A Radiation-Resistant Rod-Shaped Bacterium, Deinobacter grandis gen. nov., sp. nov., with Peptidoglycan Containing Ornithine

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Four similar strains of gram-negative, red or pink, radiation-resistant, rod-shaped bacteria were isolated from animal feces and freshwater fish. Cell division was a simple type. The deoxyribonucleic acid guanine-plus-cytosine (G + C) base ratio was 69 mol%, and menaquinone (MK-8) was found in their respiratory chain. The predominant cellular fatty acids were C16:1 and C18:1. Ornithine was the diamino acid in the peptidoglycan, and the peptidoglycan type of a representative strain was the ornithine-glycine2 (A3P) type. They stained gram-negative (by the Hucker modification staining method), and they showed an unusual cell wall structure similar to that of the genus Deinococcus. Their chemotaxonomic features were also very close to those of Deinococcus species. The ribonuclease T1 catalog of 16S ribosomal ribonucleic acid showed a close relationship to those of Deinococcus species. It is proposed that they be named Deinobacter grandis gen. nov., sp. nov.; the designated type strain is KS 0485 (= IAM 13005).

In the study of the characterization of organisms that might belong to the Flavobacterium-Cytophaga complex (14), we found that four red or pink pigment-producing bacteria did not have meso-diaminopimelic acid. We studied and attempted to identify these strains because the lack of meso-diaminopimelic acid is unusual in gram-negative bacteria (16). As shown by Woese and his co-workers (18, 19), the ribosomal ribonucleic acid (rRNA) oligonucleotide catalogs provide a powerful method for allowing recognition of phylogenetic relationships of unusual bacteria of unknown affiliation. In this case, the catalogs of the organism suggested a relationship to the genus Deinococcus, which was further supported by the peptidoglycan typing.

This paper deals with phenotypic and chemotaxonomic characterizations of the four strains. As a result of this analysis, they are assigned to a new genus, Deinobacter, and a single new species, Deinobacter grandis, is proposed here.

MATERIALS AND METHODS

Bacterial strains. Deinobacter grandis KS 0460 was isolated in May 1977 from feces of Elephas maximus raised in the Ueno Zoological Garden, Tokyo, Japan. D. grandis KS 0485, KS 0488, and KS 0492 were isolated in June 1977 from freshwater fish raised in the Freshwater Fisheries Research Laboratory, Hino, Tokyo, Japan. Strain KS 0485 was isolated from the intestines of Cyprinus carpio, strain KS 0488 was isolated from the skin of Cyprinus carpio, and strain KS 0492 was isolated from the skin of Anguilla japonica. All the strains were initially isolated on YP agar (14) containing 0.25% yeast extract, 0.25% peptone, 0.125% NaCl, and 1.5% agar at 30°C.

Cell morphology and phenotypic characteristics. Cell division was observed for up to 24 h by the slide culture method (13), and bacteria were cultured at 26°C on yeast extract-peptone agar composed of 1.0% yeast extract, 1.0% peptone, and 1.5% agar. Electron microscopic studies were carried out by the previously described method (8) with some modifications. Cells grown on yeast extract-peptone agar for 24 h were fixed with 3% glutaraldehyde in 0.05 M potassium phosphate buffer (pH 7.0) for 2 to 3 h. The cells fixed with glutaraldehyde were postfixed with aqueous 1% OsO4 in a refrigerator overnight. After the cells were washed in distilled water, they were stained in 0.5% aqueous uranyl acetate for 2 h and embedded in agar blocks. The cells were dehydrated by passing them through a series of increasing concentrations of ethanol and absolute acetone for embedding in Spurr resin. Thin sections were made by a Porter-Blum MT-2 ultramicrotome. The thin sections were stained with uranyl acetate and lead citrate and were viewed in a JEOL 200CX electron microscope. Phenotypic characteristics were determined by previously described methods (14, 15).

Radiation sensitivity. Radiation sensitivity was tested by using 150 kCi of 60Co (dose rate, 6.0 x 105 rads per h) by the method of Ito et al. (11) with a little modification. The cells were irradiated in 0.067 M phosphate buffer (pH 7.0) at a concentration of 108 cells per ml at ca. 20°C. A 10-ml volume of cell suspension that was poured into a 20-ml tube was either in equilibrium with nitrogen or bubbled with air through a capillary tube. Deinobacter grandis KS 0485, KS 0488, and KS 0492 were cultured for 24 h to provide cells instead of the usual 40-h cultivation because of their rapid growth.

Amino acid composition and amino acid linkage of the peptidoglycan. Peptidoglycan was purified by the method of Schleifer and Kandler (16). Amino acid composition was analyzed with an amino acid analyzer (Hitachi). Amino acid linkage of the peptidoglycan of strain KS 0485 was determined by the method of Schleifer and Kandler (16).

16S rRNA cataloging. The ribonuclease (RNase) T1 catalog of 16S rRNA of strain KS 0485 was analyzed by the method described by Stackebrandt et al. (17). The catalog was compared with catalogs of Deinococcus species reported by Brooks et al. (2) and representatives of various eubacterial...
groups. The catalogs of eubacteria stored by C. R. Woese were used for the comparison.

RESULTS

Cell morphology and phenotypic characteristics of *Deinobacter grandis*. All the strains stained gram negative (by the Hucker modification of the Gram staining method [4]) and were rod shaped. The cells were 0.6 to 1.2 \( \mu \)m by 1.5 to 4.0 \( \mu \)m in Gram-stained preparations from YP agar [14]. No variation in cell morphology was observed in old cultures. All the strains were nonmotile. They showed a simple type of cell division (13) (Fig. 1). Thin sections (Fig. 2) showed a cell wall structure containing an inner, thick, homogeneous layer (10 to 20 nm) just outside of the plasma membrane, and outside of that was a more lightly stained, looped, and possibly membranous layer (30 to 50 nm thick). This resembles in many ways the appearance of the class of walls illustrated by Brooks et al. (2). The thickness and structure of the inner homogeneous layer, presumed to be peptidoglycan, might be thick enough to lead to a gram-positive reaction, but the cells were gram negative.

All the strains were strictly aerobic. The colonies on YP agar after 3 days were circular, ca. 2 mm in diameter, smooth, convex, entire, and opaque and did not show spreading growth. Colonies of strain KS 0460 were pink, and those of the other three strains were red. Fluorescent pigment was not produced on King A and B media (12). The results of tests for nitrate reduction, hydrolysis of gelatin, hydrolysis of starch, catalase, and deoxyribonuclease (DNase), and hydrolysis of esculin were positive. Tests for nitrate respiration, \( \text{H}_2\text{S} \) production, indole production, aerobic and anaerobic acid production from glucose, growth in the defined medium (with carbon sources of D-glucose, acetate, succinate, and L-glutamate), and hydrolysis of Tween 80, urease, and oxidase were negative. They grew at 37 and 42°C but not at 5°C. They grew in 1% but not 5% NaCl broth. Tween 60 was hydrolyzed by one strain, KS 0460.

![Fig. 1. Phase photomicrograph of *Deinobacter grandis* KS 0485 growing on yeast extract-peptone agar at 26°C. Bar, 5 \( \mu \)m.](image1)

![Fig. 2. Thin section of cells of *Deinobacter grandis* KS 0485 showing the unusual wall profile.](image2)

![Fig. 3. Gamma irradiation sensitivity of *Deinobacter grandis*. (A) Strain KS 0485. (B) Strain KS 0460. The air bubbling (▲) and anaerobic (●) conditions are compared.](image3)
Radiation sensitivity. The four strains of Deinobacter grandis were radiation resistant, giving $D_{10}$ values greater than 0.1 megarad. Two profiles of radiation sensitivity were found. The sensitivity of strains KS 0485, KS 0488, and KS 0492 was enhanced by air bubbling during irradiation ($D_{10}$ values from 0.10 to 0.11 megarad) in comparison with nitrogen equilibrium (anaerobic) conditions ($D_{10}$ values from 0.15 to 0.17 megarad). The resistance of strain KS 0485 (0.15 to 0.17 megarad) was higher than that of the other strains. The oxygen enhancement effect for the sensitivity was very evident ($S_{AB} = 0.58$ to 0.68) in comparison with all other recorded catalogs from the eubacteria.

**DISCUSSION**

The four strains studied here were gram negative for the Hucker modification of Gram staining. However, the cell wall structure did not correspond to the profiles of most gram-negative or -positive bacteria (7). The structure, which showed a lightly stained, looped, membranous layer outside of the presumed peptidoglycan, resembles that of Deinococcus species (1, 2), although a macromolecular array external to the membranous layer and fenestration of the peptidoglycan were not observed. The type of cell wall structure of Deinococcus species and the organism mentioned here is highly unusual (2). The structure might cause confusing results in the Gram reaction, since the organisms studied here were gram negative, whereas all the species of the genus Deinococcus are described as gram positive. The difference of the response to the Gram reaction between Deinococcus species and the organism that we studied cannot exclude the close relationship shown by electron microscopic observation of cell thin sections and chemotaxonomic and phylogenetic analyses. Apparent similarities between Deinococcus species and the organism that we studied were found in cellular fatty acid composition mainly with C15:0, C15:1, and C16:1, high G + C content, peptidoglycan type (Orn-Gly2), and the RNase T1 catalog of 16S rRNA. Those chemotaxonomic characterizations and phylogenetic analyses have been used widely for bacterial classification at high taxonomic levels.

In general, phenotypic characterization is important for the taxonomy of bacteria because of their very simple morphologies. However, there are no good phenotypic characters to distinguish the genus Deinococcus and the orga-

**TABLE 1. DNA base composition and cellular fatty acid composition of the strains of Deinobacter grandis**

<table>
<thead>
<tr>
<th>Strain</th>
<th>G + C (mol%)</th>
<th>% of total fatty acids*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>i-13</td>
<td>13:0</td>
</tr>
<tr>
<td>KS 0485</td>
<td>68.7</td>
<td>1</td>
</tr>
<tr>
<td>KS 0460</td>
<td>69.2</td>
<td>1</td>
</tr>
<tr>
<td>KS 0488</td>
<td>68.4</td>
<td>1</td>
</tr>
<tr>
<td>KS 0492</td>
<td>69.4</td>
<td>1</td>
</tr>
</tbody>
</table>

*Abbreviations for fatty acids: i, iso; others are straight chains (carbon atoms:double bonds).
nism that we studied from gram-positive bacteria (2) and gram-negative bacteria (present study). Chemotaxonomic characterization (cellular fatty acid composition, quinone system, G + C content, peptidoglycan type, and polar lipid profile [3]) can show the peculiarities of this group. However, chemotaxonomic characterization can show only the difference of cellular constituents, and it cannot show phylogenetic relationships which may be revealed by the sequencing of genetically stable genes like 16s rRNA. Although the RNase T1 cataloging of 16S rRNA is a partial sequencing analysis, it gives enough information to designate the higher bacterial taxa (18, 19). By use of this technique, the genus _Deinococcus_ and the organism that we studied grouped together at a very high similarity, and the catalog of _Deinococcus_ species shows that this group arose from a very ancient lineage of eubacteria (2). We can conclude that the organism is the closest relative of the genus _Deinococcus_ found up to now and that it has almost no specific relationship with any other bacteria.

Among three _Deinococcus_ species, _Deinococcus radiodurans_ is specifically related to strain KS 0485 (Fig. 7), and this strain is included in the cluster of _Deinococcus_ species in the dendrogram. Besides the morphological difference, there are no significant differences between the organism and the genus _Deinococcus_. From this point of view, the four strains could be included in the genus _Deinococcus_. However, the morphological characteristics have been generally evaluated as important taxonomic criteria. The distribution of deinococci in nature is not known very well, and these organisms should be characterized more. Until many more strains of the deinococcus group are collected and are characterized in detail, a reasonable taxonomic step is to separate the four strains from the genus _Deinococcus_ at the genus level for inclusion in the _Deinococcaceae_ family. We propose here the new genus _Deinobacter_ for aerobic and red or pink pigment-producing rods with ornithine-containing peptidoglycan. The type species is _Deinobacter grandis_, and only this species is included in this genus.

The genus _Deinobacter_ is difficult to distinguish from, gram-negative rods by morphological and phenotypic characteristics because of their simple morphologies and lack of distinctive characteristics. Chemotaxonomic characteristics are useful to distinguish the genus _Deinobacter_. The presence of branched-chain fatty acids is very unusual in gram-
negative high-G + C bacteria. *Xanthomonas* species (9, 10) and myxobacteria (5) have branched-chain fatty acids as major fatty acids. However, those species have hydroxy fatty acids, whereas strains of the genus *Deinobacter* do not. The lack of hydroxy fatty acids is correlated with the absence of lipopolysaccharides, namely, the cell wall structure. This characteristic is of biological importance, and it must be used as a taxonomic criterion. The peculiarity in polar lipid composition was not examined for the genus *Deinobacter*; however, since the genus *Deinobacter* is the closest relative of the genus *Deinococcus*, a similar peculiarity in polar lipid composition may be expected.

**Description of *Deinobacter* gen. nov.** *Deinobacter* gen. nov. (Dei. no. bac'ter. Gr. adj. deinos strange or unusual; M.L. n. bacter masculine equivalent of Gr. neut. n. bacterium a rod; M.L. masc. n. Deinobacter unusual rod). Cells are rod shaped and gram negative. Nonmotile in the hanging drop. Cell division is simple binary fission in one plane. Chemoorganotrophs (metabolism, respiratory). Red or pink water-insoluble pigment is usually produced. Aerobic. Optimal growth temperature is 30 to 35°C. Most strains are resistant to gamma radiation, and the D90 value of the strains is higher than 0.1 megarad. Electron micrographs of a thin section indicate the presence of unusual cell wall structure with a lightly stained, looped, membranous layer external to the peptidoglycan-like layer. Interpeptide bridge of the peptidoglycan contains glycine, and peptide subunit contains alanine, glutamic acid, and ornithine. DNA base composition (G + C) approximates 69 mol%. Quinone system is MK-8. In cellular fatty acids, C15:1 and C16:1 are predominantly found. Type species is *Deinobacter grandis*.

**Deinobacter grandis** sp. nov. *Deinobacter grandis* sp. nov. (gran’dis L. adj. grandis large). The cells are rod shaped, ranging from 0.6 to 1.2 μm by 1.5 to 4.0 μm. No variation in cell morphology is observed in old cultures. Nonmotile in the hanging drop. Cell division is in a single plane by binary fission. Spreading growth is not observed on YP agar. Staining behavior is gram negative. Chemoorganotrophs (metabolism, respiratory). Red or pink water-insoluble pigment is produced. Fluorescent pigment is not produced on King A and B media. Aerobic. Most of the strains hydrolyze gelatin, starch, and esculin but not Tween 80. Nitrate is reduced to nitrite. Catalase and DNase are positive. Nitrate respiration, H2S production, indole production, urease, and oxidase are negative. Acid and gas are not produced under either aerobic or anaerobic conditions. Do not grow in the defined medium with carbon sources of D-glucose, acetate, succinate, or L-glutamate. Grow at 37 and 42°C but not at 5°C. Grow in 1% but not 5% NaCl broth. Radiation resistant to 1.0 megarad of gamma radiation. Cell wall consists of two layers. Peptidoglycan type is Orn-Gly2 (A3β). DNA base composition (G + C) approximates 69 mol%. Quinone system is MK-8. Predominant cellular fatty acids are C15:1 and C16:1. Branched-chain fatty acids of iso-C15:0, iso-C17:0, and iso-C17:1 are found. The type strain of *Deinobacter grandis* is KS 0485, which is deposited in the Institute of Applied Microbiology, University of Tokyo, Tokyo, Japan, under the accession number IAM 13004.

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**LITERATURE CITED**


