Deinococcus deserti sp. nov., a gamma-radiation-tolerant bacterium isolated from the Sahara Desert

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Two gamma- and UV-radiation-tolerant, Gram-negative, rod-shaped bacterial strains, VCD115^T and VCD117, were isolated from a mixture of sand samples collected in the Sahara Desert in Morocco and Tunisia, after exposure of the sand to 15 kGy gamma radiation. Phylogenetic analysis based on 16S rRNA gene sequences and DNA–DNA hybridizations showed that VCD115^T and VCD117 are members of a novel species belonging to the genus *Deinococcus*, with *Deinococcus grandis* as its closest relative. The DNA G+C contents of VCD115^T and VCD117 are 59·8 and 60·6 mol%, respectively. The major fatty acids (straight-chain 15 : 1, 16 : 1, 17 : 1 and 16 : 0), polar lipids (dominated by phosphoglycolipids and glycolipids) and quinone type (MK-8) support the affiliation to the genus *Deinococcus*. The strains did not grow on rich medium such as trypticase soy broth (TSB), but did grow as whitish colonies on tenfold-diluted TSB. The genotypic and phenotypic properties allowed differentiation of VCD115^T and VCD117 from recognized *Deinococcus* species. Strains VCD115^T and VCD117 are therefore identified as representing a novel species, for which the name *Deinococcus deserti* sp. nov. is proposed, with the type strain VCD115^T (=DSM 17065^T=LMG 22923^T).

Various bacterial species have the capacity to survive under conditions that are commonly considered as extreme, for example in environments experiencing high pressure or high salt concentrations. In our laboratory, we are studying bacteria that live in the upper sand layers of deserts, where they are exposed to cycles of high and low temperatures, and to cycles of desiccation and hydration. De- and rehydration may cause DNA damage in these bacteria, and in order to survive they probably possess efficient DNA-repair mechanisms. Ionizing radiation causes similar types of DNA damage including double-strand breaks, which are the most deleterious to the organism (Mattimore & Battista, 1996). Bacteria belonging to the genus *Deinococcus*, in particular the well-studied *Deinococcus radiodurans*, have the distinctive feature of being the most radiation-tolerant of vegetative cells. *D. radiodurans* can withstand doses of radiation a thousand times higher than a human can. It can survive doses of radiation that do not exist naturally on Earth. Therefore, it is likely that this radiation tolerance is related to the bacterial response to natural non-radioactive DNA-damaging conditions such as desiccation (Makarova *et al.*, 2001). At the time of writing, eight recognized species belong to the genus *Deinococcus* (Ferreira *et al.*, 1997; Rainey *et al.*, 1997; Suresh *et al.*, 2004). Three other species have been described very recently, '*Deinococcus frigens*', '*Deinococcus saxicola*' and '*Deinococcus marmoris*' (Hirsch *et al.*, 2004). Only *D. radiodurans* R_1^T has been studied extensively. Its genome has been sequenced (White *et al.*, 1999), and analyses of the transcriptome (Liu *et al.*, 2003; Tanaka *et al.*, 2004) and proteome (Lipton *et al.*, 2002) have been

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains VCD115^T and VCD117 are AY876378 and AY876379, respectively.

Levels of 16S rRNA gene sequence similarity and DNA–DNA relatedness between strains VCD115^T and VCD117 and rates of survival after gamma and UV irradiation are available as supplementary material in IJSEM Online.

reported. However, there is not yet a precise explanation of how *D. radiodurans* repairs its damaged genome and thus why it is so radiotolerant (Edwards & Battista, 2003; Narumi, 2003).

Two novel radiation-tolerant strains, VCD115^T and VCD117, were obtained after exposure of a mixture of desert sand samples, collected in the Sahara in Morocco and Tunisia, to a dose of 15 kGy gamma radiation (4·2 kGy h^{-1}, ^{60}Co source; CEA Cadarache), followed by isolation of surviving colony-forming bacteria on agar plates containing tenfold-diluted trypticase soy broth (TSB/10; 3 g l^{-1}) (Bacto; Becton Dickinson). The type strains *Deinococcus grandis* DSM 3963^T, *Deinococcus indicus* DSM 15307^T and *D. radiodurans* DSM 20539^T (=$R_1{}^T$) were obtained from the DSMZ. Single carbon-source assimilation tests were performed on RCV medium (Weaver *et al.*, 1975), adjusted to pH 7·5, and supplemented with 0·5 g ammonium sulfate l^{-1}, 0·05 g yeast extract l^{-1} and 1 % stock vitamin solution [4 μg ml^{-1} each of thiamine, riboflavin, pyridoxine, biotin, folic acid, nicotinic acid, pantothenic acid, L-(+)-ascorbic acid and cyanocobalamin]. Carbon sources were added at a final concentration of 1 g l^{-1}. Negative controls did not include the carbon source. Positive controls were TSB/10 and the RCV medium supplemented with trypton (1·7 g l^{-1}) and yeast extract (0·3 g l^{-1}). For all other tests, bacterial strains were cultivated at 30 °C in TSB/10 or on agar plates containing the same medium. For Biolog GN2 plates, cells were resuspended in basal RCV medium (without yeast extract and vitamins). API 20 NE strips (bioMérieux) were used according to the instructions of the manufacturer. Catalase activity was tested by putting a drop of 3 % hydrogen peroxide solution on a colony. Bubble formation was used to indicate a positive reaction. Susceptibility to antibiotics was analysed on agar plates containing 2·5, 10, 15 and 25 μg antibiotic ml^{-1}. To determine the survival rate after exposure to gamma radiation, cultures were grown to an OD_{600} of about 0·5, irradiated at the desired dose, diluted serially and plated. Percentage survival was determined by comparing with unirradiated cultures. To determine the survival rate after exposure to UV radiation, serial dilutions of cultures (OD_{600} of about 0·5) were spread on TSB/10 plates. Plates without their cover lids were immediately exposed to UV (UV-C, 254 nm) for the desired dose and subsequently incubated at 30 °C. The UV dose was monitored by using a VLX-3W radiometer (Bioblock Scientific).

The *rrs* (16S rRNA) genes were amplified from colonies by PCR using the primers fD1 (5′-AGAGTTTGATCCTGGCTCAG-3′, positions 8–27 on the *Escherichia coli rrs* gene) and S17 (5′-GTTACCTTGTTACGACTT-3′, positions 1492–1509 on the *E. coli rrs* gene), and the entire PCR fragment was sequenced. This resulted in sequences of 1406 and 1407 bp for VCD115^T and VCD117, respectively. The new sequences were added and aligned by reference to a database of 120 000 already aligned and analysed (neighbour-joining) 16S rRNA gene sequences. Subsequently, BLAST queries against the latest release of the Bacteria division of GenBank allowed us to verify that no closely related sequence was missing in the database. The 50 most closely related sequences were selected, *Thermus aquaticus* and *Meiothermus ruber* were added as outgroups, and alignments between these sequences were refined manually using SEAVIEW (Galtier *et al.*, 1996). A first phylogenetic analysis using the neighbour-joining method and conserved domains of the sequences produced an initial tree. We then retained sequences only for type strains, which led to 12 sequences. Phylogenetic trees were constructed according to three different methods: neighbour joining (bioNJ) (Gascuel, 1997), maximum likelihood using the Global option and maximum parsimony. The latter two programs were from PHYLIP (Phylogeny Inference Package, version 3.573c, distributed by J. Felsenstein, Department of Genome Sciences, University of Washington, Seattle, USA). For the bioNJ analysis, a matrix distance was calculated according to the Kimura two-parameter correction. Bootstrap support was determined using 1000 replications, bioNJ and Kimura two-parameter corrections. The phylogenetic trees were drawn using NJPLOT (Perrière & Gouy, 1996). Domains used to construct the final phylogenetic trees were positions 41–392 and 407–1406 of the 16S rRNA gene sequence of VCD115^T, excluding domains difficult to align among all sequences.

Extraction of genomic DNA (Earl *et al.*, 2002) and DNA–DNA hybridizations (Ezaki *et al.*, 1989; Willems *et al.*, 2001) were performed as described previously. For DNA–DNA hybridizations, four replicate wells were used and reciprocal hybridizations were carried out for all experiments. The DNA G+C contents were determined by the thermal denaturation method (Marmur & Doty, 1962) and were calculated by using the equation of Owen & Lapage (1976). *E. coli* K-12 (DNA G+C content 50·6 mol%) was used as a control. Extraction and analysis of fatty acids was performed by Dr R. M. Kroppenstedt at the DSMZ (Braunschweig, Germany) using the Sherlock Microbial Identification System (MIDI, Inc.). Polar lipid analyses were carried out by the Identification Service of the DSMZ and Dr B. J. Tindall, DSMZ. Quinone analysis was also performed by the DSMZ.

The 16S rRNA genes of the new strains VCD115^T and VCD117 showed a high level of similarity (99·7 %; see Supplementary Table S1 in IJSEM Online). According to phylogenetic analysis of the 16S rRNA gene sequences, strains VCD115^T and VCD117 form a robust clade within the genus *Deinococcus* and this clade cannot be grouped consistently with any recognized species, suggesting that these two strains represent a novel species of *Deinococcus* (Fig. 1). Percentages of 16S rRNA gene sequence similarity with other available sequences were calculated by parsing the result of BLAST analyses on the Bacteria division with the options 'no filter' and 'W=7' (NCBI Standalone BLAST). Summing for similarities over High Scoring Pairs gave 90·5 and 90·7 % similarity with *D. grandis* for strain VCD115^T and VCD117, respectively, with up to 95 % similarity when

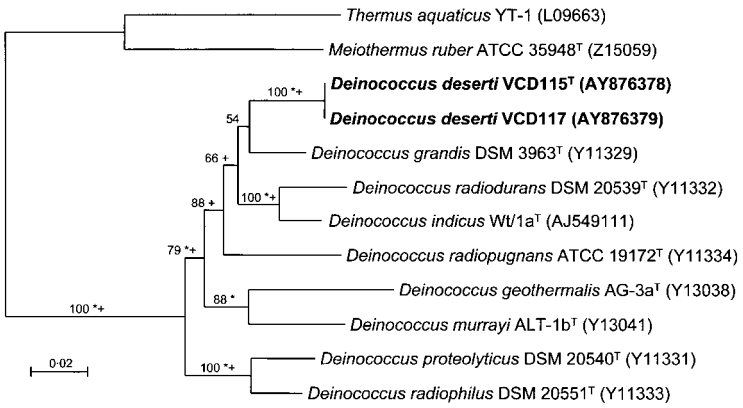

Fig. 1. Unrooted phylogenetic tree based on 16S rRNA gene sequence analysis, showing the relationship between the *Deinococcus deserti* strains and other *Deinococcus* species. The topology shown was obtained using the bioNJ algorithm with a Kimura two-parameter correction for the distances. Percentages of bootstrap support (1000 replications) are indicated as well as branches also retrieved by maximum parsimony (+) and maximum likelihood (*, $P<0{\cdot}01$); branches retrieved by all three methods should be considered as the only robust clades identified by this analysis. GenBank accession numbers are given in parentheses.

excluding the 5′ part of the sequences. The *rrs* genes of the recently isolated species '*D. frigens*', '*D. marmoris*' and '*D. saxicola*', which were not included in the phylogenetic tree, are most similar to *Deinococcus radiopugnans* (Hirsch *et al.*, 2004), which is distant from the VCD115^T/VCD117 clade (Fig. 1). The 16S rRNA genes of VCD115^T and VCD117 possess the signature nucleotides C, G, T, G, T, A, G, C and C at positions 657, 749, 757, 1050, 1208, 1421, 1429, 1471 and 1479 (*E. coli* 16S rRNA gene sequence numbering, GenBank accession number J01695), respectively, characteristic of the genus *Deinococcus* (Rainey *et al.*, 1997). A signature nucleotide (C) was also reported at position 584 by Rainey *et al.* (1997), but the more recently described species *Deinococcus geothermalis*, *Deinococcus murrayi* and *D. indicus*, as well as strains VCD115^T and VCD117, have a G at this position.

Genomic DNA of strains VCD115^T and VCD117 was cross-hybridized and hybridized with that of *D. grandis*, *D. indicus* and *D. radiodurans*. Between strains VCD115^T and VCD117, 78 % reassociation was found (80 and 76 % when DNA of strains VCD115^T and VCD117 was used as probe, respectively). By using the criterion of at least 70 % hybridization for definition of a species (Wayne *et al.*, 1987), we conclude that strains VCD115^T and VCD117 belong to the same species. A level of <20 % was found in all other DNA hybridization combinations (see Supplementary Table S1 in IJSEM Online). These results indicate that VCD115^T and VCD117 are members of a novel species, for which the name *Deinococcus deserti* sp. nov. is proposed. The DNA G+C contents were determined to be 59·8 and $60{\cdot}6\pm0{\cdot}5$ mol% for VCD115^T and VCD117, respectively. The DNA G+C content of other deinococcal species ranges from 59·4 to 70·0 mol% (Hirsch *et al.*, 2004).

Cells of *D. deserti* VCD115^T and VCD117 are non-motile rods. Most of the cells were present as pairs, but chains of four cells were also regularly observed. Cells of *D. grandis* and *D. indicus* are also rod-shaped (Suresh *et al.*, 2004), whereas those of all other members of the genus are spherical (Rainey *et al.*, 1997; Hirsch *et al.*, 2004). Colonies of VCD115^T and VCD117 were whitish on TSB/10 plates, unlike other *Deinococcus* species which were red-pigmented on this growth medium. However, when growth was obtained on RCV plates (for the carbon source assimilation tests, see below), the two *D. deserti* strains were faintly pink-pigmented. Colonies of *D. radiodurans*, *D. indicus* and *D. grandis*, in comparison with those of *D. deserti*, were strongly red-pigmented on these RCV plates. These results suggest that expression of genes involved in pigment formation may be regulated in VCD115^T and VCD117, whereas it is apparently constitutive in the other strains. Alternatively, unlike for the other *Deinococcus* strains, pigment biosynthesis in VCD115^T and VCD117 may require an exogenous compound as co-factor. As with cells of *D. indicus* and *D. grandis*, those of *D. deserti* stain Gram-negative, whereas those of other *Deinococcus* species stain Gram-positive (Suresh *et al.*, 2004; Hirsch *et al.*, 2004). *D. deserti* was routinely grown at 30 °C in or on TSB/10. Growth was also observed at 37 °C and slow growth at 23 °C, but not at 45 °C. Growth occurred between pH 6 and pH 9 with an optimum pH of about 7·5. Unlike the other *Deinococcus* type strains, the two *D. deserti* strains did not grow on plates containing the rich media LB, TGY (a trypton/glucose/yeast extract medium often used for growth of *Deinococcus* species; Brim *et al.*, 2003) or undiluted TSB.

For the single carbon-source utilization assays, the results were almost identical for VCD115^T and VCD117 (Table 1). Good growth was observed on D-glucose, D-cellobiose, maltose, D-fructose, D-sorbitol, D-mannitol, starch and Casamino acids; moderate growth was observed on L-glutamate, acetate, D-galactose, sucrose, L-alanine, succinate and (though not for VCD117) L-histidine; no growth was observed on D-xylose, L-arabinose, *myo*-inositol, glycerol, D-ribose, lactose or L-tryptophan. On Biolog GN2 plates, utilization of additional carbon sources was observed, including D-psicose, succinic acid monomethyl ester, turanose, D-mannose and L-proline. The results of the carbon-utilization tests were very similar, but not identical, between *D. deserti* and the closely related *D. grandis*, but more differences were observed with the other species with rod-shaped cells, *D. indicus* (Table 1). On API 20 NE strips, which were incubated for up to 9 days, strain VCD115^T was

Table 1. Phenotypic differences between *D. deserti* strains VCD115^T and VCD117, *D. grandis* and *D. indicus*

Strains: 1, *D. deserti* VCD115^T; 2, *D. deserti* VCD117; 3, *D. grandis* DSM 3963^T; 4, *D. indicus* DSM 15307^T. +, Positive; −, negative; (+), weakly positive; NT, not tested. On RCV, all strains were (weakly) positive for utilization of D-cellobiose, maltose, sucrose, L-glutamate, L-alanine, succinate, starch and Casamino acids. On Biolog GN2 plates, *D. deserti* and *D. grandis* were (weakly) positive for D-glucose, sucrose, D-fructose, maltose, D-mannose, D-psicose, L-proline, dextrin and succinic acid monomethyl ester.

Characteristic	1	2	3	4
DNA G+C content (mol%)	60	61	69	66
Growth on rich media (TSB, LB, TGY)	−	−	+	+
Colony colour on TSB/10	Whitish	Whitish	Red	Red
Carbon-source utilization on RCV medium				
D-Glucose, D-fructose, D-sorbitol, D-mannitol	+	+	+	−
D-Xylose, L-arabinose, lactose	−	−	−	(+)
Glycerol	−	−	+	+
L-Histidine	(+)	−	(+)	(+)
Carbon-source utilization on Biolog GN2				
Turanose, D-galactose	+	+	−	NT
Lactulose, glycerol, methyl β-D-glucoside	−	−	+	NT
Formic acid	+	−	−	NT

positive for protease and weakly positive for β-glucosidase and β-galactosidase. VCD117 was positive for protease and β-galactosidase and weakly positive for β-glucosidase. Protease production was confirmed by the observation of halo formation on plates containing 1 % skimmed milk. VCD115^T and VCD117 tolerated up to 25 μg spectinomycin and nalidixic acid ml^{-1}, and weak growth was observed at up to 10 μg bacitracin ml^{-1}. VCD115^T tolerated up to 0·5 % NaCl (added to TSB/10, which contains 0·05 % NaCl), but only weak growth of VCD117 was observed in the presence of 0·5 % NaCl.

The fatty acid composition was determined for VCD115^T, VCD117 and the closest relative *D. grandis*, grown under the same conditions. Major fatty acids were straight chains 15 : 1ω6*c*, 16 : 1ω7*c*, 17 : 1ω8*c* and 16 : 0 (Table 2), which are also predominant in most other *Deinococcus* species (Hirsch *et al.*, 2004). Smaller amounts of iso-branched fatty acids were also found. Qualitative and quantitative differences between the strains, in particular between *D. deserti* and *D. grandis*, were also observed. For example, a 16 : 1 iso-branched fatty acid was found in VCD115^T and VCD117 but not detected in *D. grandis*. The polar lipid composition of VCD115^T was found to be dominated by three phosphoglycolipids (with one of them as the major polar lipid) and four glycolipids, which is typical for *Deinococcus* species (Thompson *et al.*, 1980). Two phospholipids and one aminophospholipid were also detected. The major respiratory quinone of VCD115^T was menaquinone 8 (MK8).

Survival of cultures after exposure to increasing doses of gamma radiation was analysed for strains VCD115^T and VCD117, and compared with *D. radiodurans* R$_1^T$ and *E. coli* MC4100 (Supplementary Table S2). At the lowest dose tested (2·5 kGy), *E. coli* did not survive, whereas almost no loss in survival of strains VCD115^T and R$_1^T$ was observed. VCD117 appeared to be somewhat less tolerant than VCD115^T; exposure of cultures to 2·5, 5 and 7·5 kGy resulted in survival of 60, 15 and 6 %, respectively, for VCD117, compared with 95, 94 and 23 %, respectively, for VCD115^T. Survival of VCD115^T was comparable to that of R$_1^T$. Strains VCD115^T and VCD117 also appeared to be extremely tolerant to UV radiation, with VCD115^T being much more tolerant than VCD117 and *D. radiodurans* R$_1^T$ (Supplementary Table S2). Whereas *E. coli* did not survive the lowest dose tested (250 J m^{-2}), 73 and 11 % survival was observed at this dose for VCD115^T and VCD117, respectively, and more than 1 % of the VCD115^T cells survived after exposure to UV doses as high as 750 J m^{-2}.

To summarize, the phenotypic characteristics of strains VCD115^T and VCD117 are very similar, supporting the conclusion from the phylogenetic analysis and DNA–DNA hybridizations that they belong to the same species. Nevertheless, VCD115^T is more tolerant to gamma and UV radiation than is VCD117. It is highly likely that tolerance to radiation depends on numerous gene products and other factors such as growth conditions. Some of the genes that contribute to radiotolerance may be less active or even inactivated or absent in VCD117 compared with VCD115^T. Gene inactivation may occur by an insertion sequence (IS) element, as exemplified by a DNA damage-sensitive *D. radiodurans* strain that contained an IS in the *uvrA* gene (Narumi *et al.*, 1997). After *D. grandis* and *D. indicus*, VCD115^T and VCD117 belong to a third *Deinococcus* species with rod-shaped cells. Unlike other

Table 2. Fatty acid composition of strains VCD115^T, VCD117 and *D. grandis* DSM 3963^T

Values are percentages of the total fatty acid content. The three major fatty acids for each strain are shown in bold. −, Not detected.

Fatty acid	VCD115^T	VCD117	*D. grandis*
10 : 0 iso	0·24	−	−
12 : 0 iso	0·24	0·75	−
13 : 0 iso	−	−	0·89
13 : 0	−	−	0·66
14 : 0 iso	−	0·59	−
14 : 1ω5*c*	−	−	0·35
14 : 0	−	0·71	0·82
15 : 1 iso*/13 : 0 3-OH	−	−	0·95
15 : 0 iso	0·33	0·67	9·88
15 : 0 anteiso	−	−	0·28
15 : 1ω8*c*	1·6	1·25	1·34
15 : 1ω6*c*	**7·77**	3·60	**17·24**
15 : 0	3·25	6·70	9·26
16 : 1 iso*	5·16	4·33	−
16 : 0 iso	5·67	6·16	0·97
16 : 1ω9*c*	2·72	3·56	0·75
16 : 1ω7*c*	**29·39**	**35·23**	**19·23**
16 : 1ω5*c*	0·52	−	1·97
16 : 0	7·07	**12·11**	**9·90**
17 : 1ω9*c* iso	5·39	4·85	1·01
17 : 1 anteiso*/17 : 1 iso*	−	−	1·71
17 : 1 anteiso*	−	3·31	−
17 : 1ω9*c* anteiso	0·23	−	−
17 : 0 iso	1·65	1·72	8·13
17 : 1ω8*c*	**14·14**	**6·91**	2·95
17 : 1ω6*c*	4·88	2·07	4·10
17 : 0	4·08	2·46	7·59
17 : 0 10-methyl	0·71	−	−
18 : 1 iso*	2·87	1·26	−
18 : 0 iso	0·86	0·63	−
18 : 1ω9*c*	0·57	0·46	−
18 : 1ω7*c*	0·65	0·67	−

*Double bond position and/or configuration uncertain.

Deinococcus species, VCD115^T and VCD117 do not grow on rich media and are not (on TSB/10) or are only faintly (on RCV) pink-pigmented. Carbon-source utilization and fatty acid composition of VCD115^T and VCD117 allow further differentiation from *D. grandis* and *D. indicus*. Phylogenetic, chemotaxonomic and physiological differences with the other *Deinococcus* species support the description of a novel species for strains VCD115^T and VCD117.

Description of *Deinococcus deserti* sp. nov.

Deinococcus deserti (des.er′ti. L. gen. n. *deserti* of a desert).

Cells are non-motile and rod-shaped. Cell division occurs by constriction. Gram-negative. Whitish, smooth, circular, uniform-edged colonies of 0·5–1 mm after 72 h at 30 °C on TSB/10 plates. Faintly pink-pigmented on RCV plates. Growth is observed up to 0·5 % NaCl. Generation time of the type strain in TSB/10 is 3 h. No growth is observed on plates containing rich media. Carbon source utilization is indicated in Table 1. Major fatty acids are 15 : 1ω6*c*, 16 : 1ω7*c*, 17 : 1ω8*c* and 16 : 0. Phosphoglycolipids and glycolipids are the major polar lipids. Major respiratory quinone is MK8. Strictly aerobic, and positive for protease and catalase. Resistant to spectinomycin and nalidixic acid, but sensitive to ampicillin, carbenicillin, tetracycline, kanamycin, gentamicin, streptomycin, chloramphenicol and rifampicin. Tolerates high doses of gamma and UV radiation. DNA G+C content is 59·8 mol% for the type strain.

The type strain, VCD115^T (=DSM 17065^T=LMG 22923^T), was isolated from gamma-irradiated mixed sand samples from the Sahara Desert in Morocco and Tunisia. Strain VCD117 is a reference strain.

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