Description of four novel psychrophilic, ionizing radiation-sensitive *Deinococcus* species from alpine environments

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Five psychrophilic bacterial strains were isolated from soil samples collected above the treeline of alpine environments. Phylogenetic analysis based on 16S rRNA gene sequences indicated that these organisms represent four novel species of the genus *Deinococcus*; levels of sequence similarity to the type strains of recognized *Deinococcus* species were in the range 89.3–94.7%. Strains PO-04-20-132^T, PO-04-20-144, PO-04-19-125^T, ME-04-01-32^T and ME-04-04-52^T grew aerobically, with optimum growth at 10 °C and at pH 6–9. The major respiratory menaquinone was MK-8. The fatty acid profiles of strains PO-04-20-132^T, PO-04-20-144, PO-04-19-125^T and ME-04-01-32^T were dominated by 16:1 ω 7c, 17:0 iso and 15:1 ω 6c, whereas 16:1 ω 7c, 17:0 cyclo and 16:0 predominated in strain ME-04-04-52^T. The DNA G + C contents of strains PO-04-20-132^T, PO-04-19-125^T, ME-04-01-32^T and ME-04-04-52^T were 63.2, 63.1, 65.9 and 62.6 mol%, respectively. Strains PO-04-20-132^T, PO-04-19-125^T, ME-04-01-32^T and ME-04-04-52^T had gamma radiation D₁₀ (dose required to reduce the bacterial population by 10-fold) values of ≤ 4 kGy. These four strains showed sensitivity to UV radiation and extended desiccation as compared with *Deinococcus radiodurans*. On the basis of the phylogenetic analyses, and chemotaxonomic and phenotypic data, it is proposed that strains PO-04-20-132^T (=LMG 24019^T=NRRL B-41950^T; *Deinococcus radiomollis* sp. nov.), PO-04-19-125^T (=LMG 24282^T=NRRL B-41949^T; *Deinococcus claudionis* sp. nov.), ME-04-01-32^T (=LMG 24022^T=NRRL B-41947^T; *Deinococcus altitudinis* sp. nov.) and ME-04-04-52^T (=LMG 24283^T=NRRL B-41948^T; *Deinococcus alpinitundrae* sp. nov.) represent the type strains of four novel species of the genus *Deinococcus*.

At the time of writing, the genus *Deinococcus* comprised 25 recognized species, three of which were isolated from

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains PO-04-20-132^T, PO-04-20-144, PO-04-19-125^T, ME-04-01-32^T and ME-04-04-52^T are EF635404, EF635405, EF635406, EF635407 and EF635408, respectively.

A two-dimensional thin-layer chromatograph of total polar lipids, graphs showing the effect of gamma radiation, UV radiation and desiccation on the survival of the new isolates, and a table detailing the fatty acid compositions of the new isolates and recognized *Deinococcus* species are available as supplementary material with the online version of this paper.

low-temperature environments, namely Antarctic soils or rocks (Hirsch *et al.*, 2004). The optimal growth temperatures of species of the genus *Deinococcus* fall across a broad range from 9 to 50 °C. The majority of species are reported to have optimal growth temperatures in the mesophilic range. Exceptions are three slightly thermophilic species, namely *Deinococcus geothermalis*, *D. murrayi* and *D. maricopensis* (Ferreira *et al.*, 1997; Rainey *et al.*, 2005), and three psychrophilic species, namely *Deinococcus frigens*, *D. marmoris* and *D. saxicola* (Hirsch *et al.*, 2004).

The most studied characteristic of members of the genus *Deinococcus* is their ability to survive exposure to ionizing

radiation and to repair their DNA following damage caused by radiation (gamma or UV) or desiccation. All species of the genus *Deinococcus* for which the characteristic has been tested have shown resistance to levels of ionizing radiation to which they would never be exposed in the natural environment. The type species of the genus, *Deinococcus radiodurans* (strain R1^T), displays a D₁₀ (dose required to reduce the bacterial population by 10-fold) value of 11.5 kGy following exposure to gamma radiation at a dose rate of 2.57 kGy h⁻¹.

During the course of a study to examine the culturable bacterial diversity in alpine regions, a number of samples were collected from Pico de Orizaba, Mexico, and Mount Evans, CO, USA. These surface (upper 2 cm) soil samples were collected by using a sterile scoop from above the treeline at high altitudes (>3500 m), transported to the laboratory and stored at ambient temperature until processing. The samples were designated PO-04-19 (5002 m), PO-04-20 (5065 m), ME-04-01 (4003 m) and ME-04-04 (3596 m). Serial dilutions were plated on a number of nutrient growth media, including 1/10-strength plate count agar (PCA; Difco). The dilutions were made in liquid media of the same composition as the agar plates. Plates were incubated at 10 and 25 °C for up to 40 and 20 days, respectively. CFU values (per gram of soil) for these samples were in the range 3.3×10^3 – 1.73×10^7 . Analysis of partial 16S rRNA gene sequences of five of the bacterial strains isolated, designated PO-04-20-132^T, PO-04-20-144, PO-04-19-125^T, ME-04-01-32^T and ME-04-04-52^T, indicated that they were members of the genus *Deinococcus*, and these were characterized further.

Almost-complete 16S rRNA gene sequences comprising between 1436 and 1440 nt were determined for the five strains as described by Rainey *et al.* (1996). The 16S rRNA gene sequences were aligned against representative reference sequences of members of the genus *Deinococcus* and related taxa by using MEGA version 3.1 (Kumar *et al.*, 2004). The method of Jukes & Cantor (1969) was used to calculate evolutionary distances. Phylogenetic dendrograms were constructed and bootstrap analyses were performed by using algorithms contained in the PHYLIP package (Felsenstein, 1993). Comparison with recognized species of the genus *Deinococcus* showed that these five novel strains fell within the radiation of this genus and represented two novel lineages (Fig. 1). Levels of 16S rRNA gene sequence similarity to the type strains of recognized species of the genus *Deinococcus* were in the range 89.3–94.7%. Four of the novel strains, PO-04-20-132^T, PO-04-20-144, PO-04-19-125^T and ME-04-01-32^T, formed a coherent cluster supported by a bootstrap value of 100% whereas the fifth strain, ME-04-04-52^T, represented a distinct and separate lineage (Fig. 1). Strains PO-04-20-132^T and PO-04-20-144 shared 100% 16S rRNA gene sequence similarity. Levels of 16S rRNA gene sequence similarity between the four strains (PO-04-20-132^T, PO-04-20-144, PO-04-19-125^T and ME-04-01-32^T) clustering together were in the range 96.6–100%. Strain ME-04-04-52^T had 16S rRNA gene

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences showing the relationships of strains ME-04-01-32^T, ME-04-04-52^T, PO-04-19-125^T, PO-04-20-132^T and PO-04-20-144 and recognized *Deinococcus* species. The dendrogram was constructed from distance matrices according to the neighbour-joining method. Numbers at branching points represent bootstrap percentages based on 1000 replicates. *Truepera radiovictrix* RQ-24^T (Albuquerque *et al.*, 2005) was used as the outgroup. Bar, 2 inferred nucleotide substitutions per 100 nt.

sequence similarities in the range 89.3–93.3% to the type strains of recognized *Deinococcus* species. With such low levels of 16S rRNA gene sequence similarity to other lineages, the branching point of strain ME-04-04-52^T was not well supported by bootstrap analyses and it thus represents a well-separated lineage (Fig. 1).

DNA for determination of the genomic G + C content was isolated as described by Cashion *et al.* (1977). The DNA G + C contents of strains PO-04-20-132^T, PO-04-19-125^T, ME-04-01-32^T and ME-04-04-52^T were determined by HPLC (Mesbah *et al.*, 1989), and was 63.2, 63.1, 65.9 and 62.6 mol% respectively. These values are within the range reported for recognized *Deinococcus* species (59.4–71.1 mol%).

Cultures used for polar lipid analysis were grown on nutrient agar (Difco) at 14 °C for 15 days. Harvesting of cultures, extraction of lipids, two-dimensional TLC, and staining of the plates with molybdophosphoric acid, ninydrin and α -naphthol were performed as described by Prado *et al.* (1988). Lipoquinones were extracted from

freeze-dried cells, purified by TLC and separated by HPLC as described by Tindall (1989). Cultures for fatty acid analysis were grown in liquid medium (comprising, per litre: 0.5 g tryptone, 0.25 g yeast extract and 0.1 g glucose; pH 6) in a rotary shaker at 120 r.p.m. for 12 days at 10 °C. Fatty acid methyl esters were obtained from fresh wet biomass by saponification, methylation and extraction as described by Kuykendall *et al.*, (1988), and were separated, identified and quantified as described by Moreira *et al.* (2000).

Strains PO-04-20-132^T, PO-04-19-125^T, ME-04-01-32^T and ME-04-04-52^T had fairly similar polar lipid patterns, consisting primarily of glycerophospholipids, aminophospholipids and glycolipids (see Supplementary Fig. S1 available in IJSEM Online). These novel organisms contained one major glycerophospholipid with a similar mobility to the well-characterized 2'-O-(1,2-diacyl-*sn*-glycero-3-phospho)-3'-O-(α -galactosyl)-N-D-glyceroyl alkylamine of *D. radiodurans* (Anderson & Hansen, 1985), a major glycolipid and a major aminophospholipid. Other minor polar lipids were also detected in the novel organisms. It is interesting to note that we did not detect aminolipids in the type strain of *D. marmoris* (Supplementary Fig. S1).

The fatty acid profiles of strains PO-04-20-132^T, PO-04-19-125^T and ME-04-01-32^T were dominated by 16:1 ω 7c (26.2–36.8%), 17:0 iso (14.6–19.5%) and 15:1 ω 6c (6.9–10.9%), the combination of which allows these strains to be distinguished from all other recognized species of the genus *Deinococcus*. However, the fatty acid profiles of these strains were very similar and could not be used to distinguish them from each other. (see Supplementary Table S1 available in IJSEM Online). The fatty acid profile of ME-04-04-52^T, dominated by 17:0 cyclo (33.7%) and 16:1 ω 7c (38.5%), allowed this strain to be distinguished from the type strains of all recognized species of the genus *Deinococcus*, and also from strains PO-04-20-132^T, PO-04-19-125^T and ME-04-01-32^T. Moreover, strain ME-04-04-52^T lacked branched-chain iso and anteiso fatty acids, which are present in most strains of recognized *Deinococcus* species.

The vast majority of species of the genus *Deinococcus* have high levels of unsaturated fatty acids, irrespective of the presence or absence of branched-chain components. The novel strains had lower proportions of saturated short straight-chain or unsaturated chain fatty acids than many other mesophilic or slightly thermophilic species of the genus. The type strains of the psychrophilic species *D. saxicola* and *D. frigens* have, by contrast, extremely high levels of unsaturated fatty acids, but the type strain of the mesophilic species *Deinococcus hopiensis* also possesses similar levels of these fatty acids. The major respiratory quinone of the five new isolates was menaquinone 8 (MK-8), consistent with data for all other *Deinococcus* strains.

Cell morphology and motility were examined by phase-contrast microscopy after cultivation on agar plates. Colonies on 1/10-strength PCA were light pink. Cells of

the new isolates were Gram-positive but differed in morphology and cell size. Cells of strains PO-04-20-132^T, PO-04-20-144 and ME-04-01-32^T were rods, 2.4–2.9 μ m long and 1.2–1.5 μ m wide. Coccoid morphology was observed for strain ME-04-04-52^T, cells of which were 2.4 μ m in diameter. Cells of strain PO-04-19-125^T were spherical or short rods of 2.3 $\times$ 1.54 μ m. Cells of all the novel strains were non-motile and spores were not observed.

The temperature range for growth was determined on 1/10-strength PCA plates incubated for 10 days at temperatures between 2 and 30 °C. The optimum growth temperature for all five strains was 10 °C, with growth occurring between 5 and 25 °C but not at 2 or 30 °C. The pH range for growth was determined at 10 °C on buffered 1/10-strength PCA plates; the media buffered between pH 4 and pH 10 were prepared as described by Ferreira *et al.* (1997). Strains PO-04-20-132^T, PO-04-20-144, PO-04-19-125^T, ME-04-01-32^T and ME-04-04-52^T grew between pH 5 and pH 10 with the exception that strain ME-04-01-32^T was unable to grow above pH 9.

Tests for catalase, cytochrome oxidase, and hydrolysis of starch, casein and gelatin were performed as described by Smibert & Krieg (1981). All strains were cytochrome oxidase-negative and catalase-positive. None of the five strains was capable of hydrolysis of starch, casein or gelatin. Single-carbon source assimilation tests were performed in a defined medium solidified with deionized water-washed agar as described by Rainey *et al.* (2005). Growth was examined visually on plates incubated at 10 °C for up to 20 days. Negative control plates did not include the carbon source. Positive control cultures were grown on both 1/10-strength PCA and solidified Degryse medium 162 (Degryse *et al.*, 1978). The novel strains utilized a number of substrates for growth, and differences in substrate utilization patterns were observed. Complete results of the phenotypic characterization of the novel isolates are provided in Table 1 and in the species descriptions below.

To determine levels of resistance to UV radiation, cells of the new isolates and the type strain of *D. radiodurans* were harvested from 1/10-strength PCA when colonies began to appear and then suspended in the corresponding liquid medium to an OD₆₀₀ of 0.2. Aliquots (1 ml) of the suspension were transferred to sterile Petri dishes and exposed to a 254-nm UVLMS-38 (UVP) light source for dosages of 200, 400, 600, 800 and 1000 J m⁻². The light source was calibrated by using a J225 (UVP) radiometer to deliver a dose of 630 μ W.s cm⁻². Following exposure, suspensions of serial dilutions were plated in triplicate and incubated at 10 °C for the new isolates and at 30 °C for *D. radiodurans*. Attention was taken to ensure that plates were kept in the dark to avoid photorepair processes. Growth after a maximum of 42 days incubation was compared with that of an unirradiated control to determine the surviving fraction. The survival of isolates after exposure to various doses of gamma radiation was determined by exposing

Table 1. Differential characteristics between the five novel isolates

Strains: 1, PO-04-20-132^T; 2, PO-04-20-144; 3, PO-04-19-125^T; 4, ME-04-01-32^T; 5, ME-04-04-52^T. w, Weak utilization of the substrate when compared with control reactions; ND, not determined.

Characteristic	1	2	3	4	5
Cell morphology	Rods	Rods	Cocci/short rods	Rods	Cocci
Cell size (µm)	2.4 × 1.2	2.7 × 1.3	2.3 × 1.5	2.9 × 1.5	2.4
Growth at pH 10	+	+	+	–	+
Utilization of:					
D-Glucose	+	+	+	–	–
Fructose	w	w	+	–	–
Mannose	+	+	+	–	–
L-Rhamnose	+	w	+	–	–
L-Arabinose	+	+	+	+	w
Ribose	–	–	–	+	–
Sucrose	+	+	+	–	–
Xylose	+	+	+	–	–
Galactose	+	+	–	+	–
Glucosamine	+	+	+	–	–
DNA G + C content (mol%)	63.2	ND	63.1	65.9	62.6

aliquots of the strains (prepared as described above for UV exposure) to gamma radiation by using a cobalt-60 source at a dose rate of 2.57 kGy h^{–1}, as described by Zimmerman & Battista (2006). The cell suspensions were exposed to doses of 2.5, 5.0, 10.0 and 15.0 kGy. Aliquots of cell suspensions not exposed to gamma or UV radiation were maintained at room temperature and serial dilutions were plated at the same time as the exposed cell suspensions. For determination of desiccation resistance, cells were prepared as described for UV radiation exposure. A 100-µl aliquot of the suspension was spotted onto each well of a polystyrene 24-well microtitre plate and dried completely before placing in a desiccation chamber (Bel-Art). The desiccation chamber was maintained at room temperature. An additional desiccation experiment was performed at 10 °C. Relative humidity was maintained below 5 % throughout, as measured by a HOBO H8 (Onset), by using silica gel desiccant (which was replaced weekly). Rehydration was performed after 2, 7, 14, 28 and 42 days by adding 1 ml of the appropriate medium, allowing resuspension of the cells for 30 min, followed by vigorous mixing. Resulting suspensions were dilution plated in triplicate and incubated at the appropriate temperature for a maximum of 42 days. Surviving fractions were calculated from titres before desiccation and after rehydration. *D. radiodurans* R1^T (DSM 20539^T) was used as a control in all gamma, UV and desiccation resistance experiments.

In contrast to all other *Deinococcus* species for which data have been reported, strains PO-04-20-132^T, PO-04-19-125^T, ME-04-01-32^T and ME-04-04-52^T showed sensitivity to gamma and UV radiation as well as prolonged desiccation (see Supplementary Fig. S2 in IJSEM Online). The D₁₀ values for each of these stresses are provided for four of the five new isolates as well as for *D. radiodurans* R1^T (Table 2). From the D₁₀ values for gamma and UV

radiation exposure and prolonged desiccation it is clear that the novel strains are significantly more sensitive to these stresses as compared with *D. radiodurans*. The most sensitive strain was PO-04-20-132^T with D₁₀ values of 2.2 kGy and 220 J m^{–2} for gamma and UV radiation, respectively. No significant difference in D₁₀ values was observed when the desiccation experiment was performed at 10 °C (data not shown).

The strains isolated in the present study can be differentiated from recognized species of the genus *Deinococcus* on the basis of their phylogenetic position. They have been shown to fall into two distinct lineages, one comprising strains PO-04-20-132^T, PO-04-20-144, PO-04-19-125^T and ME-04-01-32^T and the other strain ME-04-04-52^T. Levels of 16S rRNA gene sequence similarity between these strains and the type strains of recognized *Deinococcus* species were less than 95 % and as low as 87.9 %, indicating that they do not belong to any of the established phylogenetic clusters within the genus. The novel strains were psychrophilic, as with *D. frigens*, *D. marmoris* and *D. saxicola*, but our

Table 2. D₁₀ values for the strains isolated in the present study as compared with *D. radiodurans* after exposure to gamma and UV radiation and prolonged conditions of desiccation

Strains: 1, *D. radiodurans* R1^T; 2, PO-04-19-125^T; 3, PO-04-20-132^T; 4, ME-04-01-32^T; 5, ME-04-04-52^T.

	1	2	3	4	5
Gamma (kGy)	11.5	3.6	2.2	3.8	4.0
UV (J m ^{–2})	870	310	220	550	690
Desiccation (days)	>42	11.7	8.7	2	1.8

phylogenetic analyses showed that these strains and species shared no close relationship. The new isolates thus represent two new lineages of psychrophiles within the *Deinococcus* lineage. The novel strains can be clearly differentiated from other *Deinococcus* species on the basis of their fatty acid profiles. Strains PO-04-20-132^T, PO-04-20-144, PO-04-19-125^T and ME-04-01-32^T have high levels of 16:1 ω 7c, 17:0 iso and 15:1 ω 6c fatty acids whereas strain ME-04-04-52^T has a unique fatty acid profile containing high levels of 17:0 cyclo (Supplementary Table S1).

Strains PO-04-20-132^T, PO-04-19-125^T and ME-04-01-32^T cluster together, with 16S rRNA gene sequence similarities in the range 96.6–97.3%. Although these values could be considered to be relatively high for comparisons between the majority of species of the genus *Deinococcus*, the novel strains can be differentiated on the basis of phenotypic characteristics, including cell morphology (rods for strains PO-04-20-132^T and ME-04-01-32^T, short-rods/cocci for strain PO-04-19-125^T) and differences in substrate utilization patterns (Table 1). Strain ME-04-04-52^T is not only phylogenetically distinct from the other new isolates but has a unique fatty acid profile containing high levels of 17:0 cyclo. In addition, it can be distinguished from the other isolates by its limited substrate utilization pattern and coccoid cell morphology.

On the basis of these phylogenetic, phenotypic and chemotaxonomic properties, we suggest that strains PO-04-20-132^T, PO-04-19-125^T, ME-04-01-32^T and ME-04-04-52^T represent four novel psychrophilic, ionizing radiation-sensitive species of the genus *Deinococcus*, for which the names *Deinococcus radiomollis* sp. nov., *Deinococcus claudionis* sp. nov., *Deinococcus altitudinis* sp. nov. and *Deinococcus alpinitundrae* sp. nov., respectively, are proposed.

Description of *Deinococcus radiomollis* sp. nov. Callegan, da Costa and Rainey

Deinococcus radiomollis (ra.di'o.mol'lis. L. n. *radius* a ray; L. adj. *mollis* soft, susceptible; N.L. masc adj. *radiomollis* susceptible to radiation).

Forms rod-shaped cells 2.4–2.7 μ m long and 1.2–1.3 μ m wide. Gram-positive. Cells are non-motile; spores are not observed. Colonies on 1/10-strength PCA medium are pink-pigmented. Growth occurs between 5 and 25 °C but not at 2 or 30 °C. Optimum growth temperature is 10 °C. Grows between pH 5 and 10.0. Optimum pH for growth is 6.0–9.0. Cytochrome oxidase-negative and catalase-positive. The fatty acid profile is dominated by 16:1 ω 7c, 17:0 iso and 15:1 ω 6c. Utilizes L-arabinose, cellobiose, galactose, D-glucose, fructose, mannose, L-rhamnose, sucrose, trehalose, xylose, glucosamine and glycerol. The following substrates are not utilized: melibiose, lactose, raffinose, ribose, sorbose, acetate, citrate, glutamate, α -ketoglutarate, lactate, malate, aspartate, cysteine, alanine, asparagine, glutamine, glycine, lysine, methionine, ornithine and

proline. Starch, casein and gelatin are not degraded. The DNA G+C content of the type strain is 63.2 mol%. Sensitive to gamma and UV radiation and desiccation as compared with other species of the genus.

The type strain, PO-04-20-132^T (=LMG 24019^T=NRRL B-41950^T), was isolated from an alpine soil at 5065 m on Pico de Orizaba, Mexico. Strain PO-04-20-144 (=LMG 24018=NRRL B-41951) has been deposited as a reference strain of this species.

Description of *Deinococcus claudionis* sp. nov. Callegan, da Costa and Rainey

Deinococcus claudionis (clau.di.o'nis. N.L. gen. n. *claudionis* of Claudio, named after Gerardo Claudio Sanchez, a Mexican conservationist).

Forms spherical or short rod-shaped cells 2.3 μ m long and 1.5 μ m wide. Gram-positive. Cells are non-motile; spores are not observed. Colonies on 1/10-strength PCA medium are pink-pigmented. Growth occurs between 5 and 25 °C but not at 2 or 30 °C. Optimum growth temperature is 10 °C. Grows between pH 5 and 10.0. Optimum pH for growth is 6.0–9.0. Cytochrome oxidase-negative and catalase-positive. The fatty acid profile is dominated by 16:1 ω 7c, 17:0 iso and 15:1 ω 6c. Utilizes L-arabinose, cellobiose, fructose, D-glucose, mannose, L-rhamnose, sucrose, trehalose, xylose, glycerol and glucosamine. The following substrates are not utilized: galactose, melibiose, lactose, raffinose, ribose, sorbose, acetate, citrate, glutamate, α -ketoglutarate, lactate, malate, aspartate, asparagine, cysteine, alanine, glutamine, glycine, lysine, methionine, ornithine and proline. Starch, casein and gelatin are not degraded. The DNA G+C content of the type strain is 63.1 mol%. Sensitive to gamma and UV radiation and desiccation as compared with other species of the genus.

The type strain, PO-04-19-125^T (=LMG 24282^T=NRRL B-41949^T), was isolated from an alpine soil at 5002 m on Pico de Orizaba, Mexico.

Description of *Deinococcus altitudinis* sp. nov. Callegan, da Costa and Rainey

Deinococcus altitudinis (al.ti.tu'di.nis. L. n. *altitudo* altitude; L. gen. n. *altitudinis* of altitude).

Forms rod-shaped cells 2.9 μ m long and 1.5 μ m wide. Gram-positive. Cells are non-motile; spores are not observed. Colonies on 1/10-strength PCA medium are pink-pigmented. Growth occurs between 5 and 25 °C but not at 2 or 30 °C. Optimum growth temperature is 10 °C. Grows between pH 5 and 9.0. Optimum pH for growth is 6.0–8.0. Cytochrome oxidase-negative and catalase-positive. The fatty acid profile is dominated by 16:1 ω 7c, 17:0 iso and 15:1 ω 6c. Utilizes L-arabinose, cellobiose, galactose, trehalose, glycerol and ribose. The following substrates are not utilized: D-glucose, fructose, L-rhamnose, mannose, melibiose, lactose, raffinose, sorbose, sucrose, xylose,

acetate, citrate, α -ketoglutarate, lactate, malate, aspartate, cysteine, glutamate, alanine, asparagine, glutamine, glucosamine, glycine, lysine, methionine, ornithine and proline. Starch, casein and gelatin are not degraded. The DNA G+C content of the type strain is 65.9 mol%. Sensitive to gamma and UV radiation and desiccation as compared with other species of the genus.

The type strain, ME-04-01-32^T (=LMG 24022^T=NRRL B-41947^T), was isolated from an alpine soil at 4003 m on Mount Evans, CO, USA.

Description of *Deinococcus alpinitundrae* sp. nov. Callegan, da Costa and Rainey

Deinococcus alpinitundrae (al.pi.ni.tun'dra.e. L. adj. *alpinus* alpine; N.L. n. *tundra* the tundra; N.L. gen. n. *alpinitundrae* of the alpine tundra, of the treeless region above the treeline of high mountains).

Forms spherical cells ~2.4 μ m in diameter. Gram-positive. Cells are non-motile; spores are not observed. Colonies on 1/10-strength PCA medium are pink-pigmented. Growth occurs between 5 and 25 °C but not at 2 or 20 °C. Optimum growth temperature is 10 °C. Grows between pH 5 and 9.0. Optimum pH for growth is 6.0–9.0. Cytochrome oxidase-negative and catalase-positive. The fatty acid profile is dominated by 16:1 ω 7c, 17:0 cyclo and 16:0. Utilizes L-arabinose, cellobiose, trehalose and glycerol. The following substrates are not utilized: D-glucose, fructose, galactose, L-rhamnose, melibiose, lactose, mannose, raffinose, ribose, sorbose, sucrose, xylose, acetate, citrate, α -ketoglutarate, lactate, malate, aspartate, cysteine, alanine, glutamine, glycine, lysine, methionine, glucosamine, glutamate, asparagine, ornithine and proline. Starch, casein and gelatin are not degraded. The DNA G+C content of the type strain is 62.6 mol%. Sensitive to gamma and UV radiation and desiccation as compared with other species of the genus.

The type strain, ME-04-04-52^T (=LMG 24283^T=NRRL B-41948^T), was isolated from an alpine soil sample at 3596 m on Mount Evans, CO, USA.

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