Complete genome sequence of *Deinococcus maricopensis* type strain (LB-34)

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*Deinococcus maricopensis* (Rainey and da Costa 2005) is a member of the genus *Deinococcus*, which is comprised of 44 validly named species and is located within the deeply branching bacterial phylum *Deinococcus–Thermus*. Strain LB-34 was isolated from a soil sample from the Sonoran Desert in Arizona. Various species of the genus *Deinococcus* are characterized by extreme radiation resistance, with *D. maricopensis* being resistant in excess of 10 kGy. Even though the genomes of three *Deinococcus* species, *D. radiodurans*, *D. geothermalis* and *D. deserti*, have already been published, no special physiological characteristic is currently known that is unique to this group. It is therefore of special interest to analyze the genomes of additional species of the genus *Deinococcus* to better understand how these species adapted to gamma- or UV ionizing-radiation. The 3,498,530 bp long genome of *D. maricopensis* with its 3,301 protein-coding and 66 RNA genes consists of one circular chromosome and is a part of the Genomic Encyclopedia of Bacteria and Archaea project.

**Introduction**

Strain LB-34 (= DSM 21211 = NRRL B-23946 = LMG 22137) is the type strain of *Deinococcus maricopensis* [1]. In addition to the type strain LB-34, two more strains of this species, KR 1 and KR 23, were characterized by Rainey et al. [1]. The generic name derives from the Greek words ‘dienos’ meaning ‘strange or unusual’ and ‘coccus’ meaning ‘a grain or berry’ [2]. The species epithet is derived from the Neo-Latin word ‘maricopensis’ referring to the Maricopa Nation, a native tribe in Arizona [1]. Strain LB 34 was isolated from desert soil in Arizona and described by Rainey et al. in 2005 [1]. The genus *Deinococcus* was proposed in 1981 by Brooks and Murray [2] to separate the distinct radiation-resistant species from the genus *Micrococcus* in which those species
Deinococcus maricopensis type strain (LB-34T)

were originally classified. With the description of Deinobacter grandis by Oyaizu et al. [3], a second genus was placed to the family Deinococcaceae, and in 1997 Rainey et al. proposed to transfer Deinobacter to the genus Deinococcus, based on investigations of the phylogenetic diversity of the Deinococci as determined by 16S rRNA gene sequence analysis. In conclusion, an emended description of the genus Deinococcus was published, showing that the cells can be spherical or rod-shaped [4]. Members of the genus Deinococcus were isolated from various environmental habitats including air [5-7], arid soil [1,8-12], water and activated sludge [13-15], alpine environments [16], rhizosphere [17], Antarctica [18], hot springs [19], aquifer [20], marine fish [21] and radioactive sites [22]. Here we present a summary classification and a set of features for D. maricopensis LB-34T, together with the description of the complete genomic sequencing and annotation.

Classification and features
A representative genomic 16S rRNA sequence of strain LB-34T was compared using NCBI BLAST under default settings (e.g., considering only the high-scoring segment pairs (HSPs) from the best 250 hits) with the most recent release of the Greengenes database [23] and the relative frequencies, weighted by BLAST scores, of taxa and keywords (reduced to their stem [24]) were determined. The single most frequent genus was Deinococcus (100.0%) (114 hits in total). Regarding the three hits to sequences from members of the species, the average identity within HSPs was 99.9%, whereas the average coverage by HSPs was 97.6%. Regarding the 77 hits to sequences from other members of the genus, the average identity within HSPs was 91.5%, whereas the average coverage by HSPs was 60.5%. Among all other species, the one yielding the highest score was D. radiodurans, which corresponded to an identity of 91.2% and an HSP coverage of 88.0%. The highest-scoring environmental sequence was AY905380 (‘Extensive ionizing-radiation-resistant recovered sonoran and description nine new species genus Deinococcus obtained single mixed agricultural/open desert soil clone L14-471’), which showed an identity of 98.1% and a HSP coverage of 70.2%. The five most frequent keywords within the labels of environmental samples which yielded hits were ‘skin’ (7.7%), ‘litholog/stream’ (2.8%), ‘fossa’ (2.4%), ‘microbi’ (2.4%) and ‘forearm’ (2.1%) (136 hits in total).

Environmental samples which yielded hits of a higher score than the highest scoring species were not found.

Figure 1 shows the phylogenetic neighborhood of D. maricopensis LB-34T in a 16S rRNA based tree. The sequences of the four identical 16S rRNA gene copies in the genome differ by one nucleotide from the previously published 16S rRNA sequence (AY743274).

The cells of D. maricopensis are rod-shaped, up to 6 µm in length and 2.0 µm wide (Figure 2). D. maricopensis is a Gram-positive, non-sporo-forming bacterium (Table 1). Colonies on Rich medium are orange to pink. The cells are non-motile. The organism is chemooorganotrophic [1]. The temperature range for growth is 10°C to 45°C, with an optimum at 40°C [1]. Cytochrome oxidase and catalase activity have been observed [1]. Strains may utilize L-arabinose, cellobiose, galactose, glucose, mannose, maltose, sucrose, trehalose, glucosamine, glycerol, malate, asparagine, aspartate, glucosamine, alanine, ornithine and proline. Fructose can be used by strain KR23, but not by strain LB-34T [1]. Strain LB-34T showed similar levels of desiccation tolerance of up to four weeks as compared to D. radiodurans strain R1T. Strain LB-34T is resistant to > 10kGy, but more sensitive to ionizing radiation than strain D. radiodurans R1T [1].

Chemotaxonomy
The major cellular fatty acids of the strain LB-34T were identified as iso-C15:0, iso-C17:0 and C16:0. Menaquinone 8 (MK-8) was determined as the major respiratory quinone of the strain. Phosphoglycolipid and glycolipid pattern are similar to those of other Deinococcus species [1]. No data are available for strain LB-34T showing the peptidoglycan type of the cell wall.

Genome sequencing and annotation
Genome project history
This organism was selected for sequencing on the basis of its phylogenetic position [45], and is part of the Genomic Encyclopedia of Bacteria and Archaea project [46]. The genome project is deposited in the Genomes On Line Database [29] and the complete genome sequence is deposited in GenBank. Sequencing, finishing and annotation were performed by the DOE Joint Genome Institute (JGI). A summary of the project information is shown in Table 2.
Figure 1. Phylogenetic tree highlighting the position of *D. maricopensis* relative to the other type strains within the family *Deinococcaceae*. The tree was inferred from 1,382 aligned characters [25,26] of the 16S rRNA gene sequence under the maximum likelihood criterion [27] and rooted in accordance with the current taxonomy. The branches are scaled in terms of the expected number of substitutions per site. Numbers above branches are support values from 1,000 bootstrap replicates [28] if larger than 60%. Lineages with type strain genome sequencing projects registered in GOLD [29] are shown in blue, and published genomes in bold [30-34]. The genome of *D. radiodurans* published by White *et al.* in 1999 [35] later turned out not to be from the type strain [36].
**Deinococcus maricopensis** type strain (LB-34T)

![Scanning electron micrograph of D. maricopensis LB-34T](image)

**Figure 2.** Scanning electron micrograph of *D. maricopensis* LB-34T

**Table 1.** Classification and general features of *D. maricopensis* LB-34T according to the MIGS recommendations [37].

| MIGS ID | Property                  | Term                              | Evidence code |
|---------|---------------------------|-----------------------------------|---------------|
|         | Current classification    | Domain *Bacteria*                 | TAS [38]      |
|         |                           | Phylum *Deinococcus-Thermus*      | TAS [39]      |
|         |                           | Class *Deinococci*                | TAS [40,41]   |
|         |                           | Order *Deinococcales*             | TAS [4]       |
|         |                           | Family *Deinococcaceae*           | TAS [2,4]     |
|         |                           | Genus *Deinococcus*               | TAS [2,4]     |
|         |                           | Species *Deinococcus maricopensis*| TAS [1,42]    |
|         |                           | Type strain LB-34                 | TAS [1]       |
|         | Gram stain                | positive                          | TAS [1]       |
|         | Cell shape                | rods                              | TAS [1]       |
|         | Motility                  | non-motile                        | TAS [1]       |
|         | Sporulation               | none                              | TAS [1]       |
|         | Temperature range          | mesophile, 10°C–45°C              | TAS [1]       |
|         | Optimum temperature       | 40°C                              | TAS [1]       |
|         | Salinity                  | not reported                       |               |
| MIGS-22 | Oxygen requirement         | aerobic                           | TAS [1]       |
|         | Carbon source             | carbohydrates                     | TAS [1]       |
|         | Energy metabolism         | chemoorganotroph                  | TAS [1,2]     |
| MIGS-6  | Habitat                   | soil                              | TAS [1]       |
| MIGS-15 | Biotic relationship       | free-living                       | NAS           |
| MIGS-14 | Pathogenicity             | none                              | NAS           |
|         | Biosafety level           | 1                                 | TAS [43]      |
|         | Isolation                 | soil                              | TAS [1]       |
| MIGS-4  | Geographic location       | Sonoran Desert, Arizona, USA      | TAS [1]       |
| MIGS-5  | Sample collection time    | 1999                              | NAS           |
| MIGS-4.1| Latitude                  | 32.93                             | NAS           |
| MIGS-4.2| Longitude                 | -112.30                           | NAS           |
| MIGS-4.3| Depth                     | not reported                       |               |
| MIGS-4.4| Altitude                  | not reported                       |               |

Evidence codes - IDA: Inferred from Direct Assay (first time in publication); TAS: Traceable Author Statement (i.e., a direct report exists in the literature); NAS: Non-traceable Author Statement (i.e., not directly observed for the living, isolated sample, but based on a generally accepted property for the species, or anecdotal evidence). These evidence codes are from of the Gene Ontology project [44]. If the evidence code is IDA, then the property was directly observed by one of the authors or an expert mentioned in the acknowledgements.
Table 2. Genome sequencing project information

| MIGS ID | Property                  | Term                                                                 |
|---------|---------------------------|----------------------------------------------------------------------|
| MIGS-31 | Finishing quality         | Finished                                                             |
| MIGS-28 | Libraries used            | Three genomic libraries: one 454 pyrosequence standard library, one 454 PE library (7 kb insert size), one Illumina library |
| MIGS-29 | Sequencing platforms      | Illumina GAii, 454 GS FLX Titanium                                   |
| MIGS-31.2| Sequencing coverage      | 170.9 × Illumina; 75.4 × pyrosequence                                |
| MIGS-30 | Assemblers                | Newbler version 2.3-PreRelease-10-21-2009-gcc-4.1.2-threads, Velvet version 0.7.63, phrap |
| MIGS-32 | Gene calling method       | Prodigal 1.4, GenePRIMP                                              |
|         | INSDC ID                  | CP002454                                                             |
|         | Genbank Date of Release   | January 20, 2011                                                     |
|         | GOLD ID                   | Gc01597                                                              |
|         | NCBI project ID           | 43461                                                                |
|         | Database: IMG-GEBA        | 2503982045                                                           |
| MIGS-13 | Source material identifier| DSM 21211                                                            |
|         | Project relevance         | Tree of Life, GEBA                                                   |

Growth conditions and DNA isolation

*D. maricopensis* LB-34T, DSM 21211, was grown in DSMZ medium 736 (Rich Medium) [47] at 28°C. DNA was isolated from 0.5-1 g of cell paste using MasterPure Gram-positive DNA purification kit (Epigenet MGP04100) following the standard protocol as recommended by the manufacturer, with a modification in cell lysis by adding 20 μl lysozyme (100 mg/μl), and 10 μl mutanolysine, achromopeptidase and lysostphine, each, for 40 min at 37°C, followed by one hour incubation on ice after the MPC step. DNA is available through the DNA Bank Network [48,49].

Genome sequencing and assembly

The genome was sequenced using a combination of Illumina and 454 sequencing platforms. All general aspects of library construction and sequencing can be found at the JGI website [50]. Pyrosequencing reads were assembled using the Newbler assembler version 2.3 (Roche). The initial Newbler assembly consisting of 58 contigs in two scaffolds was converted into a phrap assembly by [51] making fake reads from the consensus, to collect the read pairs in the 454 paired end library. Illumina GAii sequencing data (957.8 Mb) were assembled with Velvet version 0.7.63 [52] and the consensus sequences were shredded into 1.5 kb overlapped fake reads and assembled together with the 454 data. The 454 draft assembly was based on 234.5 Mb 454 draft data and all of the 454 paired end data. Newbler parameters are -consed -a 50 -l 350 -g -m -ml 20. The Phred/Phrap/Consed software package [51] was used for sequence assembly and quality assessment in the subsequent finishing process. After the shotgun stage, reads were assembled with parallel phrap (High Performance Software, LLC). Possible mis-assemblies were corrected with gap-Resolution [50], Dupfinisher [53], or sequencing cloned bridging PCR fragments with subcloning or transposon bombing (Epigenet Biotechnologies, Madison, WI). Gaps between contigs were closed by editing in Consed, by PCR and by Bubble PCR primer walks (J.-F.Chang, unpublished). A total of 255 additional reactions were necessary to close gaps and to raise the quality of the finished sequence. Illumina reads were also used to correct potential base errors and increase consensus quality using a software Polisher developed at JGI [54]. The error rate of the completed genome sequence is less than 1 in 100,000. Together, the combination of the Illumina and 454 sequencing platforms provided 246.3 × coverage of the genome. The final assembly contained 872,337 pyrosequence and 16,604,657 Illumina reads.

Genome annotation

Genes were identified using Prodigal [55] as part of the Oak Ridge National Laboratory genome annotation pipeline, followed by a round of manual curation using the JGI GenePRIMP pipeline [56]. The predicted CDSs were translated and used to search the National Center for Biotechnology Information (NCBI) nonredundant database, UniProt, TIGR-Fam, Pfam, PRIAM, KEGG, COG, and InterPro databases. Additional gene prediction analysis and functional annotation was performed.
Deinococcus maricopensis type strain (LB-34T) within the Integrated Microbial Genomes - Expert Review (IMG-ER) platform [57].

**Genome properties**

The genome consists of a 3,498,530 bp long chromosome with a G+C content of 69.8% (Figure 3 and Table 3). Of the 3,367 genes predicted, 3,301 were protein-coding genes, and 66 RNAs; 37 pseudogenes were also identified. The majority of the protein-coding genes (70.3%) were assigned with a putative function while the remaining ones were annotated as hypothetical proteins. The distribution of genes into COGs functional categories is presented in Table 4.

![Graphical circular map of the chromosome. From outside to the center: Genes on forward strand (color by COG categories), Genes on reverse strand (color by COG categories), RNA genes (tRNAs green, rRNAs red, other RNAs black), GC content, GC skew.](image)

**Figure 3.** Graphical circular map of the chromosome. From outside to the center: Genes on forward strand (color by COG categories), Genes on reverse strand (color by COG categories), RNA genes (tRNAs green, rRNAs red, other RNAs black), GC content, GC skew.
Table 3. Genome Statistics

| Attribute                              | Value       | % of Total |
|----------------------------------------|-------------|------------|
| Genome size (bp)                       | 3,498,530   | 100.00%    |
| DNA coding region (bp)                 | 3,127,041   | 89.38%     |
| DNA G+C content (bp)                   | 2,442,849   | 69.83%     |
| Number of replicons                    | 1           |            |
| Extrachromosomal elements              | 0           |            |
| Total genes                            | 3,367       | 100.00%    |
| RNA genes                              | 66          | 1.96%      |
| rRNA operons                           | 4           |            |
| Protein-coding genes                   | 3,301       | 98.04%     |
| Pseudo genes                           | 37          | 1.10%      |
| Genes with function prediction         | 2,366       | 70.27%     |
| Genes in paralog clusters              | 368         | 10.93%     |
| Genes assigned to COGs                 | 2,412       | 71.64%     |
| Genes assigned Pfam domains            | 2,495       | 74.10%     |
| Genes with signal peptides             | 1,005       | 29.85%     |
| Genes with transmembrane helices       | 662         | 19.66%     |
| CRISPR repeats                         | 0           |            |

Table 4. Number of genes associated with the general COG functional categories

| Code | value | %age       | Description                                                                 |
|------|-------|------------|------------------------------------------------------------------------------|
| J    | 160   | 6.0%       | Translation, ribosomal structure and biogenesis                             |
| A    | 0     | 0.0%       | RNA processing and modification                                              |
| K    | 188   | 7.1%       | Transcription                                                                |
| L    | 109   | 4.1%       | Replication, recombination and repair                                        |
| B    | 2     | 0.1%       | Chromatin structure and dynamics                                             |
| D    | 29    | 1.1%       | Cell cycle control, cell division, chromosome partitioning                   |
| Y    | 0     | 0.0%       | Nuclear structure                                                            |
| V    | 45    | 1.7%       | Defense mechanisms                                                           |
| T    | 195   | 7.3%       | Signal transduction mechanisms                                               |
| M    | 137   | 5.2%       | Cell wall/membrane/envelope biogenesis                                      |
| N    | 15    | 0.6%       | Cell motility                                                                |
| Z    | 1     | 0.0%       | Cytoskeleton                                                                 |
| W    | 0     | 0.0%       | Extracellular structures                                                     |
| U    | 43    | 1.6%       | Intracellular trafficking, secretion, and vesicular transport                |
| O    | 113   | 4.3%       | Posttranslational modification, protein turnover, chaperones                 |
| C    | 125   | 4.7%       | Energy production and conversion                                             |
| G    | 205   | 7.7%       | Carbohydrate transport and metabolism                                         |
| E    | 237   | 8.9%       | Amino acid transport and metabolism                                          |
| F    | 77    | 2.9%       | Nucleotide transport and metabolism                                          |
| H    | 119   | 4.5%       | Coenzyme transport and metabolism                                           |
| I    | 105   | 4.0%       | Lipid transport and metabolism                                               |
| P    | 121   | 4.6%       | Inorganic ion transport and metabolism                                       |
| Q    | 60    | 2.3%       | Secondary metabolites biosynthesis, transport and catabolism                 |
| R    | 334   | 12.6%      | General function prediction only                                             |
| S    | 238   | 9.0%       | Function unknown                                                             |
| -    | 955   | 28.4%      | Not in COGs                                                                  |
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