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ORIGINAL PAPER

Complete genome sequence of *Nibribacter radioresistens* DG15C, a radiation resistant bacterium

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Abstract

Backgrounds: The ionizing radiation from the gamma rays is damaging the genetic materials of the cells, possibly leading to cell death and cause permanent changes within daughter cells. A red pigmented, Gram-negative, aerobic, short-rod shaped, non-motile, UV and gamma radiation tolerant bacterium *Nibribacter radioresistens* DG15C was isolated from a soil sample collected in a rice field in South Korea.
Methods: The complete genome of DG15C was sequenced and assembled using Pacific Biosciences RS II system. The genome sequence was annotated using Genomes-Expert Review (IMG-ER) platform, Prodigal, and JGI GenePRIMP pipeline. The protein-coding genes were identified using Prodigal, Pfam and COG databases implemented in the IMG systems.
Results: The complete genome sequence of strain DG15C consists of a circular chromosome (4,143,738 bp) encoding 3,969 coding sequences (CDs) and 3,582 genes. The bacterium showed resistance to gamma and UVC radiations.
Conclusion: The genome annotation as confirmed the presence of gene clusters involved in the toxicity resistance of radiation.

Keywords: *Nibribacter*, γ-Radiation, Radiation resistance, Nucleotide excision repair, PacBio RS II, Complete genome

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Introduction

Ionizing radiation is the energy required to remove electrons from a molecule, thereby ionizing them. Ionizing radiation is widely used in many fields such as in medicine, nuclear power stations, nuclear research centers, industries and many other areas. Exposure to ionizing radiation causes damage to living cells which end up in mutation, skin allergies, cancer, and death. Radiation risk has been epidemiologically studied in occupationally exposed populations and animal experiments since the radiation studies are limited in Korea[1]. The irradiation damages the cellular integrality by producing reactive oxygen species (ROS), which leads to cell damage[2-4], but the radiation resistant bacterial species belonged to *Rufibacter*[5], *Deinococcus*[6], *Spirosoma*[7], and *Hymenobacter*[8,9], contains the genomic features to survive in such tough conditions.

The proteome endures and maintains life, whereas the genome ensures the perpetuation of life by renewing the proteome, by a process of repairing, replicating, and expressing the genome to produce protein[10]. The resistant bacteria contain enzymes involved in the nucleotide excision repair (NER) pathway that restores the damaged DNA after irradiation. Therefore, genes play a key role in repairing the single-strand breaks (SSB) and double-strand breaks (DSB) caused by the irradiation. Hence the genomic study of radiation-resistant bacteria is more significant and some of the DNA repairing genes are also characterized[11]. To understand the genomic level of radiation resistance and survival strategies after gamma irradiation, we undertook the whole genome sequencing of *Nibribacter radioresistens* DG15C was isolated from a soil sample collected in a rice field in South Korea and report the complete genome sequence in this study. Our genomic

study of radiation-resistant bacteria will help in future to treat radiation diseases via gene manipulation techniques.

Materials & Methods

Analysis of gamma and UV radiation resistance

The survival curve of strain DG15C, *D. radiodurans* $R1^T$ (=DSM 20539^T) and *E. coli* K12 (=KCTC 1116) after exposure to gamma and UVC radiations was quantified using the early stationary phase cells. The cells were grown on TGY broth (1% tryptone, 0.1% glucose, 0.5% yeast extract Difco Laboratories, Detroit, Mich, USA) and exposed 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.

After treatment, the cells were diluted into microplates and plated in triplicate on TGY agar plates, then incubated[12-15].

Genome project history

The genomic DNA was extracted using a DNA extraction kit (Solgent, Korea) according to the manufacturer protocol. The genome sequence of *Nibribacter radioresistens* DG15C was deposited at DDBJ/EMBL/GenBank under the accession number CP014960. The genome project for *Nibribacter radioresistens* DG15C is listed in the Genome OnLine Database (GOLD) as project Gs0114044. The complete genome sequencing and annotation were carried out using Pacific Biosciences RS II platform. The genome sequence project information as shown in Table 3 with the association with MIGS (ver. 2.0) identifiers[16].

Genome sequencing and assembly

The library was constructed by Pacific Biosciences RS II sequencing method manual. A total of 187,957 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 4,143,738 (Table 3).

Genome annotation

The gene prediction and functional annotation were performed within the Integrated Microbial Genomes–Expert Review (IMG-ER) platform, Prodigal and JGI GenePRIMP pipeline[17]. The tRNAScan-SE tool[18] was used to find tRNA genes. Ribosomal RNA genes and ncRNA were predicted using RNAmmer_ENREF_39[19] and Infernal[20]. 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 TIGRfam, Pfam and COG databases implemented in the IMG systems.

Results

Analysis of radiation resistance

Nibribacter radioresistens DG15C was isolated from a soil sample collected in a rice field in South Korea (GPS; N36°18′71″ E127°9′87″)[21]. The strain DG15C is a Gram-negative, red pigmented, aerobic, short-rod shaped, non-motile, UV and gamma radiation tolerant bacterium (Figure 1). Strain DG15C showed moderate survival characteristics for gamma- and UVC radiation compares with that of *Deinococus radiodurance* R1[21]. The D_{10} value of strain DG15C is 6 kGy and 400 J/m^2, respectively for gamma and UCV radiation, meanwhile *Deinococcus radiodurance* showed over 12 kGy and 700 J/m^2.

Genomic properties

The complete genome of DG15C consists of a circular chromosome of 4,143,738 bp with the GC content of 49.96%. The complete sequence analysis showed 3,582 genes (3,529 protein-coding genes and 53 RNA genes) and 8 rRNA operons. The 2,541 genes were annotated to contain putative functions and remaining genes are classified as hypothetical or converted hypothetical proteins. Also, we assorted 1,978 genes into 25 COGs (Cluster of Orthologues Groups)[22] as shown in Table 1 and Figure 2.

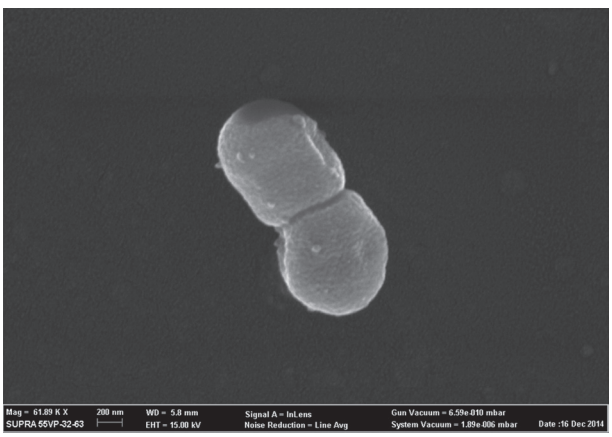

Figure 1. Scanning electron micrograph of *Nibribacter radioresistens* DG15C. The cells were grown on R2A agar for 3 days at 25°C. Bar = 200 nm.

Table 1. Number of genes associated with COG functional categories

Code	Value	%	Description
J	63	8.96	Translation
A	14	1.99	RNA processing and modification
K	3	0.43	Transcription
L	16	2.28	Replication, recombination, and repair
B	4	0.57	Chromatin structure and dynamics
D	6	0.85	Cell cycle control, mitosis, and meiosis
Y	–	–	Nuclear structure
V	6	0.85	Defense mechanisms
T	9	1.28	Signal transduction mechanisms
M	12	1.71	Cell wall/membrane biogenesis
N	–	–	Cell motility
Z	–	–	Cytoskeleton
W	–	–	Extracellular structures
U	8	1.14	Intracellular trafficking and secretion
O	55	7.82	Posttranslational modification, protein turnover, chaperones
C	79	11.24	Energy production and conversion
G	57	8.11	Carbohydrate transport and metabolism
E	106	15.08	Amino acid transport and metabolism
F	33	4.69	Nucleotide transport and metabolism
H	44	6.26	Coenzyme transport and metabolism
I	40	5.69	Lipid transport and metabolism
P	30	4.27	Inorganic ion transport and metabolism
Q	18	2.56	Secondary metabolites biosynthesis, transport and catabolism
R	88	12.52	General function prediction only
S	12	1.71	Function unknown
–	1023	81.6	Not in COGs

*COG: Cluster of Orthologue Genes

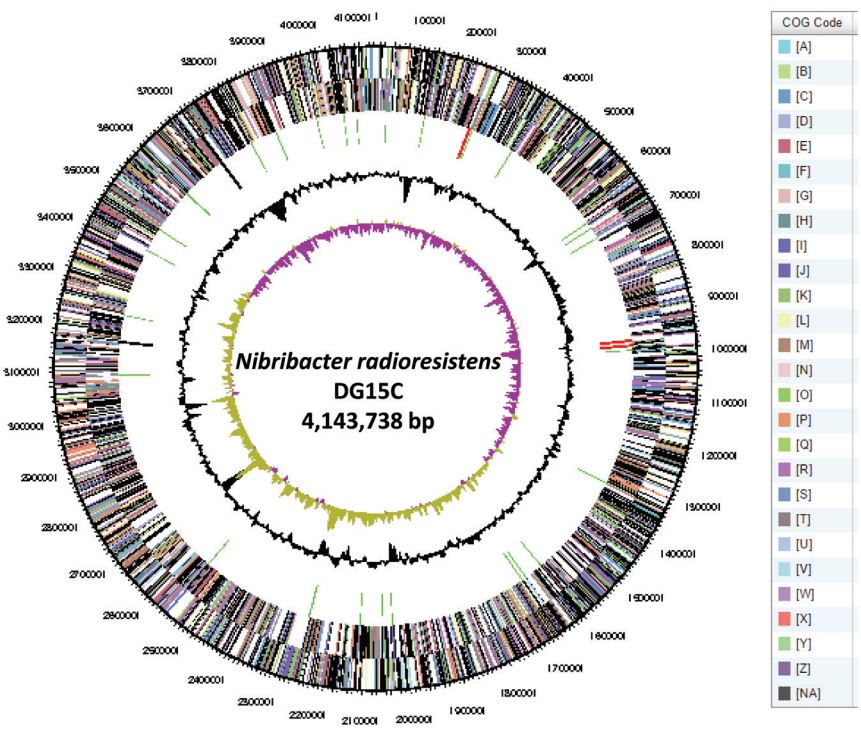

Figure 2. Graphical circular map of *Nibribacter radioresistens* DG15C. From external of the map to the interior side: color by COG categories and RNAs on the forward strand, genes on the forward strand, genes on the reverse strand, color by COG categories and RNAs on opposite strand, GC content, GC skew.

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	145×
MIGS-30	Assemblers	PacBio SMRT Analysis 2.3.0
	NCBI accession	CP010776
	GOLD ID	Gs0114044
	NCBI bioproject ID	PRJNA273388
MIGS-13	Source material identifier	DG15C

*MIGS: Minimum Information about a Genome Sequence

Table 3. Genome Statistics

Attribute	Value	% of total[a]
Genome size (bp)	4,143,738	100.00
DNA coding region (bp)	3,603,213	88.96
DNA G + C content (bp)	2,038,994	49.21
No. of contigs	1	100.00
Total genes	3,582	100.00
RNA genes	53	1.48
rRNA operons	8	0.13
Protein-coding genes	3,529	98.52
Pseudo genes	0	0.0
Genes with function prediction	2,541	70.94
Genes assigned to COGs	1,978	55.22
Genes assigned Pfam domains	2,687	75.01
Genes with signal peptides	578	16.14
Genes with transmembrane helices	827	23.09

[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

DNA repair pathways

The NER pathway involves a protein complex which includes excinuclease UvrABC having subunits A, B and C were found in *Nibribacter radioresistens* DG15C. The UvrABC excinuclease complex recognizes the structural changes caused by UV damage in DNA and repair by creating dual incisions 5′ and 3′ to the damaged site[23]. The strain DG15C genome also confirmed for the presence of UV damage repair endonuclease (UvdE) coding gene with 86% amino acid sequence similarity to the UVDE protein of *D. radiodurans* R1[11], which aids in complete UV resistance.

The complete genome analysis of the new strain also reveals the key genes for the DNA recombination repair pathways enzymes (i.e., *recA*, *recF*, *recJ*, *recN*, *recO*, *recQ*, *recR*, *ssb ruvA*, *ruvB*, and *ruvC*) that perform central role in nucleic acid metabolism and DNA mismatch repair proteins (i.e., MutS, MutS2, and MutL). The similar pathway was also reported in *D. rediodurans* that play a substantial role in the reconstruction of the *D. radiodurans* genome and DNA repairing[24]. The strain DG15C contains the other resistant pathway genes involved in NER pathway proteins such as three copies of excinuclease UvrABC system genes (UVrA, UVrB, and UVrC). The key enzymes in the NER pathway repair the damaged DNA by creating a dual incision at 5′ and 3′ to the damaged site[23].

Discussion

Strain DG15C^T was strictly aerobic, Gram-negative, non-spore forming, and small-rod shaped bacterium and showed the characteristic survival curve for gamma-radiation resistance, like *Deinococus radiodurance* R1^T. Strain DG15C^T showed resistant against a high dose of gamma radiation with the D_{10} value of 6 KGy comparatively, it's lower than that of *D. radioduracen* R1^T (Figure 3a). Similarly, strain DG15C^T also showed higher resistant resistance to UVC radiation (Figure 3b). This study is the first report that the member of the genus *Nibribacter* species exhibited extreme resistance to gamma and UVC radiation. This is the first report that a novel member of the genus *Nibribacter* species exhibited extreme resistance to gamma and UVC radiation. Several other radiation resistant bacterial strains are reported from the genus *Deinococcus*[6], *Rufibacter*[5], *Hymenobacter*[8,9], and *Spirosoma*[7].

Conclusion

In this study, we report the genomic features of UV and gamma radiation resistant bacterium *Nibribacter radioresistens* DG15C isolated from a soil sample collected in a rice field in South Korea[21]. Like other radiation resistant bacteria, the genus *Nibribacter radioresistens* DG15C contains key proteins involved in DNA repair process includes bacterial MutL-MutS pathway

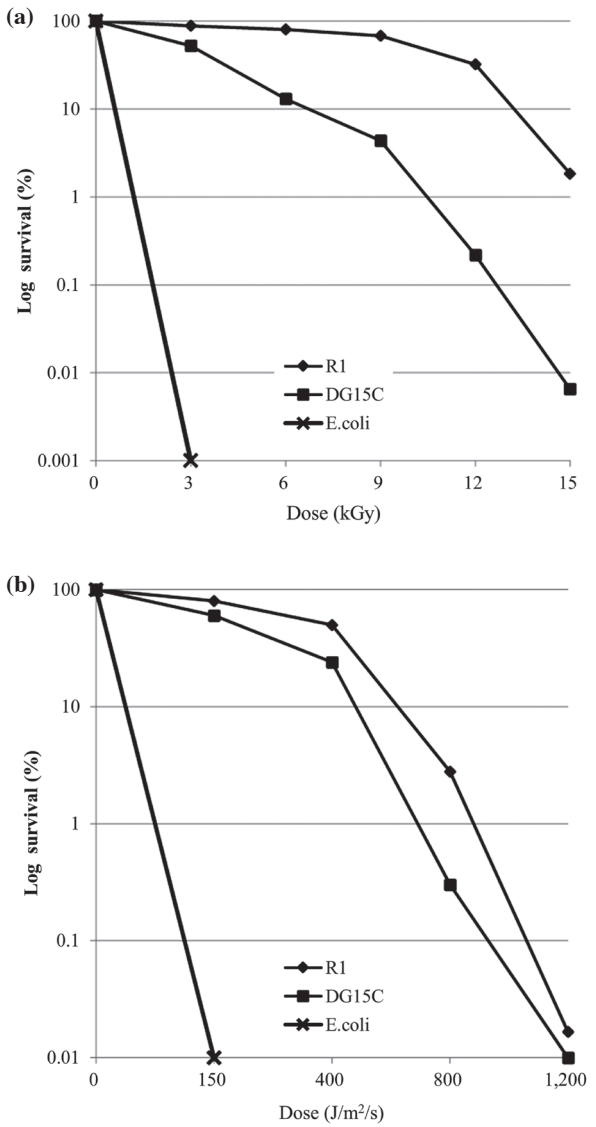

Figure 3. Representative survival curves for cells of strains DG15C^T (■), *D. radiodurans* R1^T (◆) and *Escherichia coli* K12 (✕) the following exposure to (a) gamma radiation and (b) UVC.

genes and excinuclease UVrABC pathway genes. The MutL-MutS pathway genes codes DNA mismatch repair proteins which perform a central role in the nucleic acid metabolism. The UVrABC gene has been identified as a component of nucleotide excision repair[25]. In the current study, we have reported the complete genome of the resistant strain and the main enzymes involved in the NER pathways. The ionizing radiation rapidly induces the production of reactive oxygen species (ROS) which damage protein, lipids and nucleic acids of the cells and causes cell death. The genome of the *Nibribacter radioresistens* DG15C consists of several resistance coding genes, for radiation resistance which protects the cells from the tough survival conditions. Investigation of the functional genes of these proteins will allow us a better understanding of their resistance mechanism employed by these radiation-resistant bacteria. And maybe useful for environmental bioengineering to degrade the xenobiotic chemicals near the nuclear power stations and other radiation-affected areas.

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Conflict of Interest Gayathri Subraman declares that he has no conflict of interest. Myung Kyum Kim declares that he has no conflict of interest. Ju-Young Kim declares that he has no conflict of interest. Su-Jeong Kim declares that he has no conflict of interest. Myung-Suk Kang declares that he has no conflict of interest. Sathiyaraj Srinivasan declares that he has no conflict of interest.

Human and animal rights The article does not contain any studies with human and animal and this study was performed following institutional and national guidelines.

Author contributions Gayathri Sathiyaraj and Myung Kyum Kim contributed equally.

References

1. Jin, Y. W. Epidemiological investigation of deaths among radiation workers in nuclear power plants of Korea. *J Radiat Prot* **27**, 233-237 (2002).
2. Ortiz de Orue Lucana, D., Wedderhoff, I. & Groves, M. R. ROS-Mediated Signalling in Bacteria: Zinc-Containing Cys-X-X-Cys Redox Centres and Iron-Based Oxidative Stress. *J Signal Transduct*, Article ID 605905, 9 pages (2012).
3. Waldeck, W. *et al*. ROS-mediated killing efficiency with visible light of bacteria carrying different red fluorochrome proteins. *J Photochem Photobiol B* **109**, 28-33 (2012).
4. Yu, S.-L. & Lee, S.-K. Ultraviolet radiation: DNA damage, repair, and human disorders. *Mol Cell Toxicol* **13**, 21-28 (2017).
5. Kim, M. K. *et al*. Complete genome sequence of Hymenobacter sp. DG25B, a novel bacterium with gamma-radiation resistance isolated from soil in South Korea.

J Biotech **217**, 98-99 (2016).
6. Kim, M. K. *et al*. Complete genome sequence of Deinococcus swuensis, a bacterium resistant to radiation toxicity. *Mol Cell Toxicol* **11**, 315-321 (2015).
7. Kim, M. K., Back, C. G., Jung, H. Y. & Srinivasan, S. Complete genome sequence of Spirosoma radiotolerans, a gamma-radiation-resistant bacterium isolated from rice field in South Korea. *J Biotech* **208**, 11-12 (2015).
8. Kim, M. K. *et al*. Complete genome sequence of Hymenobacter sedentarius DG5BT, a bacterium resistant to gamma radiation. *Mol Cell Toxicol* **13**, 199-205 (2017).
9. Srinivasan, S., Lee, S.-Y., Kim, M. K. & Jung, H.-Y. Complete genome sequence of Hymenobacter sp. DG25A, a gamma radiation-resistant bacterium isolated from soil. *Mol Cell Toxicol* **13**, 65-72 (2017).
10. Krisko, A. & Radman, M. Biology of extreme radiation resistance: the way of Deinococcus radiodurans. *Cold Spring Harb Perspect Biol* **5**, a012765 (2013).
11. Earl, A. M., Rankin, S. K., Kim, K. P., Lamendola, O. N. & Battista, J. R. Genetic evidence that the uvsE gene product of Deinococcus radiodurans R1 is a UV damage endonuclease. *J Bacteriol* **184**, 1003-1009 (2002).
12. Srinivasan, S. *et al*. Deinococcus radioresistens sp. nov., a UV and gamma radiation-resistant bacterium isolated from mountain soil. *Antonie van Leeuwenhoek* **107**, 539-545 (2015).
13. Cha, S., Srinivasan, S., Seo, T. & Kim, M. K. Deinococcus soli sp. nov., a gamma-radiation-resistant bacterium isolated from rice field soil. *Curr Microbiol* **68**, 777-783 (2014).
14. Srinivasan, S., Lee, J. J., Lim, S., Joe, M. & Kim, M. K. Deinococcus humi sp. nov., isolated from soil. *Int J Syst Evol Microbiol* **62**, 2844-2850 (2012).
15. Srinivasan, S., Kim, M. K., Lim, S., Joe, M. & Lee, M. Deinococcus daejeonensis sp. nov., isolated from sludge in a sewage disposal plant. *Int J Syst Evol Microbiol* **62**, 1265-1270 (2012).
16. Field, D. *et al*. The minimum information about a genome sequence (MIGS) specification. *Nat Biotech* **26**, 541-547 (2008).
17. Markowitz, V. M. *et al*. IMG ER: a system for microbial genome annotation expert review and curation. *Bioinformatics* **25**, 2271-2278 (2009).
18. Lowe, T. M. & Eddy, S. R. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res* **25**, 955-964 (1997).
19. Lagesen, K. *et al*. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Res* **35**, 3100-3108 (2007).
20. Nawrocki, E. P., Kolbe, D. L. & Eddy, S. R. Infernal 1.0: inference of RNA alignments. *Bioinformatics* **25**, 1335-1337 (2009).
21. Joo, E. S. *et al*. Spirosoma pulveris sp. nov., a bacterium isolated from a dust sample collected at Chungnam province, South Korea. *J Microbiol* **53**, 750-755 (2015).
22. Tatusov, R. L. *et al*. The COG database: an updated version includes eukaryotes. *BMC Bioinformatics* **4**, 41 (2003).
23. Petit, C. & Sancar, A. Nucleotide excision repair: from E. coli to man. *Biochimie* **81**, 15-25 (1999).
24. BATTISTA, J. R. & COX, M. M. in *Radiation Risk Estimates in Normal and Emergency Situations* (eds Arrigo A. Cigna & Marco Durante) 341-359 (Springer Netherlands, 2006).
25. Daly, M. J. A new perspective on radiation resistance based on Deinococcus radiodurans. *Nat Rev Microbiol* **7**, 237-245 (2009).