Deinococcus ficus sp. nov., isolated from the rhizosphere of Ficus religiosa L.

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A pale-pink strain (CC-FR2-107) from the rhizosphere of the sacred tree Ficus religiosa L. in Taiwan was investigated by using a polyphasic taxonomic approach. The cells were Gram-positive, rod-shaped and non-spore-forming. Phylogenetic analyses using the 16S rRNA gene sequence of the isolate indicated that the organism belongs to the genus Deinococcus, the highest sequence similarities being found with Deinococcus grandis (96·1%), Deinococcus radiodurans (94·3%), Deinococcus radiopugnans (93·2%), Deinococcus indicus (93·0%), Deinococcus proteolyticus (92·5%), Deinococcus murrayi (92·4%) and Deinococcus geothermalis (90·7%). The DNA–DNA relatedness with respect to D. grandis DSM 3963T was 17·9%. Chemotaxonomic data revealed that strain CC-FR2-107 contains only menaquinone MK-8 as the respiratory quinone, unknown phosphoglycolipids as the predominant polar lipids and 16 : 1ω7c, 17 : 1ω8c and 17 : 1ω6c iso as the predominant fatty acids. The biochemical and chemotaxonomic properties demonstrate that strain CC-FR2-107 represents a novel species, for which the name Deinococcus ficus sp. nov. is proposed. The type strain is CC-FR2-107 (= CCUG 53391T = CIP 108832).

At the time of writing, the genus Deinococcus comprises nine species with validly published names, Deinococcus radiodurans (the type species), D. erythromyxa (transferred to the genus Kocuria by Rainey et al., 1997), D. geothermalis, D. grandis, D. indicus, D. murrayi, D. proteolyticus, D. radiophilus and D. radiopugnans. These species and the taxonomy of the genus have been extensively studied (Suresh et al., 2004; Ferreira et al., 1997; Rainey et al., 1997; Brooks & Murray, 1981). In addition, the names of nine further species have recently been effectively published: Deinococcus hokokamensis, D. navajonensis, D. hopiensis, D. apachensis, D. maricopensis, D. pimenensis, D. yavapaiensis, D. papagoneis and D. sonorensis (Rainey et al., 2005) (these names have subsequently been validly published). Furthermore, three species have been described whose names have not yet been validly published; ‘Deinococcus frigens’, ‘D. saxicola’ and ‘D. marmoris’ (Hirsch et al., 2004). 16S RNA gene sequence data for an additional species, Deinococcus deserti, are already available (this name has since been validly published; de Groot et al., 2005). Several novel Deinococcus strains have been isolated from soils, desert soil, foods, faeces and dust, and have been characterized in detail; there are additional data on their extreme resistance to UV light, gamma radiation and desiccation, which is a distinctive characteristic of this genus, being present in almost every species. In members of the genus Deinococcus, ionizing radiation and desiccation induce similar types of DNA damage, and it has been proposed that resistance to unnaturally large amounts of ionizing radiation is a consequence of the ability to repair desiccation-induced DNA damage (Mattimore & Battista, 1996). Recently, the extensive diversity of this genus was recorded, and nine novel and extremely ionizing radiation-resistant bacteria isolated from desert soil have been described (Rainey et al., 2005). Although the aforementioned properties of members of this genus are well characterized, the functional roles of Deinococcus in rhizosphere soil or in plant growth promotion remain largely unexplored.

During screening for effective plant-growth-promoting rhizobacteria from the rhizosphere of the tree Ficus religiosa L., a pale-pink-pigmented bacterium was isolated on nutrient
agar. This strain (CC-FR2-10^7) was maintained and sub-cultured on nutrient agar at 30 °C for 48 h. The 16S rRNA gene sequence, fatty acid methyl ester composition of whole-cell hydrolysates, respiratory quinones and polar lipids were then determined. Additional phenotypic analyses were performed: biochemical tests, carbon-source utilization (Biolog GP2), API ZYM enzyme profiles (bioMérieux), API 20E (bioMérieux), UV radiation resistance and DNA–DNA relatedness to *D. grandis* (the most closely related species in terms of 16S rRNA gene sequence similarity).

Cultural and morphological characteristics were observed on nutrient agar and Degryse agar (Degryse et al., 1978). Flexirubin-like pigments were observed by flooding the plates with 20 % (w/v) KOH (Fautz & Reichenbach, 1980). The Gram reaction was tested by using the modified method of Cowan (1974). Motility was tested under a microscope, using cells grown for 3 days in motility-test, semi-solid medium at 30 °C. Motility was tested under a microscope, using cells grown for 3 days in motility-test, semi-solid medium at 30 °C. Fluorescence was tested after 48 h by means of plating on King’s B agar. The pH range for growth was tested in Degryse medium as described by Ferreira et al. (1997).

Strain CC-FR2-10^7 was Gram-positive and formed visible (about 2 mm), pale-pink colonies after 48 h at 30 °C. No growth was observed at temperatures above 42 °C. The colonies were translucent and shiny with entire edges. A pale-pink pigment was produced on nutrient agar: this pigment was non-diffusible, non-fluorescent and did not change upon the addition of 20 % KOH. Oxidase activity was tested for by using oxidase reagent (bioMérieux) according to the instructions of the manufacturer. The cells of strain CC-FR2-10^7 were oxidase-positive, non-motile, non-spore-forming rods. Strain CC-FR2-10^7 was able to grow well on nutrient agar and Degryse agar. Optimum growth was observed at alkaline pH; the strain could tolerate, and grow at, pH 10.

Physiological characterization and additional biochemical tests were performed to assess the carbon-source utilization pattern, using Biolog GP2 plates, and the hydrolysis of 19 substrates was investigated using the API ZYM system and API 20E according to the methods outlined by the manufacturer (bioMérieux). UV irradiation was carried out according to the methods outlined by Hirsch et al. (2004), under a 254 nm UV lamp; *Escherichia coli* served as a control.

The fatty acid pattern of strain CC-FR2-10^7 was determined using the method described by Kämpfer & Kroppenstedt (1996). The pattern is compared with those of some representative *Deinococcus* species in Supplementary Table S1 available in IJSEM Online. Strain CC-FR2-10^7 had a fatty acid profile typical of members of the genus *Deinococcus*.

The respiratory quinones were extracted and analysed by HPLC as described by Tindall (1990) and Altenburger et al. (1996). The quinone system of strain CC-FR2-10^7 consisted solely of menaquinone MK-8. This corresponds with other *Deinococcus* species, all of which contain MK-8 as the major quinone (Embley et al., 1987; Ferreira et al., 1997; Oyaizu et al., 1987; Suresh et al., 2004).

Polar lipids were extracted and analysed by two-dimensional TLC according to Tindall (1990). Like other *Deinococcus* species (Embley et al., 1987; Suresh et al., 2004; Ferreira et al., 1997), strain CC-FR2-10^7 displayed a complex polar lipid profile consisting of various unknown glycolipids, phosphoglycolipids and phospholipids and an unknown aminophospholipid; an unknown phosphoglycolipid was the predominant component (Fig. 1).

The 16S rRNA gene was amplified by using a PCR with bacterial universal primers 1F and 9R (Kämpfer et al., 2003; Suresh et al., 2005). PCR products were purified from agarose gel using the QIAquick Gel extraction kit (Qiagen). The sequencing primers used were 3F (5'-CCTACGGAGGCAGCAAG-3', corresponding to positions 341–357 of *E. coli*), 4R (5'-TTACCAGCGGCTGCTGGCAC-3'; positions 533–515) and 5F (5'-AAACTCAAATGAATTGACGGGG-3'; positions 907–928) (Brosius et al., 1978; Edwards et al., 1989). Sequence analysis was performed using an ABI PRISM 310 DNA sequencer (Applied Biosystems), sequence assembly was performed using the Wisconsin Package, version 9.1 (GCG) with a Fragment Assembly System program supplied by the National Health Research Institute of Taiwan. The phylogenetic tree was constructed from the distance matrices by using the neighbour-joining method.

![Fig. 1. Polar lipid profile of strain CC-FR2-10^7. L1 and L2, unidentified polar lipids; GL1–GL5, unidentified glycolipids; PGL1–PGL4, unidentified phospholipids and PGL5–PGL8, unidentified phosphoglycerolipids; APL, unidentified aminophospholipid; PIG1 and PIG2, brick-red pigments. PIG2 stained with α-naphthol, indicating that it contained sugar structures.](image)
Trees were constructed by using neighbour joinig (Fig. 2) and maximum parsimony (see Supplementary Fig. S1 available in IJSEM Online). An almost-complete (1453 nt) 16S rRNA gene sequence of CC-FR2-10T (AY941086) was aligned with sequences deposited in GenBank, using CLUSTAL X (Thompson et al., 1997). This showed that strain CC-FR2-10T was phylogenetically most closely related to species of the genus Deinococcus. According to the gene sequence similarity calculations, the most closely related strain was D. grandis DSM 3963T (96.1%), followed by D. radiodurans DSM 20539T (94.3%), D. radiopugnans ATCC 19172T (93.2%), D. indicus Wt-1aT (93.0%), D. proteolyticus DSM 20540T (92.5%), D. murrayi DSM 11303T (92.4%) and D. geothermalis DSM 11300T (90.7%). DNA–DNA hybridization experiments were performed with strain CC-FR2-10T and the type strain of the phylogenetically most closely related Deinococcus species, D. grandis DSM 3963T. The method used was that described by Ziemke et al. (1998), except that, for nick translation, 2 μg DNA was labelled with incubation at 15°C for 3 h. Strain CC-FR2-10T showed relatively low levels of DNA–DNA hybridization with D. grandis DSM 3963T (17.9%); reciprocal analysis, 14.1%), which clearly indicated that CC-FR2-10T represents a distinct species.

Strain CC-FR2-10T utilized several carbon sources and was able to hydrolyse 12 out of 19 compounds in the API ZYM system. The results of biochemical/physiological tests are given in Table 1 and in the species description. MK-8 was the predominant respiratory quinone of CC-FR2-10T, as for other Deinococcus species, and an unknown phosphoglycolipid was the predominant polar lipid. Strain CC-FR2-10T was resistant to UV irradiation (254 nm, 8–10 cm for

Table 1. Comparison of the phenotypic characteristics of strain CC-FR2-10T and D. grandis DSM 3963T

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>D. ficus</th>
<th>D. grandis</th>
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<tr>
<td>Utilization as carbon source:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>± L-Arabinose</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>± Lactose</td>
<td>+</td>
<td>–</td>
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<tr>
<td>± D-Trehalose</td>
<td>+</td>
<td>–</td>
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<tr>
<td>± D-Xylose</td>
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<td>–</td>
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<tr>
<td>± D-Mannose</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>± D-Melibiose</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>± N-Acetyl-D-glucosamine</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>± D-Sorbitol</td>
<td>+</td>
<td>–</td>
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</table>

Fig. 2. Phylogenetic tree, based on 16S rRNA gene sequences available from the EMBL database (accession numbers are given in parentheses), constructed after multiple alignment of the data by CLUSTAL X (Thompson et al., 1997). Distances (distance options according to the Kimura-2 model) and clustering with the neighbour-joining method were obtained by using the software package MEGA, version 2.1 (Kumar et al., 2001). Bootstrap values based on 1000 replications are listed as percentages at branching points. Bar, 0.05 K value. A maximum-parsimony tree is available as Supplementary Fig. S1 in IJSEM Online.

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10 min), as are many other *Deinococcus* species (Brooks & Murray, 1981).

On the basis of the results of this polyphasic taxonomic analysis and radiation-resistance studies, it is clear that strain CC-FR2-10\(^{T}\) represents a novel species of the genus *Deinococcus*, for which the name *Deinococcus ficus* sp. nov. is proposed.

**Description of *Deinococcus ficus* sp. nov.**

*Deinococcus ficus* (fi’cus. L. n. ficus a fig tree and the name of a botanical genus; L. gen. n. ficus of Ficus, referring to the isolation of the type strain from the rhizosphere of *Ficus religiosa* L.).

Cells are Gram-positive, non-motile, non-spore-forming rods. Aerobic, oxidase-positive and show good growth after 48 h on nutrient agar and tryptic soy agar at 37 °C. Colonies on nutrient agar are smooth, pale pinkish, circular, translucent and shiny with entire edges; colonies become mucoid. Pink pigmentation is non-diffusible, non-fluorescent and does not change upon the addition of 20% KOH. Unable to grow at 5 or 42 °C. Growth occurs at pH 5–5.10. Resistant to UV irradiation (254 nm, 8–10 cm for 10 min). Major cellular fatty acids are 16:1ω7c, 17:1ω8c, 17:1ω9c iso, 16:0, 17:0 iso and 15:0 ω6c. MK-8 is the predominant lipoquinone. An unknown phospholipoglycolipid is the predominant polar lipid. The following compounds are utilized as sole carbon sources (i.e. produce positive results in the Biolog system): dextrin, Tewsens 40 and 80, N-acetyl-D-glucosamine, N-acetyl-β-d-mannosamine (weakly), L-arabinose, D-fructose, L-fucose, D-galactose, D-galacturonic acid, D-glucuronic acid, α-D-glucose, α-D-lactose, maltose, maltotriose, D-mannitol, D-mannose, D-melibiose, methyl α-D-galactoside, methyl β-D-galactoside, methyl β-D-glucoside, D-raffinose, L-rhamnose, D-ribose, D-sorbitol, stachyose, sucrose, D-trehalose, D-xylene, acetic acid, β-hydroxybutyric acid, D-malic acid, L-malic acid, pyruvic acid methyl ester, succinic acid, L-malic acid, propionic acid, pyruvic acid, succinic acid, α- and γ-methyl succinic acid, α- and γ-methyl aspartic acid, α- and γ-aspartic acid, α- and γ-glutamic acid, α- and γ-serine, putrescine (weakly), glycerol, adenine, 2-deoxyadenosine, inosine, thymidine, uridine, adenosine 5’-monophosphate, thymidine 5’-monophosphate, uridine 5’-monophosphate, D-fructose 6-phosphate, α-D-glucose 1-phosphate, D-glucose 6-phosphate, Dl-α-glycerol phosphate. Positive for β-galactosidase, acetoin production, gelatinase, mannosidase, oxidation and cytochrome oxidase activity, alkaline phosphatase, butyrate esterase, caprylate esterase, leucine arylamidase, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase, β-glucosidase, α-mannosidase and α-fucosidase.

The type strain, CC-FR2-10\(^{T}\) (=CCUG 53391\(^{T}\) = CIP 108832\(^{T}\)), was isolated from the rhizosphere of *Ficus religiosa* L.


