Deinococcus aquatilis sp. nov., isolated from water

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A pale-pink strain (CCUG 53370^T) from water was investigated by a polyphasic taxonomic approach. The cells stained Gram-positive and were rod-shaped and non-spore-forming. Analyses using the 16S rRNA gene sequence of the isolate showed that the organism belongs to the genus *Deinococcus*, with the highest sequence similarities to the type strains of *Deinococcus ficus* (94.4%), *Deinococcus navajonensis* (94.3%) and *Deinococcus mumbaiensis* (94.3%). Chemotaxonomic data revealed that CCUG 53370^T contains exclusively menaquinone MK-8 as the respiratory quinone and a complex polar lipid profile consisting of different unidentified glycolipids and polar lipids, two unknown phospholipids and three unknown phosphoglycolipids. As in other deinococci, one of these phosphoglycolipids was predominant in the profile, and it was identified in *Deinococcus radiodurans* as 2'-O-(1,2-diacyl-*sn*-glycero-3-phospho)-3'-O-(α -galactosyl)-*N*-D-glyceroyl alkylamine. Predominant fatty acids were C_{16:1 ω 7c}, C_{17:1 ω 8c} and C_{17:1 ω 9c}. Biochemical and chemotaxonomic properties demonstrate that strain CCUG 53370^T represents a novel species, for which the name *Deinococcus aquatilis* sp. nov. is proposed. The type strain is CCUG 53370^T (=CCM 7524^T).

At present, the genus *Deinococcus* comprises more than 30 species. The taxonomy of the genus has been described extensively (Suresh *et al.*, 2004; Hirsch *et al.*, 2004; Ferreira *et al.*, 1997; Rainey *et al.*, 1997, 2005), and the number of species is still growing (Asker *et al.*, 2009; Callegan *et al.*, 2008; de Groot *et al.*, 2005; Lai *et al.*, 2006; Rainey *et al.*, 2007; Shashidhar & Bandekar, 2006; Zhang *et al.*, 2007). Several novel *Deinococcus* strains have been isolated and well characterized from soils, including desert soil, foods, faeces and dust, with additional data on their extreme resistance to UV and gamma radiation and desiccation.

A pale-pink-pigmented bacterium was isolated from industry water on nutrient agar. This strain (CCUG 53370^T) was maintained and subcultured on nutrient agar at 30 °C for 48 h and subsequently investigated for 16S rRNA gene sequence, fatty acid methyl ester composition of whole-cell hydrolysates, respiratory quinones, polar lipids, polyamines and further phenotypic characteristics.

Cultural and morphological characteristics were observed on R2A agar (Oxoid). Gram-staining was performed as described by Gerhardt *et al.* (1994). Cell morphology was observed under a Zeiss light microscope at $\times 1000$ magnification, with cells grown for 3 days at 28 °C on R2A.

Strain CCUG 53370^T stained Gram-positive and formed visible (about 2 mm) pale-pink colonies within 48 h at 30 °C. No growth was observed above 45 °C. The colonies were translucent and shiny with entire edges. Oxidase activity was tested using oxidase reagent (bioMérieux) according to the instructions of the manufacturer. Strain CCUG 53370^T was oxidase-positive and non-motile and consisted of non-spore-forming rods. CCUG 53370^T was able to grow well on nutrient agar and tryptone soy agar. Good growth was observed under alkaline conditions up to pH 11.

UV radiation resistance was tested as described by Hirsch *et al.* (2004). The strain showed high UV resistance in comparison with the control strain *Escherichia coli* K-12 (data not shown).

Physiological characterization and additional biochemical tests were performed according to the methods described by Kämpfer *et al.* (1991). Results are given in the species description. Strain CCUG 53370^T was unable to utilize

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CCUG 53370^T is AM940971.

Fatty acid profiles of strain CCUG 53370^T and selected relatives in the genus *Deinococcus* are available as supplementary material with the online version of this paper.

L-arabinose, lactose, trehalose, D-xylose, D-mannose, melibiose or D-sorbitol and utilized *N*-acetyl-D-glucosamine weakly; in contrast, *D. ficus* CC-FR2-10^T utilized all of these compounds (Lai *et al.*, 2006).

The fatty acid pattern (determined using the method described by Kämpfer & Kroppenstedt, 1996) for strain CCUG 53370^T is shown in Supplementary Table S1 (available in IJSEM Online) in comparison with some representative *Deinococcus* species. The organism showed a profile typical of the genus *Deinococcus*.

Respiratory quinones, polar lipids and polyamines were determined after cultivation of CCUG 53370^T in a medium containing the following (g l⁻¹): casein peptone (3), yeast extract (3), D-glucose (0.5), sodium pyruvate (0.3) and magnesium sulfate (0.024). Polar lipids were extracted and analysed by two-dimensional TLC according to Tindall (1990a, b) and Altenburger *et al.* (1996). Like members of other *Deinococcus* species (Embley *et al.*, 1987; Suresh *et al.*, 2004; Ferreira *et al.*, 1997), strain CCUG 53370^T displayed a complex profile consisting of different unidentified glycolipids and polar lipids, two unknown phospholipids and three unknown phosphoglycolipids (Fig. 1). It shared with other deinococci a predominant phosphoglycolipid, which was identified in *Deinococcus radiodurans* as 2'-O-(1,2-diacyl-*sn*-glycero-3-phospho)-3'-O-(α -galactosyl)-N-

Fig. 1. Polar lipid profile of strain CCUG 53370^T. L1–L6, Unidentified polar lipids; GL1–GL5, unidentified glycolipids; PGL1–PGL3, unidentified phosphoglycolipids; PL1, PL2, unidentified phospholipids; PIG1–PIG3, brick-red pigments. Polar lipids L4, L5, L6 and PL2 (probably corresponding to L2 of *D. ficus*) were detected after α -naphthol staining but specific colour development was not observed and hence they are not designated glycolipids. GL1 actually represents two α -naphthol spots that are not distinguishable in the image.

D-glyceroyl alkylamine (Anderson & Hansen, 1985). Certain other lipids exhibiting similar chromatographic and staining behaviour might have been detected in other deinococci. Embley *et al.* (1987) reported the presence of two lipids that probably correspond to GL2 and PGL3 (Fig. 1) in four strains of *D. radiodurans* (D10, D11, D12 and D13), two strains of *Deinococcus proteolyticus* (D3 and D5), a strain of *Deinococcus radiophilus* (D16) and two strains of *Deinococcus radiopugnans* (D17^T and D18). Corresponding lipids were detected in *D. radiodurans* DSM 20539^T and *Deinococcus murrayi* AG-3a^T (Ferreira *et al.*, 1997), and counterparts of GL5 (Fig. 1) may have been found in *D. proteolyticus* (D2^T and D6), *D. radiodurans* (D12 and D13), *D. radiophilus* (D14^T and D16), *D. radiopugnans* (D17^T and D18) and *D. murrayi* AG-3a^T (Embley *et al.*, 1987; Ferreira *et al.*, 1997). The presence of PGL3, PL1 and GL5, the significantly larger amount of GL2 and the absence of certain other lipids clearly distinguished CCUG 53370^T from its nearest relative as suggested by 16S rRNA gene sequence similarity, *Deinococcus ficus*.

Respiratory quinones were extracted and analysed by HPLC as reported by Tindall (1990b) and Stolz *et al.* (2007). Strain CCUG 53370^T contained a quinone system composed exclusively of menaquinone MK-8. This is in agreement with other *Deinococcus* species, which all contain menaquinone MK-8 as the major compound (Embley *et al.*, 1987; Suresh *et al.*, 2004; Ferreira *et al.*, 1997; Lai *et al.*, 2006).

Polyamines were extracted and analysed by HPLC as described in Busse & Auling (1988) and Stolz *et al.* (2007). The polyamine pattern of strain CCUG 53370^T consisted of the predominant component spermidine [41.0 μ mol (g dry weight)⁻¹] and traces of spermine [0.8 μ mol (g dry weight)⁻¹], putrescine [0.2 μ mol (g dry weight)⁻¹] and 1,3-diaminopropane [0.2 μ mol (g dry weight)⁻¹]. This polyamine pattern is in accordance with those of other deinococci, which were also reported to contain significant amounts of spermidine only in their polyamine patterns (Hamana, 1994).

The 16S rRNA gene was analysed as described by Kämpfer *et al.* (2003). Phylogenetic analysis was performed using the software package MEGA version 3.1 (Kumar *et al.*, 2004) after multiple alignment of the data by CLUSTAL_X (Thompson *et al.*, 1997). Distances were calculated (distance options according to the Kimura-2 model) and clustering with the neighbour-joining and maximum-parsimony methods was performed by using bootstrap values based on 1000 replications. The almost-complete 16S rRNA gene sequence of the strain was compared by sequence similarity calculations using the EzTaxon server (Chun *et al.*, 2007). The results of these calculations indicated that the closest relatives of strain CCUG 53370^T were *D. ficus* CC-FR2-10^T (94.4%), *D. navajonensis* KR-114^T (94.3%), *Deinococcus mumbaiensis* CON-1^T (94.3%) and *D. radiodurans* DSM 20539^T (94.3%). All other *Deinococcus* species showed lower 16S rRNA gene sequence similarities. A phylogenetic tree is shown in Fig. 2. The

Fig. 2. Phylogenetic analysis based on 16S rRNA gene sequences available from the EMBL database (accession numbers are given in parentheses) constructed after multiple alignments of data. Alignments, distance calculation (distance options according to the Kimura-2 model) and clustering with the neighbour-joining method were performed by using the software package MEGA version 3.1 (Kumar *et al.*, 2004). Bootstrap values based on 1000 replications are listed as percentages at branching points. Bar, 0.01 substitution units (K_{nuc}).

branching pattern was confirmed by maximum-parsimony analyses (data not shown). It should be noted here that *D. ficus* and *D. mumbaiensis* are very similar in both genotype and phenotype and seem to belong to the same species. On the basis of the results of this polyphasic taxonomic study, especially phylogenetic placement within the radiation of deinococci and the presence of characteristic polar lipids, menaquinone MK-8 and spermidine predominant in the polyamine pattern, it is clear that strain CCUG 53370^T represents a novel species of the genus *Deinococcus*, for which the name *Deinococcus aquatilis* sp. nov. is proposed.

Description of *Deinococcus aquatilis* sp. nov.

Deinococcus aquatilis (a.qua.ti'lis. L. masc. adj. *aquatilis* living in water).

Cells stain Gram-positive and are non-motile, non-spore-forming rods. Aerobic and oxidase-positive. Good growth after 48 h on R2A agar, nutrient agar and tryptic soy agar at 15–36 °C. Colonies on nutrient agar are smooth, pale pinkish, circular, translucent and shiny with entire edges, becoming mucoid. Unable to grow at 5 or 42 °C. Growth occurs at pH 5.5–11. Major cellular fatty acids are C_{16:1}ω7c, C_{17:1}ω8c, iso-C_{17:1}ω9c, C_{16:0}, iso-C_{17:0} and C_{15:1}ω6c. MK-8 is the predominant lipoquinone. Presents a complex polar lipid profile, consisting of different unidentified glycolipids and polar lipids, two unknown phospholipids and three unknown phosphoglycolipids, among them a predominant phosphoglycolipid with a migration on TLC similar to that of 2'-O-(1,2-diacyl-sn-glycero-3-phospho)-3'-O-(α-galactosyl)-N-D-glyceroyl alkylamine, which has been identified in *Deinococcus radiodurans*. The polyamine pattern consists of the

predominant component spermidine and traces of spermine, putrescine and 1,3-diaminopropane. The following compounds are utilized as sole carbon sources (positive after prolonged incubation for 14 days according to the method of Kämpfer *et al.*, 1991): D-glucose, sucrose, N-acetyl-D-glucosamine, maltose and acetate. The following compounds are not utilized: D-gluconate, propionate, *cis*- and *trans*-aconitate, 4-aminobutyrate, citrate, fumarate, glutarate, DL-3-hydroxybutyrate, itaconate, DL-lactate, L-malate, mesaconate, 2-oxoglutarate, pyruvate, L-alanine, β-alanine, L-aspartate, L-leucine, L-ornithine, L-proline, L-serine, N-acetylglucosamine, L-arabinose, L-arbutin, cellobiose, D-fructose, D-galactose, D-mannose, α-melibiose, L-rhamnose, D-ribose, salicin, trehalose, D-xylose, adonitol, *myo*-inositol, maltitol, D-mannitol, D-sorbitol, putrescine, adipate, azelate, suberate, L-histidine, L-phenylalanine, L-serine, L-tryptophan, 3-hydroxybenzoate and phenylacetate. bis-*p*-Nitrophenyl (pNP) phosphate, bis-pNP phenylphosphonate and bis-pNP phosphorylcholine are hydrolysed on the basis of the method described by Kämpfer *et al.* (1991). The following compounds are not hydrolysed: pNP β-D-galactopyranoside, pNP β-D-glucuronide, pNP α-D-glucopyranoside, pNP β-D-glucopyranoside, pNP β-D-xylopyranoside, L-aniline *p*-nitroanilide (pNA), γ-L-glutamate pNA and L-proline pNA.

The type strain is CCUG 53370^T (=CCM 7524^T), isolated from water.

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