	(+)	−
pH for growth	5–7	ND	5–11	ND	ND	ND
Biolog GN2 plate substrate response for:						
Aesculin	+	ND	(+)	−	−	−
N-Acetyl-D-glucosamine	(+)	−	−	(+)	−	−
Arbutin	−	(+)	−	+	−	−
D-Fructose	(+)	(+)	+	−	+	−
D-Glucose	−	(+)	+	+	+	−
D-Mannose	(+)	(+)	−	+	+	−
Sucrose	+	−	+	−	(+)	−
D-Galactose	(+)	(+)	+	−	−	−
Maltose	+	(+)	+	−	−	−
D-Mannitol	+	(+)	−	−	−	−
Enzyme activity (API ZYM):						
Cystine arylamidase	(+)	−	(+)	+	ND	ND
α-Glucosidase	−	−	+	ND	ND	+
DNA G + C content (mol%)	54	ND	65	62	63	56

Description of *Hymenobacter xinjiangensis* sp. nov.

Hymenobacter xinjiangensis (xin.jiang.en'sis. N.L. masc. adj. *xinjiangensis* pertaining to Xinjiang, an autonomous region in north-west China).

Cells are Gram-negative, aerobic, non-spore-forming rods. Motility is not observed. Cells are approximately $0.7 \times 2\text{--}5\text{ }\mu\text{m}$ in size. Cells grow best on nutrient-reduced media such as $0.1 \times$ TSA and PYES agar. Colonies on $0.1 \times$ TSA and PYES agar are translucent, pink, circular, entire, of low convexity and rough; colony diameter is up to 1.5 cm after 5 days at 28 °C. The temperature range for growth is 4–37 °C (optimum 28 °C). Oxidase- and catalase-positive. Aesculin hydrolysis is positive. Negative for fermentation of glucose, nitrate reduction, H₂S production, citrate utilization, indole production and urease. In the API ZYM system, alkaline phosphatase, esterase C4, esterase lipase C8, leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, N-acetyl-β-glucosaminidase and α-mannosidase activity are detectable. Lipase C14, trypsin, chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase,

β-glucosidase and α-fucosidase activity are not detectable. Positive in the Biolog system for dextrin, D-cellobiose, i-erythritol, L-fucose, lactulose, maltose, D-mannitol, D-psicose, D-sorbitol, sucrose, D-trehalose, acetic acid, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, γ-hydroxybutyric acid, propionic acid, succinic acid, glucuronamide, L-alanine, glycyl L-glutamic acid, L-serine, DL-carnitine, thymidine and α-D-glucose 1-phosphate. Negative in the Biolog system for α-cyclodextrin, Tweens 40 and 80, N-acetyl-D-galactosamine, adonitol, L-arabinose, D-arabitol, gentiobiose, α-D-glucose, α-D-lactose, methyl β-D-glucoside, pyruvic acid methyl ester, succinic acid methyl ester, *cis*-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, α-hydroxybutyric acid, β-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, α-ketobutyric acid, α-ketoglutaric acid, α-ketovaleric acid, DL-lactic acid, malonic acid, quinic acid, sebacic acid, bromosuccinic acid, succinamic acid, L-alaninamide, L-alanyl glycine, L-asparagine, glycyl L-aspartic acid, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-pyrogutamic acid, D-serine, L-threonine, γ-aminobutyric acid, urocanic acid, inosine, uridine, phenylethylamine, putrescine,

Table 2. Fatty acid profile of strain X2-1g^T compared with other members of the genus *Hymenobacter*

Strains: 1, X2-1g^T; 2, *H. norwichensis* LMG 21876^T; 3, *H. rigui* IMSNU 14116^T; 4, *H. actinosclerus* CCUG 39621^T; 5, *H. aerophilus* LMG 19657^T; 6, *H. roseosalivarius* DSM 11622^T. Values are percentages of total fatty acids. Data were taken from Hirsch *et al.* (1998), Collins *et al.* (2000), Buczolits *et al.* (2002, 2006), Baik *et al.* (2006) and this study. tr, Trace (<1 %); ND, not detected/not reported.

Fatty acid	1	2	3	4	5	6
C _{14:0}	1.3	1.3	ND	ND	ND	ND
iso-C _{15:1} G	2.0	ND	ND	ND	ND	ND
Summed feature 2*	2.2	tr	ND	2.3	tr	ND
iso-C _{15:0}	19.5	27.3	34.8	22.3	10.8	8.3
anteiso-C _{15:0}	3.7	10.6	5.9	25.8	22.3	ND
iso-C _{16:1} H	1.8	1.4	ND	1.5	1.5	2.7
iso-C _{16:0}	1.0	1.2	ND	ND	tr	2.1
Summed feature 3*	20.2	23.6	13.8	13.1	21.4	29.8
C _{16:1} ω5c	10.6	13.6	15.0	3.7	7.9	23.3
C _{16:0}	6.2	2.2	6.4	ND	1.7	1.1
iso-C _{15:0} 3-OH	2.2	2.2	ND	1.6	1.6	2.7
Summed feature 4*	8.5	8.3	14.4	19.9	17.7	18.5
iso-C _{17:0} 3-OH	3.4	3.0	3.1	3.1	3.5	5.8
iso-C _{17:0}	1.8	1.6	5.0	1.8	4.5	1.7
anteiso-C _{17:0}	tr	ND	ND	tr	2.3	ND
C _{17:0} 2-OH	tr	ND	ND	2.0	1.3	ND
C _{18:1} ω9c	1.1	ND	ND	ND	ND	ND
C _{18:0}	6.5	ND	ND	ND	ND	ND
C _{18:1} ω7c	4.8	ND	ND	ND	ND	ND

*Summed feature 2 contains C_{13:0} 3-OH and/or iso-C_{15:1} I; summed feature 3 contains C_{16:1}ω7c and/or iso-C_{15:0} 2-OH; summed feature 4 contains anteiso-C_{17:1} B and/or iso-C_{17:1} I.

2-aminoethanol, 2,3-butanediol and glycerol. Sensitive to chloramphenicol, colistin sulfate, erythromycin, gentamicin, penicillin G, polymyxin B sulfate, tetracycline and vancomycin. Tolerates high doses of gamma radiation, with a D₁₀ (dose required to reduce the bacterial population by 10-fold) of 4.8 kGy. Quinone system is menaquinone MK-7. Major fatty acids (>5 %) are iso-C_{15:0} (19.5 %), C_{16:1}ω7c/iso-C_{15:0} 2-OH (20.2 %), C_{16:1}ω5c (10.6 %), C_{16:0} (6.2 %), anteiso-C_{17:1} B/iso-C_{17:1} I (8.5 %) and C_{18:0} (6.5 %); complete fatty acid data are given in Table 2. The G+C content of the type strain is 54 mol% (T_m).

The type strain, X2-1g^T (=CCTCC AB 206080^T =IAM 15452^T), was isolated from gamma-irradiated soil of the Xinjiang desert, China.

Acknowledgements

We are grateful to J. P. Euzéby for his help with nomenclature and etymology.

References

- Baik, K. S., Seong, C. N., Moon, E. Y., Park, Y.-D., Yi, H. & Chun, J. (2006). *Hymenobacter rigui* sp. nov., isolated from wetland freshwater. *Int J Syst Evol Microbiol* **56**, 2189–2192.
- Buczolits, S., Denner, E. B. M., Vybiral, D., Wieser, M., Kämpfer, P. & Busse, H.-J. (2002). Classification of three airborne bacteria and proposal of *Hymenobacter aerophilus* sp. nov. *Int J Syst Evol Microbiol* **52**, 445–456.
- Buczolits, S., Denner, E. B. M., Kämpfer, P. & Busse, H.-J. (2006). Proposal of *Hymenobacter norwichensis* sp. nov., classification of 'Taxeobacter ocellatus', 'Taxeobacter gelupurpurascens' and 'Taxeobacter chitinovorans' as *Hymenobacter ocellatus* sp. nov., *Hymenobacter gelupurpurascens* sp. nov. and *Hymenobacter chitinivorans* sp. nov., respectively, and emended description of the genus *Hymenobacter* Hirsch *et al.* 1999. *Int J Syst Evol Microbiol* **56**, 2071–2078.
- Chanal, A., Chapon, V., Benzerara, K., Barakat, M., Christen, R., Achouak, W., Barras, F. & Heulin, T. (2006). The desert of Tataouine: an extreme environment that hosts a wide diversity of micro-organisms and radiotolerant bacteria. *Environ Microbiol* **8**, 514–525.
- Collins, M. D., Hutson, R. A., Grant, I. R. & Patterson, M. F. (2000). Phylogenetic characterization of a novel radiation-resistant bacterium from irradiated pork: description of *Hymenobacter actinosclerus* sp. nov. *Int J Syst Evol Microbiol* **50**, 731–734.
- de Groot, A., Chapon, V., Servant, P., Christen, R., Saux, M. F.-L., Sommer, S. & Heulin, T. (2005). *Deinococcus deserti* sp. nov., a gamma-radiation-tolerant bacterium isolated from the Sahara Desert. *Int J Syst Evol Microbiol* **55**, 2441–2446.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Hirsch, P., Ludwig, W., Hethke, C., Sittig, M., Hoffmann, B. & Gallikowski, C. A. (1998). *Hymenobacter roseosalivarius* gen. nov., sp. nov. from continental Antarctic soils and sandstone: bacteria of the Cytophaga/Flavobacterium/Bacterioides line of phylogenetic descent. *Syst Appl Microbiol* **21**, 374–383.
- Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.
- Kumar, S., Tamura, K. & Nei, M. (2004). MEGA 3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.
- Lin, Y.-C., Uemori, K., de Briel, D. A., Arunpairojana, V. & Yokota, A. (2004). *Zimmermannella helvola* gen. nov., sp. nov., *Zimmermannella alba* sp. nov., *Zimmermannella bifida* sp. nov., *Zimmermannella faecalis* sp. nov. and *Leucobacter albus* sp. nov., novel members of the family Microbacteriaceae. *Int J Syst Evol Microbiol* **54**, 1669–1676.
- Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.
- Moaledji, K. (1986). Comparison of Gram-staining and alternate methods, KOH test and aminopeptidase activity in aquatic bacteria: their application to numerical taxonomy. *J Microbiol Methods* **5**, 303–310.
- Rainey, F. A., Ray, K., Ferreira, M., Gatz, B. Z., Nobre, M. F., Bagaley, D., Rash, B. A., Park, M. J., Earl, A. M. & other authors (2005). Extensive diversity of ionizing-radiation-resistant bacteria recovered from Sonoran Desert soil and description of nine new species of the genus *Deinococcus* obtained from a single soil sample. *Appl Environ Microbiol* **71**, 5225–5235.

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

Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Woods & N. R. Krieg. Washington, DC: American Society for Microbiology.

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.

Xie, C. H. & Yokota, A. (2003). Phylogenetic analysis of *Lamprospira hyalina* based on the 16S rRNA gene sequence. *J Gen Appl Microbiol* **49**, 345–349.