Deinococcus reticulitermitis sp. nov., isolated from a termite gut

Wen Chen,¹ Baojun Wang,² Huazhu Hong,¹ Hong Yang¹
and Shuang-Jiang Liu²

Correspondence

Hong Yang

hyang@mail.ccnu.edu.cn

¹Key Laboratory of Pesticide & Chemical Biology, Ministry of Education, College of Life Sciences, Central China Normal University, Wuhan 430079, PR China

²State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, PR China

Bacterial strain TM-1^T was isolated from the gut of a wood-feeding termite, *Reticulitermes chinensis* Snyder. Cells of strain TM-1^T were Gram-negative, spherical (1.0–2.0 µm in diameter), non-motile, non-sporulating and red-pigmented. Strain TM-1^T was resistant to UV radiation, showing 34 % survival after exposure to UV light at a dose of 100 J m⁻². Growth occurred at 20–40 °C (optimum, 30 °C), at pH 6.0–10.0 (optimum, pH 6.0–7.0), and in the presence of 0–1 % (w/v) NaCl (optimum, 0–0.4 %). Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain TM-1^T was related to members of the genus *Deinococcus*, with sequence similarities ranging from 87.0 to 94.0 %. The peptidoglycan of strain TM-1^T contained ornithine, alanine, glycine and glutamic acid. The most abundant cellular fatty acids of strain TM-1^T were summed feature 3 (C_{16:1}ω7c and/or C_{16:1}ω6c; 22.3 %) and C_{16:0} (37.5 %). MK-8 was the predominant quinone. The polar lipid profile contained three glycerophospholipids, six glycolipids, one aminolipid and three unknown lipids. DNA of the type strain had a G + C content of 65.6 mol%. Based on the phylogenetic, chemotaxonomic and phenotypic data presented, strain TM-1^T represents a novel species of the genus *Deinococcus*, for which the name *Deinococcus reticulitermitis* sp. nov. is proposed, with TM-1^T (=CGMCC 1.10218^T=NBRC 106334^T) as the type strain.

The genus *Deinococcus* (Brooks & Murray, 1981) represents a deep-branching lineage within the 'Bacteria'. At the time of writing, the genus comprised 46 species with validly published names isolated from various environments such as desert soil (Rainey *et al.*, 2005), air (Weon *et al.*, 2007; Yoo *et al.*, 2010), water (Kämpfer *et al.*, 2008), a hot spring (Ferreira *et al.*, 1997), continental Antarctica (Hirsch *et al.*, 2004) and fish (Shashidhar & Bandekar, 2009). Most species of this genus are resistant to UV and gamma radiation. However, four psychrophilic, ionizing-radiation-sensitive *Deinococcus* species, isolated from alpine environments, have been described recently (Callegan *et al.*, 2008). A UV-radiation-resistant strain, *Deinococcus xinjiangensis* X-82^T, which was isolated from a desert soil sample of Xinjiang region, China, was found to be sensitive to gamma radiation and desiccation (Peng *et al.*, 2009).

During the course of a study on the diversity of culturable bacteria in the gut of a wood-feeding termite, *Reticulitermes chinensis* Snyder (Isoptera: Rhinotermitidae), bacterial strain

TM-1^T was isolated by the serial dilution method. Termites were collected from Guizi Mountain in Wuhan, China and fed with pine wood in the laboratory for 1 year. To obtain enrichment cultures, 10 worker termites were surface-sterilized in 70 % ethanol and then washed in sterile distilled water. The entire guts were removed from the abdomen and disrupted with sterile fine-tipped forceps. The hindgut contents were suspended in 1 ml sterile phosphate buffer (PBS: 0.137 M NaCl, 10 mM Na₂HPO₄, 2.7 mM KCl and 2 mM KH₂PO₄, pH 7.4) and serially diluted. The dilution was then spread on agar plates with basal medium MM-4 modified from Brune *et al.* (1995) that contained (g l⁻¹, unless otherwise stated): NaCl, 1; KCl, 0.5; MgCl₂·6H₂O, 0.1; CaCl₂·2H₂O, 0.015; NH₄Cl, 0.3; KH₂PO₄, 0.2; Na₂SO₄, 0.15; Bacto yeast extract, 0.5; Casamino acids, 0.5; PBS, 20 mM; trace element solution SL 11 (Eichler & Pfennig, 1986), 1 ml; and seven-vitamin solution (Janssen *et al.*, 1997), 1 ml. The plates were incubated at 30 °C for 1 week. Colonies exhibiting different morphologies on the plates were picked up and subcultured to obtain pure cultures. Strain TM-1^T was isolated from a single red-pigmented colony.

The nearly complete 16S rRNA gene of strain TM-1^T (1469 bp) was amplified and sequenced as described previously (Ying *et al.*, 2007). DNA BLAST searches (Altschul *et al.*,

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain TM-1^T is HM214546.

Five supplementary figures and one supplementary table are available with the online version of this paper.

1990) of 16S rRNA gene sequences using the NCBI database showed that strain TM-1^T was phylogenetically related to members of the genus *Deinococcus*. The partial 16S rRNA gene sequence was aligned with those of species of the genus *Deinococcus* by using the program CLUSTAL_X version 1.64b (Thompson *et al.*, 1997). A maximum-likelihood phylogenetic tree (Fig. 1) was constructed using fastDNAmI (Olsen *et al.*, 1994; Felsenstein, 1981) included in PHYLIP v3.5 (Felsenstein, 1993). A neighbour-joining phylogenetic tree (Supplementary Fig. S1, available in IJSEM Online) and a maximum-parsimony phylogenetic tree (Supplementary Fig. S2, available in IJSEM Online) were constructed using MEGA

version 4.1 (Kumar *et al.*, 2004). Topology of the phylogenetic trees was evaluated by the bootstrap resampling method based on 1000 replicates.

Analysis of 16S rRNA gene sequences indicated that strain TM-1^T was phylogenetically related to members of the genus *Deinococcus* with sequence similarities ranging from 87.0 to 94.0%. In particular, strain TM-1^T was closely related to *Deinococcus gobiensis* I-0^T (94.0%) (Yuan *et al.*, 2009), *Deinococcus aquaticus* PB314^T (94.0%) (Im *et al.*, 2008) and *Deinococcus caeni* Ho-08^T (94.0%) (Im *et al.*, 2008); 16S rRNA gene sequence similarities with strains

Fig. 1. Phylogenetic tree constructed with the maximum-likelihood method based on the 16S rRNA gene sequences of TM-1^T and other *Deinococcus* species. *Truepera radiovictrix* RQ-24^T was used as an outgroup. GenBank accession numbers are given in parentheses. Bootstrap values (1000 replicates) higher than 700 out of 1000 were shown. Bar, 0.01 substitutions per nucleotide position.

of all other *Deinococcus* species were lower. Phylogenetic analysis (Fig. 1) showed that strain TM-1^T formed a unique cluster with *Deinococcus gobiensis* I-0^T in phylogenetic trees constructed with the maximum-likelihood (Fig. 1), neighbour-joining (Supplementary Fig. S1, available in IJSEM Online) and maximum-parsimony (Supplementary Fig. S2, available in IJSEM Online) methods.

Cultural and morphological characteristics were observed on MM-4 agar plates. Growth was also assessed in threefold-diluted tryptic soy broth (TSB/3; 10 g l⁻¹; Bacto; Becton Dickinson), but no growth was detected on undiluted TSB or Luria-Bertani (LB) broth. Gram-staining was performed as described by Gerhardt *et al.* (1994). Morphological observations were performed by scanning electron microscopy (FEI Quanta 200). The presence of flagella and cell mobility were examined according to Dong & Cai (2001). The basic physiological and biochemical characteristics of strain TM-1^T were investigated using routine cultivation in MM-4 broth at 30 °C. The temperature range (9, 20, 30, 40, 50 °C) was determined with a TN3F temperature-gradient incubator (Advantec). Growth at different pH (4.5–10.5 at intervals of 0.5 pH units) was tested by using MM-4 in which the pH was adjusted by the addition of 5 M NaOH or HCl and verified after autoclaving. Tolerance to NaCl was tested in MM-4 medium with 0, 0.1, 0.2, 0.4, 1.0, 2.0 and 4.0% (w/v) NaCl. Metabolic properties were determined using API 20NE test kits (bioMérieux) according to the manufacturer's instructions at 30 °C. Tests for catalase and oxidase activities and hydrolysis of starch were carried out according to Dong & Cai (2001).

Cell-wall peptidoglycan analysis was performed with a method modified from Stanek & Roberts (1974) and Dong & Cai (2001) with *D. gobiensis* I-0^T as a reference. Approximately 30 mg (wet wt) of cells was placed into a small ampoule with 1 ml absolute alcohol overnight. The alcohol was then discarded and cells were air-dried. Cell pellets were resuspended in 0.1 ml 6 M HCl. The sealed ampoule was kept at 120 °C for 20 min. Before chromatography, the TLC sheet (crystallite cellulose plate; Anhui LiangChen Silicon Material) was activated at 110 °C for 30 min. Samples of cell wall preparation and standard amino acids were applied at the base line of the TLC sheet. Ascending TLC was performed with the solvent system methanol/pyridine/acetic acid/distilled water (5.0:0.5:0.125:2.5, by vol.) for approximately 3.5 h. After the chromatogram was air-dried, spots were visualized by spraying with 0.4% ninhydrin in butyl alcohol and heating at 70 °C for 5 min.

Fatty acids were extracted, methylated and analysed in parallel with those of *D. gobiensis* I-0^T and *Deinococcus radiodurans* CGMCC 1.633^T according to the standard protocol of the Sherlock Microbial Identification System (MIDI; Microbial ID; http://www.microbialid.com/PDF/TechNote_101.pdf) except that cells were grown on TSB/3 agar plates. Menaquinones were extracted and purified according to the method of Collins (1985) and were

analysed by HPLC (Wu *et al.*, 1989) with *D. radiodurans* CGMCC 1.633^T as a reference. Polar lipids were extracted according to the method of Zhang *et al.* (2010) and separated by TLC on Merck Kieselgel 60-HPTLC by two-dimensional development as described by Ross *et al.* (1985). Phospholipids were detected with the Zinzadze reagent of Dittmer & Lester (1964). Glycolipids were detected by spraying the plate with 0.5% 1-naphthol in methanol/water (1:1, v/v) and then with sulfuric acid/ethanol (1:1, v/v), followed by heating at 120 °C for 5–10 min (Xin *et al.*, 2001). Aminolipids were detected by spraying with ninhydrin reagent (0.2 g ninhydrin in 100 ml ethanol) and heating at 120 °C. Whole lipids were detected by spraying with molybdatophosphoric acid (10 g molybdatophosphoric acid hydrate in 100 ml ethanol) followed by heating at 120–160 °C (Worliczek *et al.*, 2007). The DNA G+C content was determined by thermal denaturation (Marmur & Doty, 1962), with *Escherichia coli* K-12 as a reference.

To determine the tolerance of the isolate to UV radiation, strain TM-1^T was grown in TSB/3 liquid medium to the exponential phase; *D. radiodurans* CGMCC 1.633^T and *E. coli* K-12 were tested as positive and negative controls. Cells were recovered by centrifugation, washed with sterile PBS and serially diluted. Aliquots of 0.1 ml from each dilution were spread in triplicate on TSB/3 agar plates (with their lids open), exposed to UV light (254 nm) for dosages of 100, 200, 400, 600, 800 and 1000 J m⁻², and then incubated in the dark at 30 °C for 10 days. Relative survival was determined by comparing colony counts of irradiated cells with unirradiated cultures.

Strain TM-1^T stained Gram-negative and formed visible (about 1 mm) red-pigmented colonies within 72 h at 30 °C on MM-4 agar. The colonies were translucent and shiny with entire edges after 3 days of cultivation. Strain TM-1^T was an oxidase-negative, catalase-positive, non-motile and non-spore-forming coccus (1.0–2.0 µm in diameter) (Supplementary Fig. S3, available in IJSEM Online). It was not able to use carboxymethylcellulose for growth. Additional physiological and biochemical properties of strain TM-1^T are provided in the species description and the properties that distinguish strain TM-1^T from its closely related species are listed in Table 1.

The peptidoglycan of strain TM-1^T contained ornithine, alanine, glycine and glutamic acid, which was also observed in its phylogenetically closest relative, *D. gobiensis* I-0^T. The most abundant cellular fatty acids of strain TM-1^T were summed feature 3 (C_{16:1}ω7c and/or C_{16:1}ω6c; 22.3%) and C_{16:0} (37.5%), which are also predominant in most other *Deinococcus* species (Supplementary Table S1, available in IJSEM Online). However, some qualitative and quantitative differences in fatty acid composition could be observed between strain TM-1^T and its phylogenetically closest relatives. For example, the proportion of summed feature 3 in strain TM-1^T was similar to that observed in *D. radiodurans* CGMCC 1.633^T, *D. gobiensis* I-0^T and *Deinococcus*

Table 1. Differential phenotypic characteristics of strain TM-1^T and related members of the genus *Deinococcus*

Strains: 1, TM-1^T; 2, *D. gobiensis* I-0^T; 3, *D. radiodurans* CGMCC 1.633^T; 4, *D. aquaticus* PB314^T (Im *et al.*, 2008); 5, *D. caeni* Ho-08^T (Im *et al.*, 2008); 6, *D. aquiradiocola* TDMA-uv53^T (Asker *et al.*, 2009). For phenotypic characterization, strain TM-1^T, *D. gobiensis* I-0^T and *D. radiodurans* CGMCC 1.633^T were tested in parallel in this study with API 20NE. +, Positive; –, negative; (+), weakly positive; ND, not determined.

Characteristic	1	2	3	4	5	6
Cell morphology	Coccus	Coccus	Coccus	Rod	Rod	Rod
Colony colour*	R	PR	R	R	P	PP
Temperature growth range (°C)	20–40	15–35	30	20–37	20–37	20–40
pH growth range	6.0–10.0	7.0–8.0	ND	6.0–9.5	6.5–9.5	5.0–9.0
Oxidase activity	–	+	+	+	–	+
Starch hydrolysis	(+)	+	ND	+	+	+
Nitrate reduction	+	+	+	–	–	–
Indole formation	–	–	–	–	–	–
Arginine dihydrolase activity	–	–	–	–	–	ND
Urease activity	–	+	–	–	–	–
Aesculin hydrolysis	+	+	–	+	–	+
Gelatin hydrolysis	+	+	–	–	–	+
β-Galactosidase activity	+	+	–	+	ND	+
Glucose fermentation	+	+	+	ND	ND	ND
Assimilation of (API 20 NE):						
D-Glucose	–	+	+	+	+	+
L-Arabinose	–	–	–	–	–	+
D-Mannose	+	+	+	+	–	+
D-Mannitol	–	+	–	–	–	+
N-Acetylglucosamine	–	–	–	+	+	–
Maltose	+	+	–	+	+	+
Gluconate	–	–	–	+	+	–
Capric acid	–	–	–	–	–	–
Adipic acid	–	–	–	–	–	–
Malic acid	(+)	–	+	+	–	–
Citrate	–	–	–	–	–	–
Phenylacetic acid	–	–	–	–	–	–
DNA G + C content (mol%)	65.6	65.4	67	68.3	68.4	69.1

*R, Red; PR, pink-red; P, pink; PP, pale pink.

aquiradiocola TDMA-uv53^T but lower than that found in *D. aquaticus* PB314^T and *D. caeni* Ho-08^T. In addition, in contrast to the other five *Deinococcus* strains listed in Supplementary Table S1 (available in IJSEM Online), strain TM-1^T produced higher proportions of C_{14:0}, iso-C_{13:0}, 3-OH, iso-C_{15:1}, anteiso-C_{15:1} A and anteiso-C_{17:1}ω9c.

A complex polar lipid profile of strain TM-1^T was shown by two-dimensional TLC analysis (Supplementary Fig. S4, available in IJSEM Online). The predominant polar lipids were phosphoglycolipid (PGL₂), which was consistent with previous results for *Deinococcus* species (Anderson & Hansen, 1985; Ferreira *et al.*, 1997; Im *et al.*, 2008; Peng *et al.*, 2009). Moreover, strain TM-1^T showed unknown phosphoglycolipids PGL₁ and PGL₄, glycolipids GL₂, GL₃, GL₄, GL₅, GL₆ and GL₇, aminolipid AL, and three unknown polar lipids L₁, L₂ and L₃, which were also detected in other *Deinococcus* species (Im *et al.*, 2008; Kämpfer *et al.*, 2008). However, the unique combination of unknown polar lipids distinguished it from other *Deinococcus* species. The predominant isoprenoid

quinone of TM-1^T was MK-8, which was typical of members of the genus *Deinococcus*. The DNA G + C content was 65.6 mol%, a value that was within the range found for other species of the genus *Deinococcus* (Hirsch *et al.*, 2004).

Strain TM-1^T could tolerate UV irradiation, showing 34 % survival after exposure to UV light at a dose of 100 J m^{–2}. Strain TM-1^T and *D. radiodurans* CGMCC 1.633^T could grow at a higher dose (600 J m^{–2}), whereas *E. coli* K-12 showed no resistance at this dose (Supplementary Fig. S5, available in IJSEM Online).

Based on the above phenotypic and phylogenetic studies, it is clear that strain TM-1^T is a member of the genus *Deinococcus*. However, strain TM-1^T could be distinguished from its closest relative *D. gobiensis* I-0^T by differences in oxidase and urease activities. In addition, the assimilation of various carbon sources such as glucose, mannitol and malic acid differed in the two strains (Table 1). Considering other chemotaxonomic and phenotypic characteristics, and 16S rRNA gene sequence analysis, it is concluded that bacterial

strain TM-1^T represents a novel species of the genus *Deinococcus*; the name *Deinococcus reticulitermitis* sp. nov. is proposed.

Description of *Deinococcus reticulitermitis* sp. nov.

Deinococcus reticulitermitis (re.ti.cu.li.ter'mi.tis. N.L. n. *reticulitermitis* a termite of the genus *Reticulitermes*).

Cells are spherical, non-motile and stain Gram-negative. Colonies on MM-4 agar are red-pigmented, smooth, convex, circular, uniform-edged and 1–2 mm in diameter. No growth is detected on TSB or LB broth. Grows on MM-4 and TSB/3 at 30 °C. Growth occurs at 20–40 °C (optimum, 30 °C), at pH 6.0–10.0 (optimum, pH 6.0–7.0), and in the presence of 0–1.0 % (w/v) NaCl (optimum, 0–0.4 %). Nitrate is reduced. Aesculin, gelatin and starch are hydrolysed. β -Galactosidase is positive. Urease, oxidase and arginine dihydrolase activities are absent. Indole is not produced. Assimilates D-mannose, maltose and malic acid, but not D-glucose, L-arabinose, D-mannitol, N-acetyl-D-glucosamine, gluconate, caprate, phenylacetate, adipate, malate or citrate. The major cellular fatty acids are summed feature 3 (C_{16:1}ω7c and/or C_{16:1}ω6c), C_{16:0}, C_{14:0} and C_{18:0}. The peptidoglycan contains ornithine, alanine, glycine and glutamic acid. MK-8 is the predominant lipoquinone. The polar lipid profile contains three glycopospholipids, six glycolipids, one aminolipid and three unknown lipids.

The type strain is TM-1^T (=CGMCC 1.10218^T=NBRC 106334^T), isolated from the gut of a wood-feeding termite, *Reticulitermes chinensis* Snyder, from China. The DNA G + C content of the type strain is 65.6 mol%.

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