Mol Cell Toxicol (2018) 14:453-457
DOI 10.1007/s13273-018-0050-0

ORIGINAL PAPER

Complete genome sequence of *Methylobacterium* sp. 17Sr1-43, a radiation-resistant bacterium

Myung-Suk Kang[1] **& Sathiyaraj Srinivasan**[2]

Received: 29 June 2018 / Accepted: 2 September 2018

Abstract

Backgrounds: The genus *Methylobacterium* is composed of a variety of pink-pigmented and facultatively methylotrophic bacteria. Most of the species of these genera have been shown to be either gamma radiation resistant or UV radiation resistant or both. Strain *Methylobacterium* sp. 17Sr1-43 was isolated from a gamma-irradiated soil sample collected at Seoul Women's University, South Korea.
Methods: The complete genome of strain *Methylobacterium* sp. 17Sr1-43 was sequenced and assembled using Pacific Biosciences RS II system. The genome sequence was annotated using JGI GenePRIMP, Prodigal, and Genomes-Expert Review (IMG-ER) platform. The protein-coding genes were identified using Pfam and COG databases implemented in the IMG and NCBI systems.
Results: The complete genome of strain *Methylobacterium* sp. 17Sr1-43 was found to comprise a complete circular chromosome of 5,539,695 bp, with 5,103 coding sequences (CDs) and 5,186 genes. Many identified genes involved either in DNA repair or the cellular response to ionization radiation. However, contributions by genes involved in cell wall structure/function, cell division, and intermediary metabolism were also evident. Some identified genes were previously have been associated with IR resistance or recovery from IR exposure, including the RecBCD pathway and UmuCD system.
Conclusion: The new strains of *Methylobacterium* sp. 17Sr1-43 showed both gamma and UV-C irradiation resistance, and their complete genome sequence annotation features correspondingly showed the presence of the genes involved in the radiation-resistance.

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

Introduction

Microorganisms have an evolved mechanism to maintain genomic integrity in the face of extreme environmental stresses. The class of extremophiles, like bacterium *Deinococcus radiodurans*[1], shows exceptional resistance to the high doses of ionizing radiation (IR). The repair of damaged DNA and other damaged cellular components is critical for cells to survive exposure to ionizing radiation. The ionizing irradiation damages the protein, DNA, and other cellular macromolecules by producing reactive oxygen species (ROS), which leads to cell death both by direct and indirect damages[2-4]. Absorption of IR causes the direct damage by the DNA molecule, which can lead to strand breakage and chemical alterations of bases. In contrast, indirect damage occurs when reactive oxygen species (ROS), such as hydroxyl radicals, which are formed once water absorbs IR, will interact with DNA. Hydroxyl radicals produce single-strand DNA breaks and the double-strand DNA breaks (DSBs) can occur when two IR-induced single-strand DNA breaks are in close together. DSBs are the most lethal form of DNA damage because they stop the DNA replication. The extremophiles can repair both the DSBs and other DNA damages by utilizing recombinational DNA repair and nonhomologous end joining using general metabolism.

[1]Microorganism Resources Division, National Institute of Biological Resources, Incheon 22689, Republic of Korea
[2]Department of Bio & Environmental Technology, College of Natural Science, Seoul Women's University, Seoul 01797, Korea
Correspondence and requests for materials should be addressed to S. Sathiyaraj (✉drsrini@swu.ac.kr)

It is likely that genes involved in IR survival are also involved in conserving DNA integrity under normal conditions.

Therefore, 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 hard conditions. *Methylobacterium* sp. are also proven to show resistance against radiations. However, their completely sequenced IR resistant genomes, with published information regarding their IR resistance, are relatively limited in number.

To study the genomic level of radiation resistance and survival strategies after gamma irradiation, we undertook the whole genome sequencing of *Methylobacterium* sp. 17Sr1-43, isolated from the soil sample collected in Seoul Women's University, South Korea (N 37°62′79.44″, E 127°09′04.85″) and their complete genome sequence was reported.

Materials & Methods

Analysis of gamma and UV radiation resistance

The gamma and UV radiation resistance was analyzed using TGY broth (1% tryptone, 0.1% glucose, 0.5% yeast extract Difco Laboratories, Detroit, Mich, USA). The survival curve after exposure to gamma and UVC radiations was quantified using y stationary growth phase (~10^9 CFU/mL). Cells were exposed us-

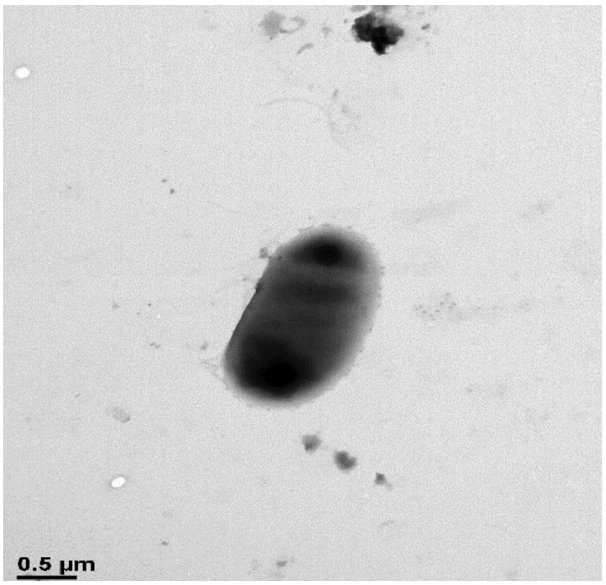

Figure 1. Transmission electron micrograph of *Methylobacterium* sp. 17Sr1-43. The cells were grown on R2A agar for 3 days at 25°C. Bar = 0.5 μm.

Table 1. Number of genes associated with COG functional categories.

Code	Value	% age	Description
J	187	7.45	Translation
A	0	–	RNA processing and modification
K	132	4.9	Transcription
L	102	4.3	Replication, recombination, and repair
B	3	0.2	Chromatin structure and dynamics
D	23	0.9	Cell cycle control, mitosis, and meiosis
Y	0	–	Nuclear structure
V	86	3.5	Defense mechanisms
T	101	4.3	Signal transduction mechanisms
M	210	9.0	Cell wall/membrane biogenesis
N	19	0.7	Cell motility
Z	0	–	Cytoskeleton
W	12	0.1	Extracellular structures
U	13	0.5	Intracellular trafficking and secretion
O	125	4.8	Posttranslational modification, protein turnover, chaperones
C	157	6.2	Energy production and conversion
G	175	7.0	Carbohydrate transport and metabolism
E	210	8.9	Amino acid transport and metabolism
F	75	2.7	Nucleotide transport and metabolism
H	141	5.6	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	10.4	General function prediction only
S	124	5.1	Function unknown
–	1,826	42.2	Not in COGs

*COG: Cluster of Orthologue Genes

ing a cobalt-60 based gamma irradiator, with irradiation strength of approximately 100 kCi (3.7 PBq) at a dose rate of 70 Gy/min. *D. radiodurans* R1^{T} (=DSM 20539^{T}) and *E. coli* K12 (=KCTC 1116) were used as positive and negative control strains, respectively. After radiation, cells were diluted in microplates and plated in triplicate on TGY agar plates[10-13].

Genome project history

DNA was extracted using a DNA extraction kit (Solgent, Korea) according to the manufacturer's protocol. The genome sequence of strain 17Sr1-43 was deposited at DDBJ/EMBL/GenBank under the accession number CP010785. Genome sequencing and annotation were carried out using Pacific Biosciences RS II platform. The genome sequence project information is shown in Table 3 with the association of MIGS (ver. 2.0) identifiers[14].

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	161×
MIGS-30	Assemblers	PacBio SMRT Analysis 2.3.0
	NCBI accession	CP029551
	GOLD ID	
	NCBI bio-project ID	PRJNA224116
MIGS-13	Source material identifier	17Sr1-43

*MIGS: Minimum Information about a Genome Sequence

Table 3. Genome Statistics.

Attribute	Value	% of total[a]
Genome size (bp)	5,539,695	100.00
DNA coding region (bp)	4,894,601	88.45
DNA G+C content (bp)	2,818,543	70.4
No. of contigs	1	100.00
Total genes	5,186	100.00
RNA genes	83	1.76
rRNA operons	18	0.13
Protein-coding genes	4,922	98.24
Pseudogenes	181	3.1
Genes with function prediction	3,383	80.51
Genes assigned to COGs	3,025	72.63
Genes assigned Pfam domains	5,521	83.79
Genes with signal peptides	406	9.66
Genes with transmembrane helices	1008	23.99

[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

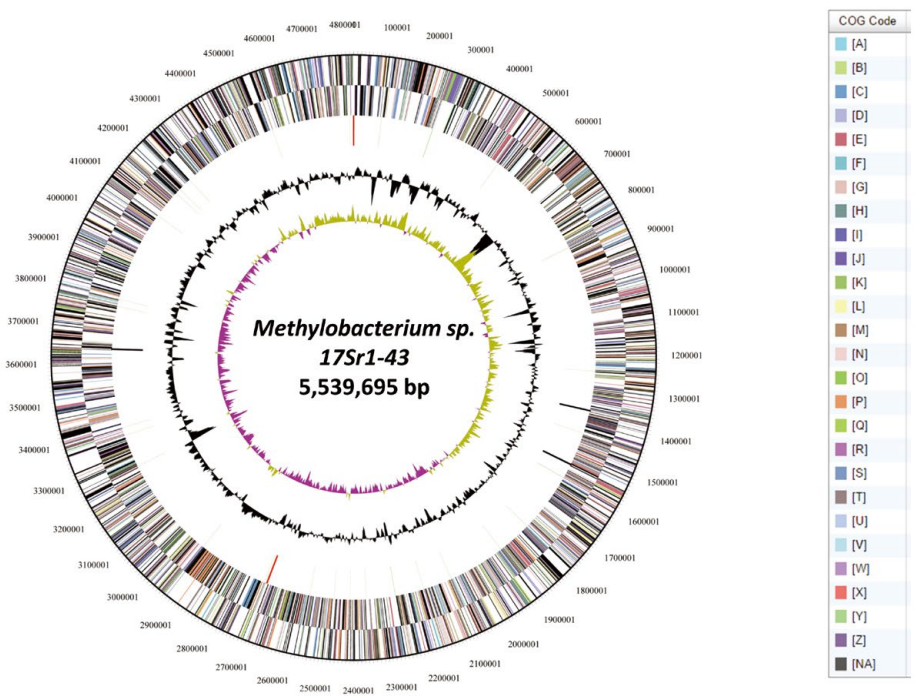

Figure 2. Graphical circular map of *Methylobacterium* sp. 17Sr1-43. From external of the map to the interior side: color by COG categories and RNAs on the forward strand, genes on the forward strand, genes on the reverse strand, color by COG categories and RNAs on opposite strand, GC content, GC skew.

Genome sequencing and assembly

A library was constructed according to Pacific Biosciences RS II sequencing method manual. The 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 functional annotation and gene prediction were performed within the Integrated Microbial Genomes-Expert Review (IMG-ER) platform, Prodigal and JGI GenePRIMP pipeline[15]. The tRNA genes were detected using tRNAScan-SE tool[16]. Ribosomal RNA genes and ncRNA were predicted using RNAmmer_EN-REF_39[17] and Infernal[18]. 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 databases implemented in the IMG systems.

Results

Analysis of radiation resistance

Methylobacterium sp. 17Sr1-43 was isolated from a soil sample collected in Seoul Women's University, South Korea (GPS; N 37°62′79.44″, E 127°09′04.85″). The 17Sr1-43 is a Gram-negative, pink-pigmented pigmented and short-rod shaped. The D_{10} value of strain 17Sr1-43 is 5 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 a new strain *Methylobacterium* sp. 17Sr1-43 consists of a circular chromosome of 5,539,695 bp with the GC content of 70.4%. The complete sequence analysis showed 5,186 genes, 61 tRNA genes, and 18 rRNA genes. The 3,125 genes were annotated to contain putative functions, and remaining genes are classified as hypothetical or converted hypothetical proteins. Also, we assorted 3,343 genes into 25 COGs (Cluster of Orthologues Groups)[19] as shown in Table 1 and Figure 2.

DNA repair pathways

The *Methylobacterium* sp. 17Sr1-43 coded for the DNA repair protein complex RecBCD and UmuCD pathways. The RecBCD enzyme is a helicase-nuclease that initiates the repair of double-stranded DNA breaks by homologous recombination[20]. The genes involved RecBCD pathway are *RecD*, *RecB*, and *RecC*. The UmuCD pathway genes are *RecA*, *LexA*, *Ssb*, *UmuC*, and *UmuD*, respectively.

Discussion

The complete genome analysis of the new strain 17Sr1-43 reveals the presence of critical genes for the DNA recombination repair pathways, which perform the central role in nucleic acid metabolism and DNA mismatch repair proteins. The analogous pathway also reported in *D. radiodurans*, which play a significant role in DNA repair mechanism[21]. The *Methylobacterium* sp. 17Sr1-43 contains the radiation resistant pathway such as RecBCD and UmcCD, these pathway genes are involved in DNA repair mechanism[22]. RecBCD is a multi-functional enzyme complex that processes DNA ends resulting from a double-strand break[23]. One key function of the RecBCD enzyme is to rescue broken replication forks via homologous, or template-directed, recombinational DNA repair[20]. This vital repair process allows the completion of DNA replication and bacterial cell division. The link between RecBCD and recombination was first demonstrated by the absence of exonuclease V activity from the *recB* and *recC* strains of *E. coli*. Subsequently, it was shown that mutations in the *recB* or *recC* gene lead to defects in conjugational, transductional, and phage recombination; a loss of SOS induction; sensitivity to DNA-damaging agents that cause DSBs; and low cell viability[24,25]. The RecBCD pathway homologous genes also reported in *Deinococcus radiodurans*[26]. This study exposed the radiation resistance of *Methylobacterium* sp, and the presence of genes involved in the ionizing radiation (IR) resistance has been proved as the key mechanism for cellular protection against radiation.

Conclusion

In this study, we report the *Methylobacterium* sp. 17Sr1-43, UV and gamma radiation-resistant bacterium isolated from Seoul Women's University, in South Korea. Like the other radiation resistance bacteria, the genus *Methylobacterium* sp. 17Sr1-43 contains vital proteins involved in DNA repair processes are bacterial RecBCD dependent pathway genes. The RecBCD enzyme plays a dual role in DNA repair mechanism; 1) act as a helicase that unwinds, or separates the strands of DNA, and 2) nuclease, which makes single-stranded

nicks in DNA.

Acknowledgements This work was supported by a research grant from Seoul Women's University (2018) and by a grant from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR201601113).

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.

References

1. Ul Hussain Shah, A. M. *et al*. A Mur regulator protein in the extremophilic bacterium Deinococcus radiodurans. *PloS One* **9**, e106341 (2014).
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. 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).
11. 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).
12. 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).
13. 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).
14. Field, D. *et al*. The minimum information about a genome sequence (MIGS) specification. *Nat Biotechnol* **26**, 541-547 (2008).
15. Markowitz, V. M. *et al*. IMG ER: a system for microbial genome annotation expert review and curation. *Bioinformatics* **25**, 2271-2278 (2009).
16. 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).
17. Lagesen, K. *et al*. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Res* **35**, 3100-3108 (2007).
18. Nawrocki, E. P., Kolbe, D. L. & Eddy, S. R. Infernal 1.0: inference of RNA alignments. *Bioinformatics* **25**, 1335-1337 (2009).
19. Tatusov, R. L. *et al*. The COG database: an updated version includes eukaryotes. *BMC Bioinformatics* **4**, 41 (2003).
20. Dillingham, M. S. & Kowalczykowski, S. C. RecBCD Enzyme and the Repair of Double-Stranded DNA Breaks. *Microbiol Mol Biol Rev* **72**, 642-671 (2008).
21. 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).
22. Jia, H. *et al*. Rotations of the 2B sub-domain of E. coli UvrD helicase/translocase coupled to nucleotide and DNA binding. *J Mol Biol* **411**, 633-648 (2011).
23. Wilkinson, M., Chaban, Y. & Wigley, D. B. Mechanism for nuclease regulation in RecBCD. *eLife* **5**, e18227 (2016).
24. Leung, W. Y., Chung, L. H., Kava, H. W. & Murray, V. RecBCD (Exonuclease V) is inhibited by DNA adducts produced by cisplatin and ultraviolet light. *Biochem Biophys Res Commun* **495**, 666-671 (2018).
25. Yang, L. *et al*. Alteration of chi recognition by RecBCD reveals a regulated molecular latch and suggests a channel-bypass mechanism for biological control. *Proc Natl Acad Sci U S A* **109**, 8907-8912 (2012).
26. Wigley, D. B. Bacterial DNA repair: recent insights into the mechanism of RecBCD, AddAB and AdnAB. *Nat Rev Microbiol* **11**, 9-13 (2013).