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ORIGINAL PAPER

Complete genome sequence of *Rufibacter* sp. DG31D, a bacterium resistant to gamma and UV radiation toxicity

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Abstract The ionizing radiation toxicity becomes a major concern for the modern world, recent years, several special interest has been given to the research for the radiation resistant and the mechanisms of which the radiation resistant bacteria survive after the irradiation. In the current study, we have isolated strain DG31D was isolated from gamma ray-irradiated soil sample and showed resistant to gamma and UV radiation. The aim of this study is to understanding the radiation resistant mechanisms and their genomic features of the strain DG31D, which can be potentially used for the biotechnological application to degrade harmful soil contamination near the nuclear power stations and other radiation-affected areas. Strain DG31D showed resistant to UV and gamma radiation with D_{10} value of 10 kGy. The genome comprised of 4,820,793 bp with the G+C content of 51.4%. It contains the genomic features of enzymes involved in the nucleotide excision repair (NER) pathway that protect the damaged DNA.

Keywords *Rufibacter*, Radiation resistance

The family *Cytophagaceae* belongs to the phylum *Bacteroides*, consist of 30 valid bacterial genera including *Rufibacter*[1]. The family *Cytophagaceae* contains several reported species with gamma and UV radiation resistance from the genus *Hymenobacter*[2-9]. The species of *Rufibacter* have been isolated from a soil sample that has been reported to have resistant to UV radiation. *Rufibacter tibetensis* isolated from Tibet soil was reported to have UVC radiation resistant[1]. Two other species *Rufibacter roseus*[10] and *Rufibacter immobilis*[11] have been isolated from radiation-polluted soil and high-altitude saline lake, respectively. In this study, we report a complete genome sequence and mechanism of the radiation resistant of a novel *Rufibacter* strain isolated from a gamma ray-irradiated soil sample collected in Seoul city (GPS; N 35°33′38″E 126°53′44″), South Korea. This is the first genome sequence report of a gamma and UV radiation resistant novel *Rufibacter* strain that is isolated from irradiated soil. Strain DG31D was characterized based on the polyphasic taxonomic techniques, and the phylogenetic position of the strains was confirmed by 16S rRNA gene sequence analysis.

The ionizing radiation such as gamma ray and UV rays damage the cellular integrality by producing reactive oxygen species (ROS)[12,13], which leads to cell death. The ROS generated by ionizing radiation cause severe damages to the intracellular proteins and lipids, which was controlled by the manganese ion complex. The radiation resistant bacteria, such as *Deinococcus radiodurans* and *Deinococcus geothermalis* known to maintain higher quantities of manganese ions and protect the protein oxidation during the ionizing radiation[21]. There are few reports about the genome features of radiation resistant bacterium have been published from the genus *Deinococcus*[21] and *Hymenobacter*. The radiation resistant bacterium contains the enzymes involved in the nucleotide excision repair (NER) pathway that protect

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the damaged DNA. The genome also contains reported to contains other genes involved in the efficient removal of double-strand breaks (DSB) caused by the ionizing radiations.

Strain DG31D showed higher resistant to gamma radiation with the D_{10} value of 10 kGy. Therefore, for the better understand of the genomic insights of radiation resistance and understanding its survival strategies with ionizing radiation, we undertook the whole genome sequencing of the bacterium. The genome annotation also confirms the presence of key enzymes involving in the nucleotide excision repair (NER) pathway that protect the damaged DNA from gamma and UV radiation.

Results & Discussion

Morphology and physiology

The strain DG31D was isolated from a soil sample collected at Seoul and the cells were Gram-negative, aerobic, non-spore-forming rods with 0.3-0.8 μm in diameter and 1.5-2.4 μm in length (Figure 1). The colonies were circular, opaque with an entire margin and red colored on R2A agar medium.

Radiation resistant Analysis

The isolated strain, DG31D, showed the characteristic survival curve for gamma-radiation resistance, similar to *Deinococus radiodurance* $R1^T$ (Figure 2a). Strain DG31D showed lower resistance (52.2% and 81.0% survival) to 3 kGy gamma radiation compared with the 88% survival of *D. radioduracen* $R1^T$. Similarly, strain DG31D showed less resistance to UV-C radiation (Figure 2b). The genus *Rufibacter* was first isolated from a UVC-irradiated soil sample[1]. This study is the first report that *Rufibacter* species exhibit gamma radiation resistance.

16S rRNA gene analysis

Two copies of the 16S rRNA gene were found in the genome were identical (1498) and compared with the closely related taxa obtained from GenBank[14]. Strain DG31D was considered to belong to the genus *Rufibacter*, with the closest relatives being *R. tibetensis* NRRL B-51285^T (96.4%) and *Nibribacter koreensis* GSR3061^T (94.5%). A phylogenic tree based on the neighbor-joining method clearly showed strain DG31D

Figure 1. Scanning electron micrograph of *Rufibacter* sp. DG31D, The cells were grown on R2A agar for 3 days at 25°C. Bar, 1 μm.

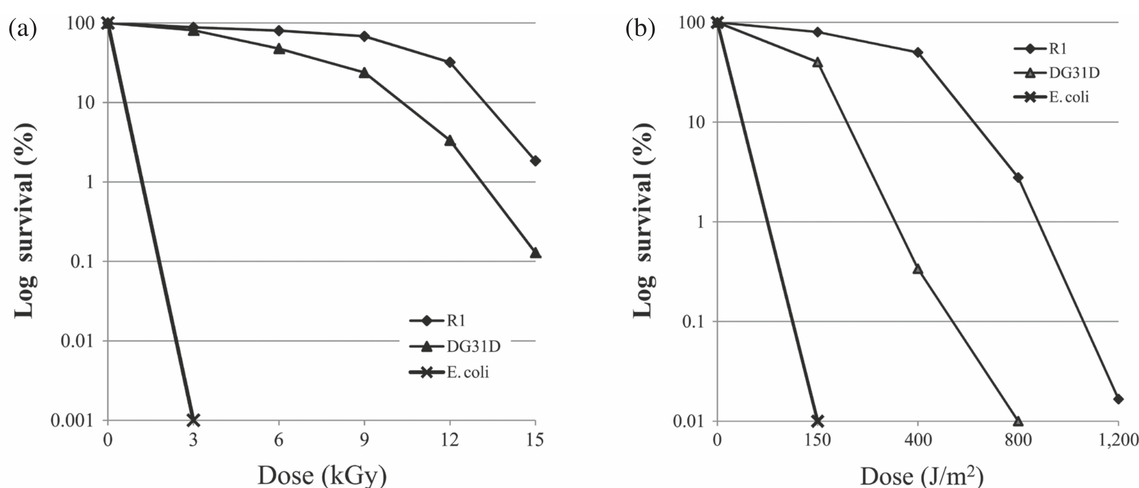

Figure 2. Representative survival curves for cells of strains DG31D (▲), *D. radiodurans* R1 (◆) and *Escherichia coli* K12 (X) the following exposure to (a) gamma radiation (b) and UVC.

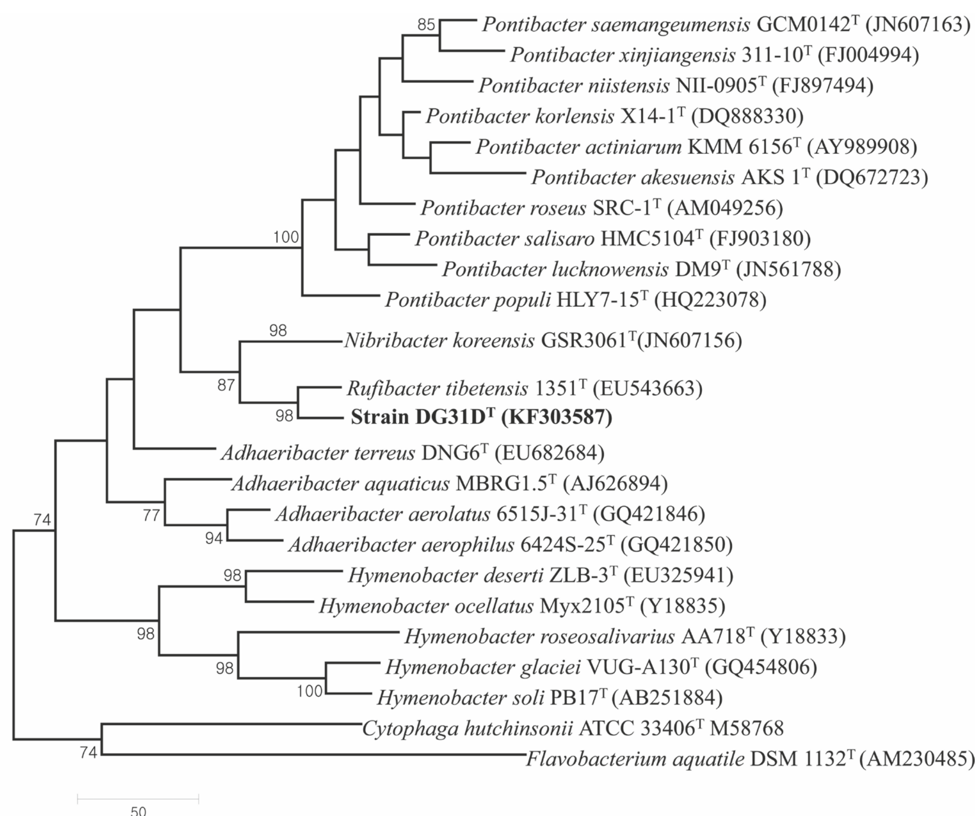

Figure 3. Neighbor-joining phylogenetic tree based on the 16S rRNA gene sequences of strains DG31D^T and related taxa. Numbers at branch nodes represent bootstrap values (>50%) obtained by a percentage of 1,000 replicates. Closed circles indicate the corresponding nodes recovered by the maximum-parsimony algorithm.

to belong to two different genera, with high bootstrap values (Figure 3).

Genome properties

The genome of strain DG31D consists of a circular chromosome of the size 4,820,793 bp with the GC content of 51.35%. A total of 3,937 genes were predicted, among them 3,886 genes are protein-coding genes, 51 RNA genes and 942 are pseudogenes. 2,944 genes were assigned to have putative functions and remaining genes are annotated as hypothetical or converted hypothetical proteins. A total of 2,243 genes is categories into Cluster of Orthologues Groups (COGs)[15] and they are presented in Table 1 and Figure 4.

Pathway prediction of DNA recovery

The complete genome of the strain DG31D was annotated, and the genomic features revealed the key enzymes involved in the DNA recovery after the ionizing radiation rehabilitation and the presence of genes for manganese transport. The damaged DNA is repaired using UvrABC pathway and RecA and MutS mediated pathways. The cluster of genes involving in the nucleotide excision repair (NER)[16-18] are present in the genome, which include two copies of excinuclease ABC subunit A, an excinuclease ABC subunit A and excinuclease ABC subunit C[19]. Similarity, the ReaA and Muts mediated pathway genes such as DNA mismatch repair protein (MutS;), RecA protein and protein implicated in DNA repair function with RecA and Muts.

Conclusions

The molecular mechanism underlying DNA repair and the key enzymes involved in the repair process has been discussed. The ionizing radiation toxicity rapidly induces the production of reactive oxygen species (ROS) which damage protein, lipids and nucleic acids of the cells leads to cell death. *The strain* DG31D showed high resistance to gamma radiation toxicity[20] with the D10 value more than 10 kGy. The radiation resistant bacteria have the repair mechanism using UvrABC pathways (nucleotide excision repair; NER) which prevent the DNA damage after the ionizing ra-

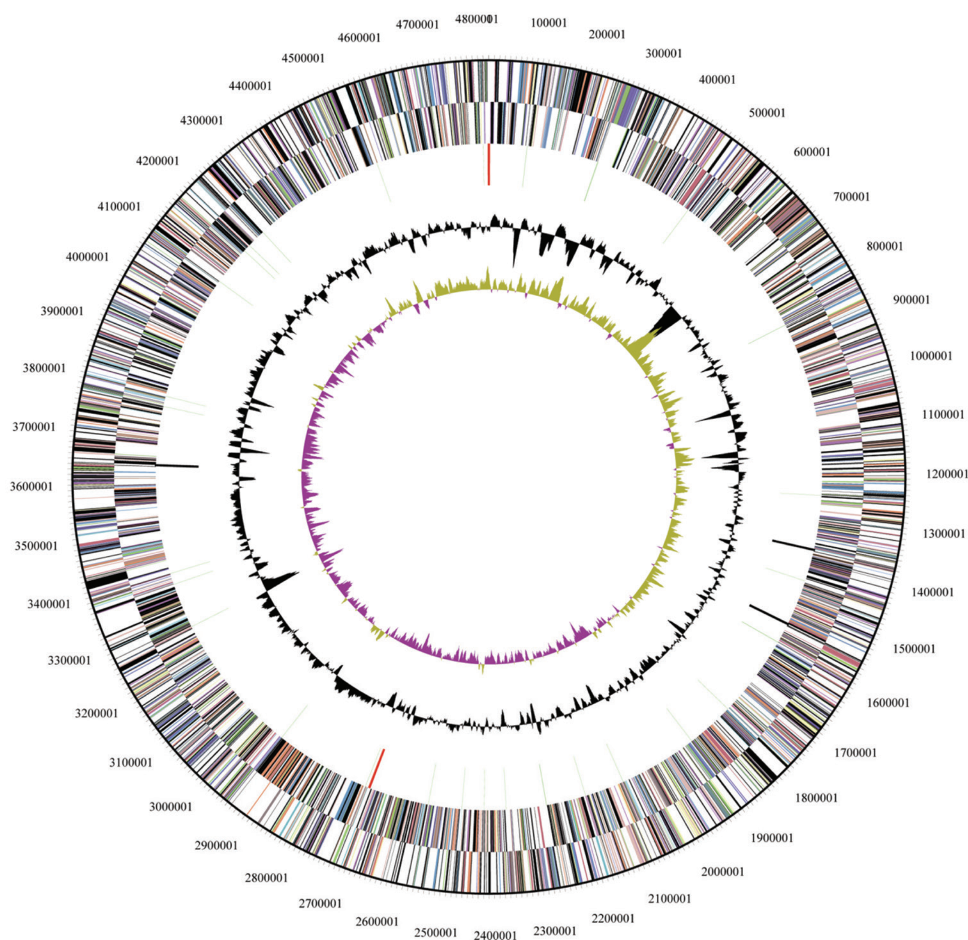

Figure 4. Graphical circular map of *Rufibacter* sp. DG31D. From outside to the center: color by COG categories and RNAs on forward strand, genes on forward strand, genes on reverse strand, color by COG categories and RNAs on reverse strand, GC content, GC skew.

diation toxicity[21]. The radiation resistant mechanisms of the radiation toxicity resistant and their key enzymes can be potentially used for environmental bioengineering to degrade the xenobiotic chemicals near the nuclear power stations and other radiation-affected areas.

Materials & Methods

Isolation and growth conditions

The strain DG31D was isolated from gamma ray-irradiated soil, 1 g soil sample was irradiated by gamma radiation (5 kGy) using a cobalt-60 gamma irradiator (point source, AECL, IR-79, Nordion, Canada). After irradiation, the soil sample was suspended in 10 mL of saline (0.85% (w/v) NaCl), and the mixture was serially diluted. Each of the diluents was spread onto ten-fold diluted R2A agar plates in 100 μL aliquots (Difco, USA), and incubated at 25°C for one week. Single colonies on the plates were selected and transferred to new R2A plates, then incubated for an additional 3 days. Two purified strains were tentatively identified using 16S rRNA gene sequencing through the EzTaxon-e server[22] (http://eztaxon-e.ezbiocloud.net). Cells were preserved in R2A supplemented with 20% (v/v) glycerol at −80°C.

Gamma and UV radiation resistant analysis

For measurement of the survival rate after exposure to gamma and UVC radiation, the early stationary phase (~10^9 CFU/mL) of bacteria cultured in TGY broth (Difco Laboratories, Detroit, Mich, USA) was used. The bacterial cultures were irradiated using a cobalt-60 based gamma irradiator, with irradiation strength of approximately 100 kCi (3.7 PBq) at a dose rate of 70 Gy/min.

Table 1. Number of genes associated with the 25 general COG functional categories.

Code	Value	% age	Description
J	192	7.82	Translation
A	0	−	RNA processing and modification
K	113	4.6	Transcription
L	98	3.99	Replication, recombination, and repair
B	1	0.04	Chromatin structure and dynamics
D	23	0.94	Cell cycle control, mitosis, and meiosis
Y	0	−	Nuclear structure
V	86	3.5	Defense mechanisms
T	106	4.32	Signal transduction mechanisms
M	223	9.08	Cell wall/membrane biogenesis
N	19	0.77	Cell motility
Z	0	−	Cytoskeleton
W	1	0.04	Extracellular structures
U	13	0.53	Intracellular trafficking and secretion
O	120	4.89	Posttranslational modification, protein turnover, chaperones
C	153	6.23	Energy production and conversion
G	173	7.04	Carbohydrate transport and metabolism
E	220	8.96	Amino acid transport and metabolism
F	68	2.77	Nucleotide transport and metabolism
H	138	5.62	Coenzyme transport and metabolism
I	113	4.6	Lipid transport and metabolism
P	145	5.9	Inorganic ion transport and metabolism
Q	66	2.69	Secondary metabolites biosynthesis, transport and catabolism
R	256	10.42	General function prediction only
S	124	5.05	Function unknown
−	1,694	43.03	Not in COGs

* Abbreviation; COG − Cluster of Orthologues Genes.

For comparison of resistance levels with other species, *D. radiodurans* R1^T (=DSM 20539^T) and *E. coli* K12 (=KCTC 1116) were used as positive and negative control strains, respectively. After irradiation, cells were diluted in microplates and plated in triplicate on TGY agar plates, then incubated[23-26]. Similarly, for the UVC resistant analysis, the cells were serially diluted in saline (0.85% NaCl), spotted on TGY agar plates, and placed on a UVC ultraviolet cross-linker (UVP, CX-2000, CA, USA) at 254 nm. The UVC dose was 20 J/m^2/s, and different radiation doses were achieved by adjusting the total exposure time. The plates were incubated for 3 days prior to enumeration of colonies[27,28].

DNA isolation

The genomic DNA was extracted using a genomic DNA purification kit (Promega) and cleaned using a PowerClean DNA Clean-Up Kit (MO BIO) according to the standard protocol recommended by the manufacturer.

Phylogenic analysis

The 16S rRNA gene sequences were edited with the BioEdit program[29]. Multiple alignments were performed with the CLUSTAL X program[30], after which the evolutionary distances were calculated using the Kimura two-parameter model[31]. The phylogenetic tree was constructed using the neighbor-joining methods[32] in the MEGA5 Program[33], with bootstrap values based on 1,000 replications[34]. A maximum-parsimony (MP) method[35] using the min-mini heuristic algorithm was also used for the construction of the phylogenetic tree.

Genome project history

The genome sequence of strain DG31D was deposited at DDBJ/EMBL/GenBank under the accession number CP010777. The genome project for DG31D is listed in the Genome OnLine Database (GOLD) as project Gp0109415. Genome sequencing and annotation were carried out using Pacific Biosciences RS II platform. The genome sequence project information was shown in Table 2 with the association with MIGS (ver. 2.0)

Table 2. Genome sequencing project information.

MIGS ID	Property	Term
MIGS-31	Finishing quality	Finished
MIGS-28	Libraries used	PacBio library
MIGS-29	Sequencing platforms	Pacific Biosciences RS II
MIGS-31.2	Sequencing coverage	106×
	Assemblers	PacBio SMRT Analysis 2.3.0
MIGS-30	INSDC ID	CP010777
	GOLD ID	Ga0064125
	NCBI project ID	273395
MIGS-13	Source material identifier	DG31D

* Abbreviation; MIGS, Minimum Information about a Genome Sequence

Table 3. Genome Statistics.

Attribute	Value	% of Total[a]
Genome size (bp)	4,820,793	100.00%
DNA coding region (bp)	4,109,092	85.24%
DNA G+C content (bp)	2,475,257	51.35%
No. of contigs	1	
Total genes	3,937	100.00%
RNA genes	51	1.30%
rRNA operons	7	0.18%
Protein-coding genes	3,886	98.70%
Pseudo genes	942	23.93%
Genes with function prediction	2,944	74.78%
Genes assigned to COGs	2,243	56.97%
Genes assigned Pfam domains	3,054	77.57%
Genes with signal peptides	678	17.22%
Genes with transmembrane helices	913	23.19%

[a] The total is based on either the size of the genome in base pairs or the total number of protein-coding genes in the annotated genome.
Abbreviation; bp, base pair; DNA, deoxyribonucleic acid; RNA, ribonucleic acid

identifiers[36].

Genome sequencing and assembly

A library was constructed according to Pacific Biosciences RS II sequencing method manual. The 87,887 sequencing reads were obtained and were assembled using the PacBio SMRT Analysis (version, 2.3.0) with default options. The final assembly resulted in 1 contig generating corresponding genome size of 4,820,793 (Table 3).

Genome annotation

The functional annotation and gene prediction was performed within the Integrated Microbial Genomes-Expert Review (IMG-ER) platform, Prodigal and JGI GenePRIMP pipeline[37]. The tRNAScan-SE tool[38] was used to find tRNA genes. Ribosomal RNA genes and ncRNA were predicted using RNAmmer_ENREF_39[39] and Infernal[40]. Identification of protein coding genes was performed using Prodigal, followed by a round of manual curation using the JGI GenePRIMP pipeline. The predicted CDS were searched using the TIGR-fam, Pfam and COG data-bases implemented in the IMG systems.

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Conflict of Interest The authors declare no conflict of interest.

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