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Phylogenetic Diversity of the Deinococci as Determined by 16S Ribosomal DNA Sequence Comparison

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16S ribosomal DNA (rDNA) sequences were determined for the five species of the genus *Deinococcus* (*Deinococcus erythromyxa*, *Deinococcus proteolyticus*, *Deinococcus radiodurans*, *Deinococcus radiophilus*, and *Deinococcus radiopugnans*) and the single species of the genus *Deinobacter* (*Deinobacter grandis*). With the exception of *Deinococcus erythromyxa*, the deinococci form a coherent phylogenetic cluster which is related to the *Thermus-Meiothermus* lineage. An analysis of the 16S rDNA sequence of *Deinococcus erythromyxa* revealed that this organism is an actinomycete and a member of the genus *Kocuria*. *Deinobacter grandis* falls within the radiation of the genus *Deinococcus* and phylogenetically can be considered a member of this genus. The results of the phylogenetic analyses are consistent with chemotaxonomic data. On the basis of our data, *Deinobacter grandis* is transferred to the genus *Deinococcus* as *Deinococcus grandis* comb. nov., the description of the genus *Deinococcus* is emended accordingly, and *Deinococcus erythromyxa* is transferred to the genus *Kocuria* as *Kocuria erythromyxa* comb. nov. The description of the family *Deinococcaceae* is emended to include organisms with rod-shaped cells, and a set of 16S rDNA signature nucleotides is designated for this group. On the basis of the distinct phylogenetic position of the *Deinococcus* lineage and a set of 16S rDNA signature nucleotides, the order *Deinococcales* ord. nov. is described.

The extreme radiation resistance (1), unusual cell wall structure (21, 22, 27, 34), and lack of phylogenetic relationship to the gram-positive bacteria (25) of "*Micrococcus radiodurans*" were the stimuli for a polyphasic study of 55 strains of red-pigmented micrococci performed by Brooks et al. (3). The results obtained provided the basis for the subsequent description of the genus *Deinococcus* (2). The genus *Deinococcus* was described for "*Micrococcus radiodurans*" and other radiation-resistant cocci (2), and the following five species were placed in the new genus: *Deinococcus erythromyxa*, *Deinococcus proteolyticus*, *Deinococcus radiodurans*, *Deinococcus radiophilus*, and *Deinococcus radiopugnans* (2). The genus *Deinococcus* was differentiated from the genus *Micrococcus* and other genera of gram-positive cocci on the basis of its layered cell wall, the presence of L-ornithine in its peptidoglycan, its lack of teichoic acids, the presence of palmitoleate as its predominant fatty acid, and its resistance to gamma and UV radiation (2). The family *Deinococcaceae* was also described with the genus *Deinococcus* as the type genus (2).

On the basis of phylogenetic and taxonomic data, another genus, the genus *Deinobacter*, was described and added to the family *Deinococcaceae* (16). Although the chemotaxonomic and phylogenetic data indicated that *Deinobacter grandis* and the members of the genus *Deinococcus* are closely related, a new genus was created on the basis of the rod-shaped morphology of the new isolate (16).

16S rRNA catalog comparisons have revealed that the deinococci represent a distinct phylogenetic lineage of eubacteria (32, 33). Woese et al. (33) defined the radioresistant micrococci (i.e., *Deinococcus* species) as representatives of a distinct phylum. The phylogenetic relatedness of the genera *Deinococcus* and *Thermus* was subsequently demonstrated by comparing 16S rRNA catalogs (10), and the significance of this relationship was further investigated by analyzing nearly complete 16S rRNA sequences (29). At this time, only one complete 16S rRNA sequence has been determined for the deinococci (29). This sequence is derived from *Deinococcus radiodurans* UWO 298, which is not the type strain of the species, and has recently been used to design oligonucleotide probes which reportedly are strain, species, and genus specific (31). In order to determine the degree of phylogenetic diversity within and between the genera *Deinococcus* and *Deinobacter*, the 16S ribosomal DNA (rDNA) sequences of the type strains of the six species of these genera were determined. The results of our analyses of these sequences and our resulting taxonomic conclusions are presented below.

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MATERIALS AND METHODS

Strains investigated. The strains used in this study were obtained from DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany, or the American Type Culture Collection, Rockville, Md. The following strains were used: *Deinobacter grandis* DSM 3963^T (T = type strain), *Deinococcus erythromyxa* ATCC 187^T, *Deinococcus proteolyticus* DSM 20540^T, *Deinococcus radiodurans* DSM 20539^T, *Deinococcus radiophilus* DSM 20551^T, *Deinococcus radiopugnans* ATCC 19172^T, and *Kocuria rosea* DSM 20447^T.

16S rDNA sequence determination and phylogenetic analyses. Genomic DNA extraction, PCR amplification of the 16S rDNA, and sequencing of the PCR products were carried out as described previously (18). Sequence reaction products were electrophoresed with a model 373A automated sequencer (Applied Biosystems, Foster City, Calif.). The 16S rDNA sequences obtained in this study were manually aligned with the sequences of representatives of the main bacterial lines of descent by using the ae2 editor (14). Since some of the reference sequences obtained from the databases were not complete sequences and consisted of less than 1,400 nucleotides, two data sets were produced. One data set comprising the sequences generated in this study and the reference sequences contained information for 1,092 unambiguous nucleotide positions present in all sequences between positions 44 and 1365 (*Escherichia coli* numbering [4]). The second data set, containing sequence information for 1,459 unambiguous nucleotide positions present in the sequences between positions 28 and 1526 (*E. coli* numbering [4]), was produced by using the 16S rDNA sequences of only *Deinobacter grandis*, *Deinococcus proteolyticus*, *Deinococcus radiodurans*, *Deinococcus radiophilus*, and *Deinococcus radiopugnans*. Evolutionary distances were

TABLE 1. Levels of 16S rDNA/rRNA sequence similarity between the deinococci and their closest relatives

Species	% Sequence similarity					
	Deinobacter grandis	*Deinococcus proteolyticus*	*Deinococcus radiodurans*	*Deinococcus radiophilus*	*Deinococcus radiopugnans*	*Deinococcus erythromyxa*
Deinococcus proteolyticus	91.5					
Deinococcus radiodurans	93.7	91.1				
Deinococcus radiophilus	90.8	94.9	90.9			
Deinococcus radiopugnans	93.2	90.1	90.9	89.7		
Deinococcus erythromyxa	76.7	77.9	76.8	77.8	76.5	
Thermus thermophilus	78.3	79.0	78.1	79.2	78.5	77.8
Thermus aquaticus	78.1	79.1	77.6	79.3	78.2	77.4
Meiothermus ruber	78.9	79.1	78.3	78.6	77.8	76.2
Meiothermus chliarophilus	77.8	77.8	77.5	77.8	78.1	75.7
Arthrobacter globiformis	77.3	78.4	77.5	78.1	76.3	96.1
Micrococcus luteus	77.5	78.7	77.9	78.1	76.5	95.4
Kocuria rosea	76.6	77.9	76.6	77.7	76.3	99.9

calculated by the method of Jukes and Cantor (12). Phylogenetic dendrograms were generated by using treeing algorithms contained in the PHYLIP package (8). Tree topologies were evaluated by performing bootstrap analyses (7) of the neighbor-joining data, using 1,000 resamplings.

Chemotaxonomic determinations. The cell wall peptidoglycan, menaquinone, and fatty acid compositions of *Deinococcus erythromyxa* were determined as described previously (9).

Nucleotide sequence accession numbers. The 16S rDNA sequences obtained in this study have been deposited in the EMBL database under the following accession numbers: *Deinobacter grandis* DSM 3963^T, Y11329; *Deinococcus erythromyxa* ATCC 187^T, Y11330; *Deinococcus proteolyticus* DSM 20540^T, Y11331; *Deinococcus radiodurans* DSM 20539^T, Y11332; *Deinococcus radiophilus* DSM 20551^T, Y11333; and *Deinococcus radiopugnans* ATCC 19172^T, Y11334. The accession numbers for the sequences of the organisms used as representatives of main bacterial groups are as follows: *Agrobacterium tumefaciens* IFO 13257, D01257; *Arthrobacter globiformis* DSM 20124^T, M23411; *Bacillus subtilis* NCDO 1769, X60646; *Bacteroides fragilis* ATCC 25285^T, M61006; *Brevibacterium linens* DSM 20425^T, X77451; *Burkholderia cepacia* ATCC 25416^T, M22467; *Chlamydia psittaci* ATCC VR125, M13769; *Chlorobium limicola*, M31769; *Chloroflexus aurantiacus* ATCC 29366^T, M34116; *Clostridium butyricum* ATCC 19398^T, M59085; *Coprothermobacter proteolyticus* ATCC 35245^T, X69335; *Escherichia coli*, J01695; *Fervidobacterium nodosum* ATCC 35602^T, M59177; *Kocuria rosea* DSM 20447^T, X87756; *Meiothermus chliarophilus* DSM 9957^T, X84212; *Meiothermus ruber* ATCC 35948^T, L09672; *Microbacterium lacticum* DSM 20427^T, X77441; *Micrococcus luteus* ATCC 381, M38242; *Nesterenkonia halobia* DSM 20541^T, X80747; *Spirochaeta aurantia* ATCC 25082^T, M57740; *Streptomyces griseus* NCTC 9080, X61478; *Thermomicrobium roseum* ATCC 27502^T, M34115; *Thermotoga maritima* DSM 3109^T, M21774; *Thermus aquaticus* ATCC 25104^T, L09663; *Thermus thermophilus* ATCC 27634^T, M26923; and *Verrucomicrobium spinosum* DSM 4136^T, X90515.

RESULTS AND DISCUSSION

16S rDNA and rRNA sequence analyses. Almost complete 16S rDNA sequences (>95% of the *E. coli* sequence [4]) were determined for the type strains of the five species of the genus *Deinococcus* (*Deinococcus erythromyxa*, *Deinococcus proteolyticus*, *Deinococcus radiodurans*, *Deinococcus radiophilus*, and *Deinococcus radiopugnans*) and the only species of the genus *Deinobacter* (*Deinobacter grandis*). The 16S rDNA sequences determined in this study ranged from 1,459 to 1,479 nucleotides long. The phylogenetic position of the deinococci in relation to representatives of the main lineages in the domain *Bacteria* is shown in Fig. 1, a phylogenetic dendrogram that was constructed by using the neighbor-joining method (19). With the exception of *Deinococcus erythromyxa*, the *Deinococcus* species and *Deinobacter grandis* cluster together. *Deinococcus erythromyxa* was found to group within the actinomycete lineage, close to *K. rosea* (formerly *Micrococcus roseus* [23]). The highest level of 16S rDNA sequence similarity found for the *Deinococcus erythromyxa* sequence was the level of similarity to *K. rosea* (99.9%), and the levels of similarity to *Kocuria kristinae* and *Kocuria varians* were 95.7 and 96.6%, respectively. The levels of 16S rDNA or rRNA similarity between the other *Deinococcus* species and *Deinobacter grandis* ranged from 89.7 to 93.7% (Table 1). The 16S rDNA sequence of *Deinobacter grandis* exhibited 90.8 to 93.7% similarity to the sequences of *Deinococcus* species. The levels of 16S rDNA sequence similarity between members of the *Deinococcus-Deinobacter* group and their phylogenetic neighbors in the *Thermus-Meiothermus* group ranged from 77.5 to 79.3% (Table 1), while the levels of sequence similarity to the other taxa included in Fig. 1 were 72.1 to 78.7% (data not shown). Thus, the results of our comparative phylogenetic analyses of the 16S rDNA sequences of members of the genera *Deinococcus* and *Deinobacter* showed that *Deinobacter grandis* and all *Deinococcus* species except *Deinococcus erythromyxa* form a distinct phylogenetic lineage within the domain *Bacteria*. A distinct relationship between the *Deinococcus-Deinobacter* lineage and the *Thermus-Meiothermus* lineage was observed, and this relationship was supported by the results of bootstrap analyses (Fig. 1).

The *Deinococcus-Deinobacter* group comprises two lineages, both of which are supported by bootstrap values of 100%. One lineage contains *Deinococcus proteolyticus* and *Deinococcus radiophilus*, while the other consists of *Deinococcus radiodurans*, *Deinococcus radiopugnans*, and *Deinobacter grandis*. The relationship of *Deinobacter grandis* and *Deinococcus radiopugnans* is unstable, as indicated by the recovery of this species pair in only 58% of the phylogenetic analyses. Although *Deinobacter grandis* clusters with *Deinococcus radiopugnans* in Fig. 1, it exhibits a slightly higher 16S rDNA similarity value with *Deinococcus radiodurans* (93.7%) than with *Deinococcus radiopugnans* (93.2%). The levels of sequence similarity within the *Deinococcus-Deinobacter* cluster range from 89.7 to 94.9%.

Prior to this study, all phylogenetic conclusions regarding the distinct phylogenetic position of the *Deinococcus* lineage were based on the 16S rRNA catalog and/or sequence of a single strain, *Deinococcus radiodurans* UWO 298, which is not the type strain of the species (29, 32, 33). The 16S rRNA sequence of strain UWO 298 (29) exhibits 96.9% similarity to the 16S rRNA sequence of the type strain of *Deinococcus radiodurans*, strain UWO 288 (= DSM 20539). It should be noted that Brooks et al. (3) determined the level of DNA homology between strains UWO 288^T and UWO 298 and obtained a value of 33%, which indicates that these two strains are members of distinct genospecies.

A set of signature nucleotides for the *Deinococcus* lineage was described by Woese et al. (33); the number of nucleotides in this set was subsequently reduced as additional sequences for other phyla became available (32). The results of a com-

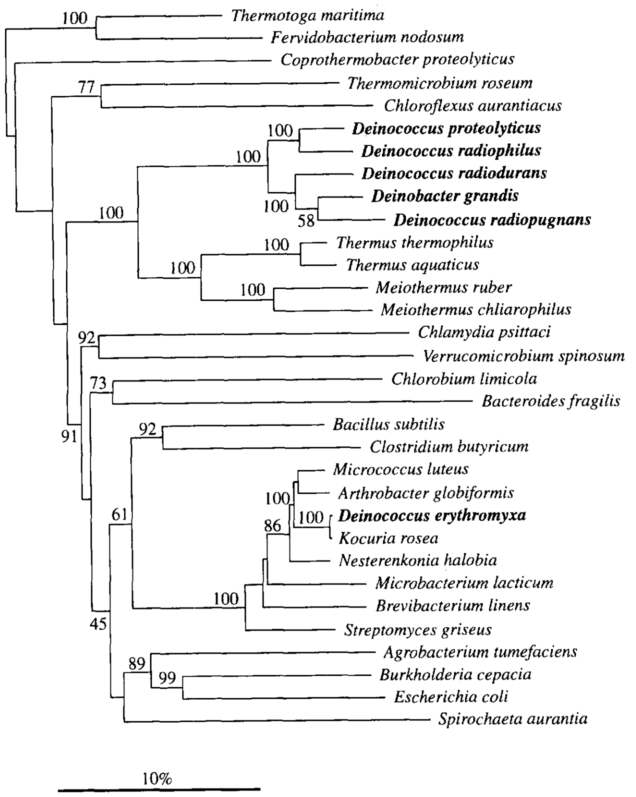

FIG. 1. Phylogenetic dendrogram based on 16S rDNA/rRNA sequence comparisons. The dendrogram was constructed by using the neighbor-joining method (19) from evolutionary distances (12). Scale bar = 10 inferred nucleotide substitutions per 100 nucleotides. The numbers at the branch points are bootstrap values determined by using the neighbor-joining data (7).

parative analysis of the 16S rDNA sequences obtained in this study and the sequences available in the public databases allowed us to identify a set of signature nucleotides that are considered unique to the *Deinococcus-Deinobacter* lineage (Table 2). This set of signature nucleotides, as well as the low levels of 16S rDNA sequence similarity to other taxa (<80%), supports the distinctness of the *Deinococcus-Deinobacter* lineage. In precedent-setting studies, other distinct lineages have been proposed as the orders *Aquificales* (5), *Planctomycetales* (20), *Thermotogales* (11), and *Verrucomicrobiales* (28) based on the results of analyses of phylogenetic data. The validity of orders which were described before the use of 16S rRNA- and DNA-based phylogenies has also been confirmed by phylogenetic analyses; examples include the orders *Chlamydiales* (30), *Spirochaetales* (17), and *Actinomycetales* (26). As pointed out by Stackebrandt et al. (24), the use of morphological, chemotaxonomic, and physiological data in descriptions of taxa above the genus level is unwise since as new organisms with novel morphotypes, chemotypes, and physiotypes are described, the descriptions become so broad as to be meaningless. On the basis of the results of the phylogenetic analyses carried out in this study and the unique set of signature nucleotides, the *Deinococcus* lineage comprising the species of the genus *Deinococcus* should be described at the level of an order, *Deinococcales* ord. nov.

TABLE 2. 16S rDNA signature nucleotide positions that distinguish members of the genera *Deinococcus* and *Deinobacter* from members of the other phylogenetic lineages within the domain *Bacteria*

Positions	Nucleotides in:	
	Deinococcus-Deinobacter lineage	Other bacteria
584-757	C-U	G-U
657-749	C-G	U/G-A/C/U
1050-1208	G-U	G-C
1421-1479	A-C/U	U/G/C-C/G/A
1429-1471	G-C	A/C-G/U

Taxonomic status of *Deinobacter grandis*. It is clear from the phylogenetic results that *Deinobacter grandis* is a member of the deinococcus lineage and exhibits higher levels of 16S rDNA sequence similarity to individual *Deinococcus* species (Table 1) than some of the *Deinococcus* species exhibit to each other. These results bring into question the distinct status of the genus *Deinobacter*. In the original description (16), the only feature of *Deinobacter grandis* that distinguished it from *Deinococcus* species was its rod-shaped morphology. It was on the basis of the rod-shaped morphology that the genus *Deinobacter* was described, since the chemotype and 16S rRNA catalog comparisons indicated that *Deinobacter grandis* was closely related to the genus *Deinococcus*.

Given the results of the complete 16S rDNA sequence analysis of all species of the genus *Deinococcus* and *Deinobacter grandis* and the previously published chemotaxonomic data, the taxonomic status of *Deinobacter grandis* requires clarification. If the separate status of the genus *Deinobacter* is to be maintained on the basis of its rod-shaped morphology and phylogenetic position, then the individual *Deinococcus* species should be reclassified in separate genera since they are as phylogenetically distinct from each other as they are from *Deinobacter grandis*. Individual *Deinococcus* species can be reclassified only on the basis of 16S rDNA sequence comparison data since these organisms are very similar in their chemotaxonomic characteristics. The alternative is to transfer *Deinobacter grandis* to the genus *Deinococcus*; this would be consistent with the results of the 16S rDNA sequence analysis and chemotaxonomic characteristics. Less emphasis can be placed on morphological characteristics at the genus level, as demonstrated recently in phylogenetic analyses and subsequent reclassification of some members of the genera *Arthrobacter* and *Micrococcus* (13). *Micrococcus agilis*, a coccus, was found on the basis of 16S rDNA sequence analysis results to fall within the radiation of the species considered to represent the genus *Arthrobacter* sensu stricto, and this position was supported by the results of a comparison of the chemotaxonomic data. *Micrococcus agilis* was transferred to the genus *Arthrobacter* as *Arthrobacter agilis*, and the genus description was emended to accommodate coccoid cells (13). We propose that *Deinobacter grandis* be transferred to the genus *Deinococcus* as *Deinococcus grandis* comb. nov.

Chemotaxonomic analyses of *Deinococcus erythromyxa*. *Deinococcus erythromyxa* possesses group A type peptidoglycan A11.6 (L-Lys–L-Ala_3). The predominant menaquinone is MK-8(H_2), and smaller amounts of MK-9(H_2), MK-7(H_2), MK-6(H_2), and MK-10(H_2) are also present. The main component of the fatty acid profile is $C_{15:0}$ anteiso; $C_{15:0}$ iso and $C_{16:1}$ occur in smaller amounts. These chemotaxonomic data are consistent with the data published previously (2, 6).

Taxonomic status of *Deinococcus erythromyxa*. When Brooks and Murray (2) described the species *Deinococcus erythromyxa*, they included it in the genus *Deinococcus* as a species incertae sedis, indicating that further investigation of its taxonomic status was required. At that time, doubts concerning the genus affiliation of this species were raised due to its two-layered cell wall and peptidoglycan type (L-Lys–L-Ala_{3-4}). These properties were in contrast to the properties of the other *Deinococcus* species, which had multilayered cell walls containing the peptidoglycan L-Orn–Gly_2. The erroneous inclusion of *Deinococcus erythromyxa* in the genus *Deinococcus* was subsequently pointed out by the same authors (15). Embley et al. (6) later carried out a comprehensive chemotaxonomic study of the *Deinococcus* species and found that *Deinococcus erythromyxa* differs from the other *Deinococcus* species in cell wall amino acid and menaquinone compositions, fatty acid profile, and polar lipid content. On the basis of these results, it was suggested that *Deinococcus erythromyxa* should be removed from the genus *Deinococcus* and that its relationship to members of the genera *Arthrobacter* and *Micrococcus* should be evaluated by nucleic acid analysis (6).

The 16S rDNA sequence comparison with representatives of the main bacterial lineages showed that *Deinococcus erythromyxa* groups with the actinomycetes and exhibits high levels of sequence similarity to members of the genus *Kocuria*. The levels of 16S rDNA sequence similarity to the *Kocuria* species are high, ranging from 95.4 to 99.9%. The high degree of 16S rDNA sequence similarity between *Deinococcus erythromyxa* and *K. rosea* (99.9%) demonstrates that *Deinococcus erythromyxa* is a member of the genus *Kocuria*. This phylogenetic placement is in complete agreement with the chemotaxonomic data previously reported for *Deinococcus erythromyxa* and members of the genus *Kocuria*. Although *Deinococcus erythromyxa* and *K. rosea* (formerly *Micrococcus roseus*) exhibit 99.9% 16S rDNA sequence similarity, previously determined DNA-DNA reassociation data supported the species status of *Deinococcus erythromyxa*. In 1980, a value of 21% was obtained for DNA-DNA reassociation between the type strains of *Deinococcus erythromyxa* (then designated *Sarcina erythromyxa*) and *K. rosea* (formerly *Micrococcus roseus*) (3). At that time, this result did not provide useful information concerning the genus status of *Deinococcus erythromyxa*, but now it can be used in combination with the results of the 16S rDNA sequence comparison to distinguish *Deinococcus erythromyxa* from its closest relative, *K. rosea*. On the basis of the results of the 16S rDNA sequence analysis, chemotaxonomic data, and the results of DNA-DNA reassociation experiments, we proposed that *Deinococcus erythromyxa* should be transferred to the genus *Kocuria* as *Kocuria erythromyxa* comb. nov.

Taxonomic conclusions. (i) Description of *Kocuria erythromyxa* (Brooks and Murray 1981) comb. nov. The description of *Kocuria erythromyxa* is the same as that given by Brooks and Murray (2). In addition to the properties described by Brooks and Murray (2) for this species, the major fatty acid is $C_{15:0}$ anteiso. The predominant menaquinone is MK-8(H_2). The type strain is strain UWO 1045 (= ATCC 187).

(ii) Description of *Deinococcus grandis* (Oyaizu et al. 1987) comb. nov. The description of *Deinococcus grandis* is the same as the description given by Oyaizu et al. (16). The type strain is strain KS 0485 (= IAM 13005).

(iii) Emendation of the genus *Deinococcus* (Brooks and Murray 1981). The transfer of a rod-shaped species to the genus *Deinococcus* requires the genus description to be emended as follows: cells are spherical or rod shaped. The remainder of the genus description is unaltered.

(iv) Emendation of the family *Deinococcaceae* (Brooks and Murray 1981). The transfer of a rod-shaped species to the genus *Deinococcus* requires the family description to be emended as follows: cells are spherical or rod shaped. The following pattern of 16S rDNA/rRNA signature nucleotide pairs define the family *Deinococcaceae*: C-U at positions 584 and 757, C-G at positions 657 and 749, G-U at positions 1050 and 1208, A-C/U at positions 1421 and 1479, and G-C at positions 1429 and 1471. The remainder of the family description is unaltered.

(v) Description of the order *Deinococcales* ord. nov. Rainey et al. *Deinococcales* (Dei.no.coc.ca'les. M. L. masc. n. *Deinococcus*, type genus of the order; *-ales*, ending denoting an order; M. L. fem. pl. n. *Deinococcales*, the *Deinococcus* order). The order contains the type family *Deinococcaceae*. The 16S rDNA/rRNA signature pattern is the same as that of the family *Deinococcaceae*.

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