

A report of unrecorded bacterial species of Korea isolated in 2016, belonging to the family *Deinococcaceae* and *Planctomycetaceae*

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In 2016, as part of a larger effort to discover indigenous prokaryotic species in Korea, we isolated the family *Deinococcaceae* and *Planctomycetaceae* as unrecorded bacterial species. From the high 16S rRNA gene sequence similarity (>98.5%) and formation of a robust phylogenetic clade with known species, it was determined that each strain was a distinct bacterial species. There are no official reports that these two species have been described in Korea; therefore, the bacterial strains of *Deinococcus* and *Blastopirellula* are described for the first time in Korea. Gram reaction, colony and cell morphology, basic biochemical characteristics, and isolation sources are also described in the species description section.

Keywords: 16S rRNA, bacterial diversity, *Deinococcaceae*, *Planctomycetaceae*, unreported species

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INTRODUCTION

In 2016, we collected diverse samples and isolated bacterial species that were unrecorded in Korea. The identified bacterial species belonged to the families *Deinococcaceae* and *Planctomycetaceae*. The present report focuses on the isolation and description of these unrecorded species.

The family *Deinococcaceae* include the genus *Deinococcus* that was proposed by Brooks & Murray (1981) on the basis of the classification of deinococci by their typical red pigmentation and high resistance to environmental hazards including extreme UV light, ionizing radiation, and desiccation. Since 1981, the number and descriptions of validated species in this genus has increased (Kim *et al.*, 2015).

The genera *Planctomyces* and *Pirella* were assigned to a new family, *Planctomycetaceae*, in 1987 by Schlesner and Stackebrandt. Members of this family are characterized by a unique morphology, the formation of buds, and a proteinaceous cell wall (Schlesner and Stackebrandt, 1987).

This study focuses on the description of two bacterial species belonging to the families *Deinococcaceae* and *Planctomycetaceae* that have not officially been reported in Korea.

MATERIAL AND METHODS

Two bacterial strains assigned to the phylum *Bacteroidetes* were isolated from sea sand. All environmental samples were processed independently, serially diluted, spread onto culture agar media R2A and marine agar (MA), and incubated at 30°C for 2-3 days (Table 1). All strains were purified as single colonies and stored as

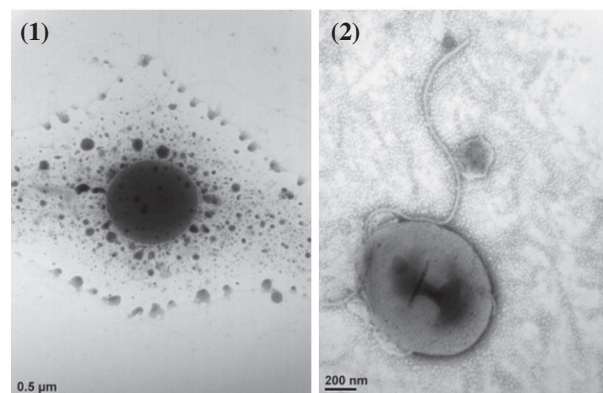
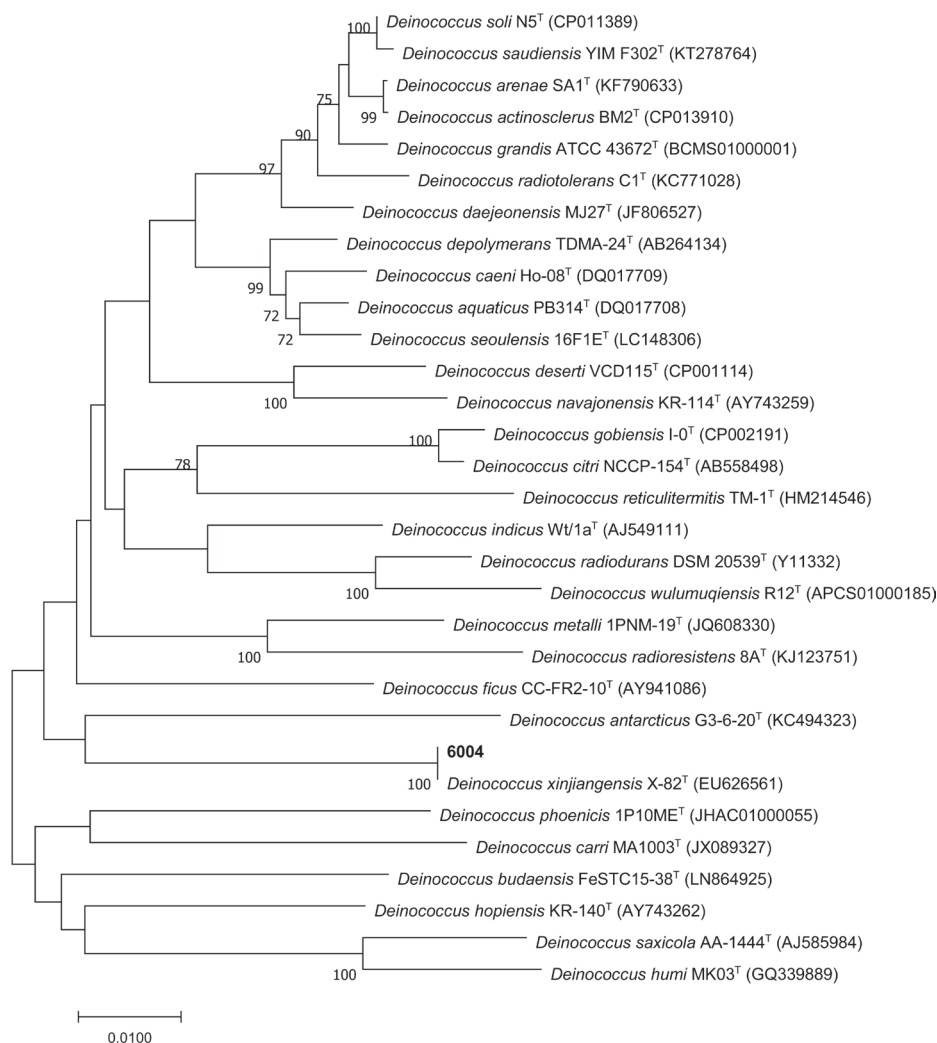


Fig. 1. Transmission electron micrographs of cells of the strains isolated in this study. The cells were cultured at their optimal growth conditions. Strains: 1, 6004; 2, CAU 1293.

Table 1. The taxonomic affiliations of isolated strains belonging to the class *Betaproteobacteria*.

Order	Family	Genus	Strain ID	NIBR ID	Most closely related species	Similarity (%)	Isolation source	Medium	Incubation conditions
<i>Deinococcales</i>	<i>Deinococcaceae</i>	<i>Deinococcus</i>	6004	NIBRBAC 000498565	<i>Deinococcus xinjiangensis</i>	100	Sediment soil	R2A	30°C, 2d
<i>Planctomycetales</i>	<i>Planctomycetaceae</i>	<i>Blastopirellula</i>	CAU 1293	NIBRBAC 000498500	<i>Blastopirellula cremea</i>	98.7	Sea sand	MA	30°C, 3d

**Fig. 2.** Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences shows the relationship between the strains isolated in this study and their relatives of the genus *Deinococcus*. Bootstrap values (>70%) are shown above nodes for the neighbor-joining methods. Bar: 0.01 substitutions per nucleotide position.

10–20% glycerol suspension at -80°C as well as lyophilized ampoules.

Colony morphology and cell size of the strains were observed by using transmission electron microscopy. Electron micrographs of the strains are shown in Fig. 1. Gram reaction was performed according to the classic Gram procedure described by Doetsch (1981). Biochem-

ical characteristics were tested by using API 20NE galleries (bioMérieux) according to the manufacturer's instructions. Genomic DNA was extracted and 16S rRNA gene was amplified by PCR with 9F and 1492R universal bacterial primers (Weisburg *et al.*, 1991). The 16S rRNA gene sequences of the related taxa were obtained from EzBioCloud server (Yoon *et al.*, 2017). The two

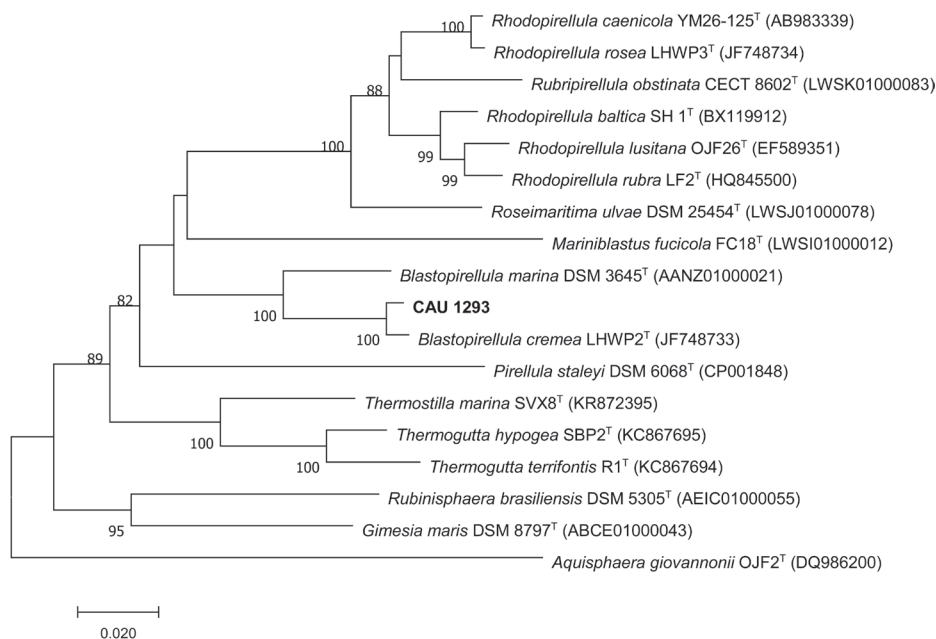


Fig. 3. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences shows the relationship between the strains isolated in this study and their relatives of the genus *Blastopirellula*. Bootstrap values (>70%) are shown above nodes for the neighbor-joining methods. Bar: 0.02 substitutions per nucleotide position.

strains and related taxa (retrieved from the NCBI database) were aligned with SINA (v1.2.11) according to the SILVA seed alignment (<http://www.arb-silva.de>; Pruesse *et al.*, 2012). The evolutionary distances were calculated using the two-parameter model (Kimura, 1983). Phylogenetic trees were constructed using the neighbor-joining method (Saitou and Nei, 1987) in MEGA7 program (Kumar *et al.*, 2016) with bootstrap values based on 1,000 replications (Felsenstein, 1985).

RESULTS AND DISCUSSION

The two strains were distributed into two orders: *Deinococcales* and *Planctomycetales*. These strains were gram-staining-positive and cocci-shaped bacteria (Fig. 1). One strain was assigned to the family *Deinococcaceae* in the order *Deinococcales* within the genus *Deinococcus* (Fig. 2). The other strain was assigned to the family *Planctomycetaceae*, order *Planctomycetales* and the genus *Blastopirellula* (Fig. 3). Here we report two unrecorded bacterial species belonging to the families *Deinococcaceae* and *Planctomycetaceae* which were isolated in Korea.

Description of *Deinococcus xinjiangensis* 6004

Cells are gram-staining-positive, non-flagellated, and cocci-shaped. Colonies are circular, entire, smooth, raised,

and pink colored after 2 days of incubation at 30°C on R2A. Positive for urease and gelatinase activities. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, esculin hydrolysis, and β -galactosidase activity. Does not utilize L-arabinose, D-mannitol, N-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain 6004 (= NIBR BAC000498565) was isolated from a sediment soil sample, Han River, Korea.

Description of *Blastopirellula cremea* CAU 1293

Cells are gram-staining-positive, flagellated, and cocci-shaped. Colonies are circular, convex, entire margins, and yellow colored after 3 days of incubation at 30°C on MA. Positive for esculin hydrolysis, gelatinase, and β -galactosidase activity. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, and urease activities. Does not utilize D-mannose, N-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain CAU 1293 (= NIBR BAC000498500) was isolated from a sea sand sample, Jeju, Korea.

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