Deinobacterium chartae gen. nov., sp. nov., an extremely radiation-resistant, biofilm-forming bacterium isolated from a Finnish paper mill

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A rod-shaped, non-spore-forming, non-motile, aerobic, oxidase and catalase-positive and radiation-resistant bacterium (designated strain K4.1^T) was isolated from biofilm collected from a Finnish paper mill. The bacterium grew as pale pink colonies on oligotrophic medium at 12 to 50 °C (optimum 37 to 45 °C) and at pH 6 to 10.3. The DNA G + C content of the strain was 66.8 mol%. According to 16S rRNA gene sequence analysis, strain K4.1^T was distantly related to the genus *Deinococcus*, sharing highest similarity with *Deinococcus pimensis* (90.0 %). In the phylogenetic tree, strain K4.1^T formed a separate branch in the vicinity of the genus *Deinococcus*. The peptidoglycan type was A3β with L-Orn–Gly–Gly and the quinone system was determined to be MK-8. The polar lipid profile of strain K4.1^T differed markedly from that of the genus *Deinococcus*. The predominant lipid of strain K4.1^T was an unknown aminophospholipid and it did not contain the unknown phosphoglycolipid predominant in the polar lipid profiles of deinococci analysed to date. Two of the predominant fatty acids of the strain, 15 : 0 anteiso and 17 : 0 anteiso, were lacking or present in small amounts in species of the genus *Deinococcus*. Phylogenetic distinctness and significant differences in the polar lipid and fatty acid profiles suggest classification of strain K4.1^T as a novel genus and species in the family *Deinococcaceae*, for which we propose the name *Deinobacterium chartae* gen. nov., sp. nov. The type strain is K4.1^T (=DSM 21458^T =HAMBI 2721^T).

The order *Deinococcales* currently consists of two families, *Deinococcaceae* (Battista & Rainey, 2001) and *Trueperaceae* (Albuquerque *et al.*, 2005). Both families contain only one genus, *Deinococcus* and *Truepera*, respectively. The genus *Truepera* holds only one species, *Truepera radiovictrix* (Albuquerque *et al.*, 2005). The genus *Deinococcus* is comprised of 39 species at the time of writing and new species are frequently described (ten in 2009). Members of the genus *Deinococcus* were isolated from environments ranging from arid soils (Hirsch *et al.*, 2004; Rainey *et al.*, 2005, 2007; de Groot *et al.*, 2005; Callegan *et al.*, 2008; Yuan *et al.*, 2009) to water (Kämpfer *et al.*, 2008; Im *et al.*, 2008) and geothermal springs (Asker *et al.*, 2008, 2009; Ferreira *et al.*, 1997). *Truepera radiovictrix* was also isolated

from a geothermal spring (Albuquerque *et al.*, 2005). *Deinococcus geothermalis* is known as a common colonizer in paper machines (Kolari *et al.*, 2003; Peltola *et al.*, 2008).

Strain K4.1^T was isolated from a biofilm growing in the wire section of a Finnish paper mill (wire water temperature 45–50 °C) producing folding boxboard. Biofilm was collected with a sterile plastic tube and transported to the laboratory on the same day. The biofilm was washed with sterile tap water to remove loosely attached bacteria. Washed pieces of the biofilm were incubated on a polystyrene 12-well plate in sterilized board mill wire water amended with 1.0 g soluble starch and 0.1 g yeast extract per litre. After 2 days of shaking at 160 r.p.m., 48 °C, freshly grown biofilm from the edges of the well was streaked on a plate of paper machine water (PMW) agar (wire water from the machine in question supplemented with 1.0 g soluble starch, 0.1 g yeast extract and 15 g agar per litre) and grown for 4 days at 48 °C. A pale pink colony (strain K4.1^T) was isolated from this plate and subcultured until pure on PMW agar plates.

Abbreviations: PMW, paper machine water; SEM, scanning electron microscopy; TEM, transmission electron microscopy.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain K4.1^T is AM988777.

Four supplementary figures and a supplementary table are available with the online version of this paper.

On R2A agar (g l⁻¹: yeast extract 0.5, meat peptone 0.5, Casamino acids 0.5, glucose 0.5, starch 0.5, K₂HPO₄ 0.3, MgSO₄ 0.05, sodium pyruvate 0.3, agar 15), strain K4.1^T grew as pale pink colonies of approximately 2 mm diameter after 2 days at 45 °C. Strain K4.1^T grew on R2A plates at 12 to 50 °C, but not at 55 °C, with an optimum between 37 and 45 °C. Growth was observed after 4 days of incubation (160 r.p.m.) at 45 °C at pH from 6.0 to 10.3, tested in increments of 0.3–0.5 pH units in R2 broth (g l⁻¹: yeast extract 0.5, meat peptone 0.5, Casamino acids 0.5, glucose 0.5, starch 0.5, K₂HPO₄ 0.3, MgSO₄ 0.05, sodium pyruvate 0.3) buffered with 12 mM MES, for pH range 5.9–6.9, CHES, for pH range 8.2–9.9, or CAPS, for pH range 9.7–11.0. Growth occurred on R2A plates with 3 % (w/v) but not with 5 % NaCl (5 days at 45 °C). No motility of the cells of strain K4.1^T was observed with a microscope and no spores were observed in 5-day-old cultures stained with malachite green. No anaerobic (Anaero Gen atmosphere generation system; Oxoid) growth was observed on medium in the absence of added carbohydrate [tryptic soy agar (TSA), g l⁻¹: casein peptone 15, soy peptone 5, NaCl 5, agar 15] for 3 days at 45 °C.

Oxidation of carbon sources by strain K4.1^T was analysed by using Phenotypic MicroArray plates PM1 and PM2A (Biolog) according to the manufacturer's instructions. Results were measured with a plate reader (OD_{600 nm}; Victor³, Perkin-Elmer) after 48 h of incubation from duplicate plates. A reaction was considered positive when the OD₆₀₀ was >0.08 units higher than that of the negative control without carbon source in both duplicate plates. Strain K4.1^T oxidized polymers of α -1,4-linked glucoses (dextrin, γ -cyclodextrin and starch) as well as α -1,3- and α -1,6-linked di- and trisaccharides (raffinose, turanose, palatinose and gentiobiose) but not 1,4-linked glucosides, sucrose, maltose or cellobiose. Strain K4.1^T oxidized pentose (D-xylose) and deoxyhexose (L-fucose) but not the monomeric hexoses fructose, mannose or glucose. These findings indicate that the pentose phosphate cycle may represent the preferred pathway for glucose utilization rather than glycolysis or that strain K4.1^T is defective in the uptake of hexoses. Starch degradation was tested on R2A agar supplemented with 10 g starch l⁻¹. Formation of a clearing zone around bacterial growth proved that strain K4.1^T is able to utilize starch. It appears that glucose-containing polymers are metabolized via cyclodextrins rather than exocellularly hydrolysed to glucose. The strain oxidized gelatin and several L-amino acids (alanine, aspartic acid, asparagine, glutamine, histidine and lysine) but not L-valine, L-glycine, L-hydroxyproline or L-phenylalanine. Of the monocarboxylic acids, the strain oxidized glycolic, propionic, butyric, caproic and *p*- and *m*-hydroxyphenylacetic acids but not benzoic or 2-hydroxybenzoic acid, or lactic or capric acid. Of the dicarboxylic acids, it oxidized succinic, fumaric, *m*-tartaric and malonic acids but not D- and L-tartaric acids. Several intermediates of the TCA cycle (acetic, pyruvic, L-malic or α -ketoglutaric acid) did not support the growth of strain K4.1^T.

Utilization of nitrogen, phosphorus and sulphur sources by strain K4.1^T were tested on Phenotypic MicroArray plates PM3B and PM4A (Biolog) using succinate as the carbon source. Inorganic nitrogen and phosphorus sources were not utilized when succinate was the carbon source. Four amino acids, three hexosamines, seven dipeptides, uric acid and alloxan were used as sole sources of nitrogen. Also, six nucleosides but not the corresponding purine (excepting guanine) or pyrimidine bases were used as sole sources of nitrogen. Nucleotide 5' monophosphates were the only compounds used as the sole sources of phosphorus when succinate was the carbon source. Strain K4.1^T did not grow on any of the tested sulphur sources when succinate was supplied as the carbon source. A list of the oxidized or utilized sources of carbon, nitrogen and phosphorus is provided in the species description.

Production of acid and gas from carbohydrates, reduction of nitrate and nitrite, and oxidase activity were tested at 45 °C as described by MacFaddin (2000). Strain K4.1^T produced acid from glucose, fructose and maltose but not from sucrose, mannitol, ribose, lactose, xylose, *myo*-inositol, sorbose or arabinose. Strain K4.1^T did not produce gas from any of the tested carbohydrates. Reference strains *Deinococcus radiodurans* DSM 20539^T and *Deinococcus geothermalis* 11300^T produced no acid or gas from any of the tested carbohydrates, which is in line with the genus description (Battista & Rainey, 2001). *Truepera radiovictrix* DSM 17093^T did not grow on this medium [standard broth base media: 1 % (w/v) peptone, 0.1 % meat extract, 0.5 % NaCl, 0.5 % agar, 0.002 % phenol red] or on other media composed of peptones without carbohydrate [3.3 × peptone yeast extract (PYE): 1 % (w/v) peptic digest of casein, 1 % yeast extract, 1.5 % agar; and TSA]. Strain K4.1^T, similarly to *Deinococcus radiodurans* DSM 20539^T and *Deinococcus geothermalis* 11300^T, was oxidase-positive (indicator *N,N,N',N'*-tetramethyl-*p*-phenylenediamine dihydrochloride) but did not reduce nitrate.

For ultrathin sections, strain K4.1^T was grown on TSA for 3 days at 37 or 45 °C, fixed in phosphate (0.1 M, pH 7.2) buffered 2.5 % glutaraldehyde for 2 h, and rinsed with phosphate buffer three times. Cells were secondary fixed in 1 % osmiumtetroxide at 4 °C for 1 h. After dehydration in a series of ethanol concentrations (50 to 70 to 96 to 100 %) and 100 % acetone, the cells were embedded in epon (Epon 812, Electron Microscopy Sciences). Ultrathin sections were cut, stained with uranium acetate and lead citrate and examined by transmission electron microscopy (TEM; JEOL 1200EX II). For field emission scanning electron microscopy (SEM), strain K4.1^T was grown in R2-broth for 3 days at 37 °C and fixed, dehydrated and examined as described previously (Raulio *et al.*, 2008).

Strain K4.1^T is a rod-shaped bacterium (Fig. 1) and cells measured from TEM images (not shown) of negatively stained cells grown on TSA for 3 days at 45 °C were 0.8–1.3 μ m wide and 1.4–2.5 μ m long. Cells were longer when grown in rich liquid medium [tryptic soy broth (TSB),

Fig. 1. Electron micrographs of strain K4.1^T. (a) Ultrathin section examined by TEM showing cell size and morphology. (b) Ultrastructure of cell surfaces examined by SEM. (c, d) Cell wall structure of strains K4.1^T and *D. geothermalis* E50051, respectively.

g l⁻¹: casein peptone 17, soy peptone 3, NaCl 5, K₂HPO₄ 2.5, glucose 2.5] at 45 °C for 1 day (3–8 µm) and filamentous (up to 40 µm long) after 2 days (Supplementary Fig. S1a and b, available in IJSEM Online).

In transmission electron micrographs the cell wall of strain K4.1^T lacked an outer membrane (Fig. 1c) and in the scanning electron micrographs the cell surface resembled that of Gram-positive bacteria; no shrinkage of any outer membrane was visible (Fig. 1b). Strain K4.1^T did not lyse in 3 % KOH and possessed no aminopeptidase. However, the cells stained Gram-negatively, even very young cultures stained after 11 h of incubation on R2A at 45 °C (data not shown).

The cell envelope of strain K4.1^T had a multilayered appearance (Fig. 1c), but was less complex than that of many species of the genus *Deinococcus* (Battista & Rainey, 2001; Ferreira *et al.*, 1997). It was very different from the cell envelope of the paper machine isolate *Deinococcus geothermalis* E50051 (Fig. 1d), which, like the type strain of *Deinococcus geothermalis* (Ferreira *et al.*, 1997), was enclosed in a capsule-like structure. Cross-sections of cells of strain K4.1^T resembled more those of *Deinococcus radiopugnans* (Battista & Rainey, 2001).

Inclusion bodies (Supplementary Fig. S2) had a yellow fluorescence when stained with DAPI (4'-6-diamidino-2-phenylindole), possibly representing polyphosphate (Tijssen *et al.*, 1982).

Resistance of strain K4.1^T to ionizing radiation was tested as described by Zimmerman & Battista (2006). One millilitre aliquots of an overnight culture grown in TSB were irradiated with doses of 0, 2, 4, 7 and 10 kGy ($\pm 10\%$) at a rate of 0.17–0.29 kGy h⁻¹. After irradiation, tenfold dilution series were prepared, appropriate dilutions plated in triplicate on R2A (strain K4.1^T, 45 °C), on DSMZ medium 53: corynebacterium agar (*Deinococcus radiodurans*, 28 °C) or on plate count agar (*Escherichia coli*,

37 °C) and the colonies counted after growth for 1–2 days. Non-irradiated controls were maintained at room temperature and plated simultaneously with the irradiated bacteria. *Deinococcus radiodurans* DSM 20539^T was used as a radiation-resistant reference and *E. coli* ATCC 51739 as a sensitive reference. Strain K4.1^T resisted radiation similarly to the type strain of *Deinococcus radiodurans*, i.e. lost less than one log of viability upon exposure to 10 kGy (Supplementary Fig. S3). This characteristic confirms relatedness of strain K4.1^T to *Truepera radiovictrix* (Albuquerque *et al.*, 2005) and *Deinococcus radiodurans* and distinguishes it from the more distantly related taxa of the genus *Thermus*, such as *Thermus aquaticus* and *Thermus thermophilus* (Albuquerque *et al.*, 2005; Omelchenko *et al.*, 2005).

The 16S rRNA gene sequence of strain K4.1^T shared highest similarities with the type strains of *Deinococcus pimensis* (90.0 %) and *Deinococcus peraridilitoris* (89.6 %) (according to similarity table analysis, www.eztaxon.org; Chun *et al.*, 2007). Levels of sequence similarity to all of the other type strains of the recognized species of the genus *Deinococcus* ranged from 89.4 % (*Deinococcus maricopenensis*) to 86.7 % (*Deinococcus hopiensis*). A phylogenetic tree reconstructed using the maximum-likelihood algorithm and including all the type species of the genera *Deinococcus* and *Truepera* demonstrated that strain K4.1^T forms a distinct line of descent branching at the base of the genus *Deinococcus* (Supplementary Fig. S4). A separate phylogenetic position of strain K4.1^T was also obtained using neighbour-joining and maximum-parsimony algorithms (Supplementary Fig. S4). Neighbour-joining and maximum-parsimony bootstrap analysis yielded high support for a phylogenetic position separate from both the genera *Deinococcus* and *Truepera* (Supplementary Fig. S4).

Polar lipids were extracted and analysed by two-dimensional thin layer chromatography as described previously (Altenburger *et al.*, 1996; Tindall, 1990a, b). Strain K4.1^T was grown for 2 days at 45 °C on 3.3 × PYE agar. *Truepera*

radiovictrix DSM 17093^T was grown on thermus agar, which is essentially 0.33 × PYE agar supplemented with FeCl₃ and trace elements, for 3 days at 45 °C. The predominant lipid of strain K4.1^T was an unknown aminophospholipid (APL1). In addition, the lipid profile consisted of two other aminophospholipids, one unknown phospholipid, four unknown glycolipids, two unknown aminolipids and one unknown polar lipid. This lipid profile is quite unusual compared to species of the genus *Deinococcus*, which have been reported to contain an unknown phosphoglycolipid as the predominant lipid (with the exception of *Deinococcus yavapaiensis*; Rainey *et al.*, 2005) and other phosphoglycolipids as well (Callegan *et al.*, 2008; Kämpfer *et al.*, 2008; Ferreira *et al.*, 1997; Embley *et al.*, 1987; Weon *et al.*, 2007; Zhang *et al.*, 2007). No phosphoglycolipids were detected in strain K4.1^T (Fig. 2a). Chromatographic analysis of a mixture of lipid extracts of strain K4.1^T and *Deinococcus grandis* on the same thin layer plate demonstrated that APL1 and the predominant phosphoglycolipid specific for deinococci exhibited almost identical chromatographic behaviour (results not shown) and thus the two lipids can only be distinguished by their different staining behaviour. However, to the best of our knowledge, the presence of the major lipid APL1 together with the absence of phosphoglycolipids distinguish strain K4.1^T from all species of the genus *Deinococcus* examined for polar lipids, including the type species of the genus, *Deinococcus radiodurans* (Embley *et al.*, 1987), and its close phylogenetic relatives that might form the core of a redefined genus *Deinococcus* such as *Deinococcus radiopugnans*, *Deinococcus ficus*, *Deinococcus deserti*, *Deinococcus frigens*, *Deinococcus*

saxicola and *Deinococcus marmoris* (Hirsch *et al.*, 2004; de Groot *et al.*, 2005; Embley *et al.*, 1987; Lai *et al.*, 2006). Reanalysis of the polar lipids of *Truepera radiovictrix* confirmed the observation that it contains a complex mixture of glycolipids and phospholipids (Albuquerque *et al.*, 2005). The polar lipid profile of *Truepera radiovictrix* was composed of eleven glycolipids, two phospholipids, one aminolipid and two lipids not staining with specific spray reagents such as ninhydrin, molybdenum blue or α -naphthol (Fig. 2b). This lipid profile was substantially different from that of strain K4.1^T. The major aminolipid of strain K4.1^T (APL1) was not detectable in *Truepera radiovictrix* and conversely, the major compound, phospholipid PL1, and several glycolipids were not found in strain K4.1^T. These data demonstrate that strain K4.1^T can unambiguously be distinguished from *Truepera radiovictrix* based on the polar lipid profile.

The peptidoglycan structure of strain K4.1^T was analysed according to Schleifer (1985). The analysis revealed the peptidoglycan type as A3 β with L-Orn-Gly-Gly, also found in representatives of the genera *Deinococcus* (Hirsch *et al.*, 2004; Embley *et al.*, 1987; Rainey *et al.*, 1997), *Thermus* and *Meiothermus* (da Costa *et al.*, 2001). DNA G+C content was determined with HPLC as 66.8 mol%. The peptidoglycan and DNA base composition were analysed at DSMZ (German Collection of Microorganisms and Cell Cultures, Germany).

Whole-cell fatty acids of strain K4.1^T were analysed from biomass grown at 28 °C [TSBA (TSB with 15 g l⁻¹ agar), 1 day] and at 45 °C [TSBA, 1 day; R2A, 2 days; thermus agar (DSMZ medium 1033), 2 days] as methyl esters

Fig. 2. Polar lipid profile of strain K4.1^T (a) and *Truepera radiovictrix* (b) after detection with molybdate phosphoric acid. APL1–3, unknown aminophospholipids; AL1,2, unidentified aminolipids; GL1–11, unidentified glycolipids; L1,2, unidentified polar lipids not detectable with any of the specific spray reagents; PL1,2, unidentified phospholipids; redPig, red pigment. Same labels in the two images do not indicate that the corresponding spots represent the same lipid.

derivatized according to the protocol of MIDI (MIDI, 2001). The main fatty acids of strain K4.1^T were 15:0 iso, 15:0 anteiso, 17:0 iso and 17:0 anteiso (Table 1). Strain K4.1^T adapted to growth at temperatures below the optimum by synthesizing unsaturated 15:1 anteiso acid. It differed from species of the genus *Deinococcus* (Tables 1, 2 and Supplementary Table S1) by the absence of straight chain unsaturated fatty acids, which are the major components in most deinococci. Unbranched fatty acids were found only in minute amounts in strain K4.1^T in contrast to the deinococci. Instead, branched fatty acids constituted over 90 % of the fatty acids at both 45 and 28 °C and in peptone rich (TSBA) and low peptone

medium (R2A). Hydroxy fatty acids (17:0 iso 3-OH and 17:0 2-OH) were also found in strain K4.1^T. Those were absent or scarce in deinococci.

Respiratory quinones were extracted and analysed as described by Tindall (1990a, b). Strain K4.1^T contained menaquinone MK-8, which is the major quinone of all genera in the phylum *Deinococcus-Thermus* (Albuquerque *et al.*, 2005; Rainey *et al.*, 2007; Miroshnichenko *et al.*, 2003).

The phylogenetic distance of strain K4.1^T to the genus *Deinococcus* is large (16S rRNA similarity 90–86.7 %) but smaller than that to the genus *Truepera* (similarity 85.3 %).

Table 1. Whole-cell fatty acids (% of total) of strain K4.1^T, *Truepera radiovictrix* DSM 17093^T and deinococci (*D. radiodurans* DSM 20539^T, *D. radiopugnans* ATCC 19172^T, *D. grandis* DSM 3963^T)

The major fatty acids (>5 %) are highlighted by means of bold type. Fatty acids that represent less than 1 % in all species are not shown. —, <0.1 %.

Fatty acid	K4.1 ^T			DSM 17093 ^T	DSM 20539 ^T	ATCC 19172 ^T	DSM 3963 ^T
	28 °C, TSBA, 24 h	45 °C, TSBA, 24 h*	45 °C, Thermus agar, 48 h*	45 °C, Thermus agar, 48 h	28 °C, TSBA, 48 h	28 °C, TSBA, 48 h	28 °C, TSBA, 48 h
10:0	—	0.4–0.6	1	0.7	—	—	—
13:0 iso	3	2	2	—	—	1	1
13:0 anteiso	0.4	0.4–0.5	0.4–0.5	—	—	—	0.3
14:0 iso	0.5	1	4	—	—	—	—
14:0	—	1	1	—	—	2	0.9
15:0 iso F	—	—	—	—	—	3	—
15:1 iso H/13:0 3-OH	3	0.5	—	—	—	—	—
15:1 anteiso A	11	0.2–0.5	—	—	—	—	—
15:0 iso	16	11–12	13	1	—	18	8
15:0 anteiso	23	27–28	42–43	38	—	—	0.4
15:1 ω 8c	—	—	—	—	—	4	1
15:1 ω 6c	—	—	—	—	1	5	20
16:0 iso	0.4	0.6	3	1	0.6	—	1
16:1 ω 9c	—	—	—	—	5	0.9	1
16:1 ω 7c	—	—	—	—	55	14	20
16:1 ω 5c	—	—	—	—	1	—	2
16:0	2	1	3	4	18	10	11
15:0 2-OH	—	—	—	—	—	—	—
17:1 iso ω 9c	—	—	—	4	—	25	1
17:1 anteiso ω 9c	—	—	—	2	—	—	—
17:0 iso	27	28	12	13	—	8	8
17:0 anteiso	7	7	9–10	18	—	—	0.4
17:1 ω 8c	—	—	—	2	4	4	4
17:1 ω 6c	—	—	—	—	4	—	4
17:0	0.2	0.6–1	1	6	2	3	11
18:0 iso	—	1	0.5	3	—	—	—
18:1 ω 9c	—	0.4	—	—	2	—	0.3
18:1 ω 7c	—	—	—	2	6	—	—
18:0	0.8	0.8	0.6–0.7	3	—	—	0.2
17:0 iso 3-OH	3	2	2	—	—	—	—
19:0 iso	1	9	—	0.4	—	—	—
19:0 anteiso	0.5	2–3	—	0.9	—	—	—

*Fatty acids were extracted and analysed twice.

fatty acids are branched 15:0 and 17:0 acids and the major polar lipid is an unknown aminophospholipid. MK-8 is the major respiratory quinone and the peptidoglycan structure is A3 β L-Orn–Gly–Gly. DNA G + C content is 67 mol%. The type species is *Deinobacterium chartae*.

Description of *Deinobacterium chartae* sp. nov.

Deinobacterium chartae (char'tae. L. gen. fem. n. *chartae* of/ from paper).

Exhibits the following properties in addition to those given in the genus description. Plate-grown cells are 0.8–1.3 μ m in width and 1.4–2.5 μ m in length, may grow to filaments up to 40 μ m in rich liquid medium. Stains Gram-negatively, but has features of Gram-positive cells. Catalase and oxidase-positive, urease-negative. Forms colonies with pale pink pigment on R2A agar. Growth occurs at 12 to 50 °C (optimum 37 to 45 °C) and between pH 6 and 10.3. Growth is inhibited in the presence of >3% (w/v) NaCl. Anaerobic growth does not occur on TSA. Produces acid from glucose, fructose and maltose. Utilizes as a carbon source: succinic acid, L-aspartic acid, glycerol, L-fucose, D-gluconic acid, D-xylose, D-glucose 6-phosphate, D-galactonic acid γ -lactone, L-asparagine, D-aspartic acid, D-glucosaminic acid, L-glutamine, *m*-tartaric acid, D-glucose 1-phosphate, glycyl L-aspartic acid, citric acid, *myo*-inositol, D-threonine, fumaric acid, bromosuccinic acid, propionic acid, glycolic acid, L-alanine, acetoacetic acid, methyl pyruvate, *p*-hydroxyphenylacetic acid, *m*-hydroxyphenylacetic acid, glucuronamide, D-galacturonic acid, phenylethylamine, chondroitin sulfate C, γ -cyclodextrin, dextrin, gelatin, glycogen, D-arabinose, arbutin, 3-O- β -D-galactopyranosyl-D-arabinose, gentiobiose, lactitol, β -methyl D-galactoside, palatinose, raffinose, salicin, D-tagatose, turanose, butyric acid, caproic acid, D-glucosamine, α -ketovaleric acid, 5-keto-D-gluconic acid, malonic acid, oxalomalic acid, L-histidine, dihydroxyacetone, melibionnic acid, *N*-acetyl-D-glucosaminitol, L-lysine and 3-hydroxy-2-butanone but not malate, cellobiose, D-galactose, L-arabinose, *N*-acetyl-D-glucosamine, D-saccharic acid, trehalose, D-mannose, dulcitol, D-serine, D-sorbitol, DL- α -glycerol phosphate, L-glutamic acid, Tween 20, D-fructose, acetic acid, α -D-glucose, maltose, 1,2-propanediol, α -ketoglutaric acid, sucrose, uridine, adonitol, mucic acid, tricarballic acid, L-threonine, L-malic acid, pyruvic acid, L-galactonic acid γ -lactone, capric acid, D-lactic acid methyl ester, L-ornithine, *N*-acetylneuraminic acid, L-phenylalanine, β -D-allose, sedoheptulosan, citraconic acid, amygdalin, citramalic acid, L-valine, glycine, DL-carnitine, D-arabitol, methyl α -D-glucoside, 2-hydroxybenzoic acid, quinic acid, *sec*-butylamine, L-arabitol, 4-hydroxybenzoic acid, D-ribono-1,4-lactone, L-homoserine, DL-octopamine, xylitol, sebacic acid, L-hydroxyproline, putrescine, inulin, 2-deoxy-D-ribose, methyl β -D-glucuronic acid, sorbic acid, i-erythritol, methyl α -D-mannoside, γ -aminobutyric acid, succinamic acid, methyl β -D-xyloside, δ -aminovaleric acid, D-tartaric acid, L-tartaric acid or 2,3-butanone. Utilizes as a

nitrogen source: L-alanine, L-glutamine, L-histidine, L-proline, D-glucosamine, D-galactosamine, D-mannosamine, adenosine, cytidine, guanine, guanosine, thymidine, uridine, inosine, uric acid, alloxan, Ala–Asp, Ala–Gln, Ala–Glu, Ala–His, Ala–Leu, Ala–Thr and Gly–Gln. Does not utilize as a nitrogen source: ammonia, nitrite, nitrate, urea, biuret, L-arginine, L-aspartic acid, glycine, L-methionine, L-valine, D-valine, L-citrulline, L-homoserine, L-ornithine, *N*-acetyl DL-glutamic acid, *N*-phthaloyl L-glutamic acid, L-pyroglytamic acid, hydroxylamine, methylamine, *N*-butylamine, ethylamine, ethanolamine, ethylenediamine, putrescine, agmatine, β -phenylethylamine, tyramine, formamide, DL-lactamide, *N*-acetyl-D-galactosamine, adenine, cytosine, uracil, parabanic acid, γ -amino-*N*-butyric acid, ε -amino-*N*-caproic acid or δ -amino-*N*-valeric acid. Utilizes as a phosphorus source: adenosine 5'-monophosphate, guanosine 5'-monophosphate, uridine 5'-monophosphate and thymidine 5'-monophosphate. Does not utilize as a phosphorus source: phosphate, trimetaphosphate, tripolyphosphate, triethyl phosphate, hypophosphite, adenosine 2'-monophosphate, adenosine 3'-monophosphate, adenosine 2',3'-cyclic monophosphate, adenosine 3',5'-cyclic monophosphate, guanosine 3'-monophosphate, guanosine 2',3'-cyclic monophosphate, guanosine 3',5'-cyclic monophosphate, cytidine 3'-monophosphate, cytidine 2',3'-cyclic monophosphate, cytidine 3',5'-cyclic monophosphate, uridine 2'-monophosphate, uridine 3'-monophosphate, uridine 2',3'-cyclic monophosphate, uridine 3',5'-cyclic monophosphate, guanosine 2'-monophosphate, DL- α -glycerol phosphate, β -glycerol phosphate, carbamyl phosphate, D-2-phosphoglyceric acid, phosphoglycolic acid, 2-deoxy-D-glucose 6-phosphate, 6-phosphogluconic acid, D-mannose 1-phosphate, D-mannose 6-phosphate, cysteamine-S-phosphate, phospho-L-arginine, O-phospho-D-serine, O-phospho-L-threonine, phosphocreatine, phosphorylcholine, O-phosphorylethanolamine, phosphonoacetic acid, 2-aminoethylphosphonic acid, thymidine 3'-monophosphate, inositol hexaphosphate or thymidine 3',5'-cyclic monophosphate. The fatty acid composition is listed in Table 1. MK-8 is the only respiratory quinone. The lipid profile consists of three unknown aminophospholipids, of which one is the predominant lipid, one unknown phospholipid, four unknown glycolipids, two unknown aminolipids and one unknown polar lipid. Peptidoglycan structure is A3 β L-Orn–Gly–Gly and the DNA G + C content of the type strain is 66.8 mol%. May be grown on R2A or TSA at 45 °C in ambient air.

The type strain, K4.1^T (=DSM 21458^T =HAMBI 2721^T), was isolated from a biofilm collected from a Finnish paper mill.

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References

- Albuquerque, L., Simões, C., Nobre, M. F., Pino, N. M., Battista, J. R., Silva, M. T., Rainey, F. A. & da Costa, M. S. (2005). *Truepera radiovictrix* gen. nov., sp. nov., a new radiation resistant species and the proposal of *Trueperaceae* fam. nov. *FEMS Microbiol Lett* **247**, 161–169.
- Altenburger, P., Kämpfer, P., Makristathis, A., Lubitz, W. & Busse, H.-J. (1996). Classification of bacteria isolated from a medieval wall painting. *J Biotechnol* **47**, 39–52.
- Asker, D., Awad, T. S., Beppu, T. & Ueda, K. (2008). *Deinococcus misasensis* and *Deinococcus roseus*, novel members of the genus *Deinococcus*, isolated from a radioactive site in Japan. *Syst Appl Microbiol* **31**, 43–49.
- Asker, D., Awad, T. S., Beppu, T. & Ueda, K. (2009). *Deinococcus aquiradiicola* sp. nov., isolated from a radioactive site in Japan. *Int J Syst Evol Microbiol* **59**, 144–149.
- Battista, J. R. & Rainey, F. A. (2001). Genus I. *Deinococcus* Brooks and Murray 1981. In *Bergey's Manual of Systematic Bacteriology* 2nd edn, vol. 1, pp. 396–403. Edited by D. R. Boone, R. W. Castenholz & G. M. Garrity. New York: Springer.
- Bruno, W. J., Socci, N. D. & Halpern, A. L. (2000). Weighted neighbor joining: a likelihood-based approach to distance-based phylogeny reconstruction. *Mol Biol Evol* **17**, 189–197.
- Callegan, R. P., Nobre, M. F., McTernan, P. M., Battista, J. R., Navarro-González, R., McKay, C. P., da Costa, M. S. & Rainey, F. A. (2008). Description of four novel psychrophilic, ionizing radiation-sensitive *Deinococcus* species from alpine environments. *Int J Syst Evol Microbiol* **58**, 1252–1258.
- Chun, J., Lee, J.-H., Jung, Y., Kim, M., Kim, S., Kim, B. K. & Lim, Y. W. (2007). EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int J Syst Evol Microbiol* **57**, 2259–2261.
- da Costa, M. S., Nobre, M. F. & Rainey, F. A. (2001). Genus I. *Thermus* Brock and Freeze 1969. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 1, pp. 404–414. Edited by D. R. Boone, R. W. Castenholz & G. M. Garrity. New York: Springer.
- de Groot, A., Chapon, V., Servant, P., Christen, R., Fischer-Le Saux, M., 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.
- Embley, T. M., O'Donnell, A. G., Wait, R. & Rostron, J. (1987). Lipid and cell wall amino acid composition in the classification of members of the genus *Deinococcus*. *Syst Appl Microbiol* **10**, 20–27.
- Ferreira, A. C., Nobre, M. F., Rainey, F. A., Silva, M. T., Wait, R., Burghardt, J., Chung, A. P. & da Costa, M. S. (1997). *Deinococcus geothermalis* sp. nov. and *Deinococcus murrayi* sp. nov., two extremely radiation-resistant and slightly thermophilic species from hot springs. *Int J Syst Bacteriol* **47**, 939–947.
- Halinen, K., Fewer, D. P., Sihvonen, L. M., Lyra, C., Eronen, E. & Sivonen, K. (2008). Genetic diversity in strains of the genus *Anabaena* isolated from planktonic and benthic habitats of the Gulf of Finland (Baltic Sea). *FEMS Microbiol Ecol* **64**, 199–208.
- Hirsch, P., Gallikowski, C. A., Siebert, J., Peissl, K., Kroppenstedt, R., Schumann, P., Stackebrandt, E. & Anderson, R. (2004). *Deinococcus frigens* sp. nov., *Deinococcus saxicola* sp. nov., and *Deinococcus marmoris* sp. nov., low temperature and draught-tolerating, UV-resistant bacteria from continental Antarctica. *Syst Appl Microbiol* **27**, 636–645.
- Im, W.-T., Jung, H.-M., Ten, L. N., Kim, M. K., Bora, N., Goodfellow, M., Lim, S., Jung, J. & Lee, S.-T. (2008). *Deinococcus aquaticus* sp. nov., isolated from fresh water, and *Deinococcus caeni* sp. nov., isolated from activated sludge. *Int J Syst Evol Microbiol* **58**, 2348–2353.
- Kämpfer, P., Lodders, N., Huber, B., Falsen, E. & Busse, H. J. (2008). *Deinococcus aquatilis* sp. nov., isolated from water. *Int J Syst Evol Microbiol* **58**, 2803–2806.
- Kolari, M., Nuutinen, J., Rainey, F. A. & Salkinoja-Salonen, M. S. (2003). Colored moderately thermophilic bacteria in paper-machine biofilms. *J Ind Microbiol Biotechnol* **30**, 225–238.
- Lai, W.-A., Kämpfer, P., Arun, A. B., Shen, F.-T., Huber, B., Rekha, P. D. & Young, C.-C. (2006). *Deinococcus ficus* sp. nov., isolated from the rhizosphere of *Ficus religiosa* L. *Int J Syst Evol Microbiol* **56**, 787–791.
- MacFaddin, J. F. (2000). *Biochemical Tests for Identification of Medical Bacteria*, 3rd edn. Philadelphia, PA: Lippincott Williams & Wilkins.
- MIDI (2001). *Sherlock Microbial Identification System Operating Manual*, version 4.0. Newark, DE: MIDI, Inc.
- Miroshnichenko, M. L., L'Haridon, S., Nercissian, O., Antipov, A. N., Kostrikina, N. A., Tindall, B. J., Schumann, P., Spring, S., Stackebrandt, E. & other authors (2003). *Vulcanithermus media-tlanticus* gen. nov., sp. nov., a novel member of the family *Thermaceae* from a deep-sea hot vent. *Int J Syst Evol Microbiol* **53**, 1143–1148.
- Omelchenko, M. V., Wolf, Y. I., Gaidamakova, E. K., Matrosova, V. Y., Vasilenko, A., Zhai, M., Daly, M. J., Koonin, E. V. & Makarova, K. S. (2005). Comparative genomics of *Thermus thermophilus* and *Deinococcus radiodurans*: divergent routes of adaptation to thermophily and radiation resistance. *BMC Evol Biol* **5**, 57.
- Peltola, M., Kanto Öqvist, C., Ekman, J., Kosonen, M., Jokela, S., Kolari, M., Korhonen, P. & Salkinoja-Salonen, M. (2008). Quantitative contributions of bacteria and of *Deinococcus geothermalis* to deposits and slimes in paper industry. *J Ind Microbiol Biotechnol* **35**, 1651–1657.
- Peng, F., Zhang, L., Luo, X., Dai, J., An, H., Tang, Y. & Fang, C. (2009). *Deinococcus xinjiangensis* sp. nov., isolated from desert soil. *Int J Syst Evol Microbiol* **59**, 709–713.
- Rainey, F. A., Nobre, M. F., Schumann, P., Stackebrandt, E. & da Costa, M. S. (1997). Phylogenetic diversity of the deinococci as determined by 16S ribosomal DNA sequence comparison. *Int J Syst Bacteriol* **47**, 510–514.
- 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.
- Rainey, F. A., Ferreira, M., Nobre, M. F., Ray, K., Bagaley, D., Earl, A. M., Battista, J. R., Gómez-Silva, B., McKay, C. P. & da Costa, M. S. (2007). *Deinococcus peraridilitoris* sp. nov., isolated from a coastal desert. *Int J Syst Evol Microbiol* **57**, 1408–1412.
- Raulio, M., Järn, M., Ahola, J., Peltonen, J., Rosenholm, J. B., Tervakangas, S., Kolehmainen, J., Ruokolainen, T., Narko, P. & Salkinoja-Salonen, M. (2008). Microbe repelling coated stainless steel analysed by field emission scanning electron microscopy and physicochemical methods. *J Ind Microbiol Biotechnol* **35**, 751–760.
- Schleifer, K. H. (1985). Analysis of the chemical composition and primary structure of murein. *Methods Microbiol* **18**, 123–156.

- Tijssen, J. P. F., Beekes, H. W. & Van Steveninck, J. (1982).** Localization of polyphosphates in *Saccharomyces fragilis*, as revealed by 4',6-diamidino-2-phenylindole fluorescence. *Biochim Biophys Acta* **721**, 394–398.
- Tindall, B. J. (1990a).** A comparative study of the lipid composition of *Halobacterium saccharovorum* from various sources. *Syst Appl Microbiol* **13**, 128–130.
- Tindall, B. J. (1990b).** Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* **66**, 199–202.
- Weon, H.-Y., Kim, B.-Y., Schumann, P., Son, J.-A., Jang, J., Go, S.-J. & Kwon, S.-W. (2007).** *Deinococcus cellulosilyticus* sp. nov., isolated from air. *Int J Syst Evol Microbiol* **57**, 1685–1688.
- Yang, Y., Itoh, T., Yokobori, S., Itahashi, S., Shimada, H., Satoh, K., Ohba, H., Narumi, I. & Yamagishi, A. (2009).** *Deinococcus aerius* sp. nov., isolated from the high atmosphere. *Int J Syst Evol Microbiol* **59**, 1862–1866.
- Yuan, M., Zhang, W., Dai, S., Wu, J., Wang, Y., Tao, T., Chen, M. & Lin, M. (2009).** *Deinococcus gobiensis* sp. nov., an extremely radiation-resistant bacterium. *Int J Syst Evol Microbiol* **59**, 1513–1517.
- Zhang, Y. Q., Sun, C. H., Li, W. J., Yu, L. Y., Zhou, J. Q., Zhang, Y. Q., Xu, L. H. & Jiang, C. L. (2007).** *Deinococcus yunweiensis* sp. nov., a gamma- and UV-radiation-resistant bacterium from China. *Int J Syst Evol Microbiol* **57**, 370–375.
- Zimmerman, J. M. & Battista, J. R. (2006).** Measuring survival in microbial populations following exposure to ionizing radiation. In *Extremophiles, Methods in Microbiology*, vol. 35, pp. 745–754. Edited by F. A. Rainey & A. Oren. London: Academic Press.