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ORIGINAL PAPER

Complete genome sequence of *Spirosoma pulveris* JSH 5-14^T, a bacterium isolated from a dust sample

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Abstract Dust particles from the deserts and semi-arid lands in northern China cause pollution that increase the burden of allergic disease particularly in the urban population of East Asia. Dust particles that carried with windstorm are associated with microbial populations, which include virus, bacteria, and fungi. *Spirosoma pulveris* JSH 5-14^T isolated from the gamma ray-irradiated dust sample collected at Nonsan, Chungnam province, South Korea and showed resistance against gamma and UV radiation. We carried out the whole genome sequencing to understand insight of radiation resistance and their mechanisms of survival. The whole genome of strain JSH 5-14^T is comprised of 7,188,680 bp (G + C content of 50.50%) including 5,896 protein-coding genes and 52 RNA genes. The genome analysis of strain JSH 5-14^T showed the presence of several genes involved in DNA repair pathways and defense mechanism against irradiation. In this study, we discuss the implication of such findings concerning other radiation resistant bacteria.

Keywords: *Spirosoma*, Dust radiation resistance, γ-Radiation

Introduction

Asian dust or yellow sand events in the East Asia are the major concern of environmental contamination and human health. Asian dust storm starts from the deserts of Northern China and scatters the dust aerosol over the regions of Korea and Japan[1-3]. High amounts of particles especially micro-dust and coarse particles ($PM_{2.5}$ and PM_{10}) are transported by high wind from the arid and semi-arid tracks of Northern Chain to Korea[4]. The mineral dust particles that carried with windstorm are associated with microbial loads, which include virus, bacteria, and fungi[5-7]. The dust particles transported by the wind, sometimes over long distances, on the air currents in higher attitude along with microbial populations. Strain *Spirosoma pulveris* JSH 5-14^T isolated from the gamma ray-irradiated dust sample collected at Nonsan, Chungnam province, South Korea and showed resistance against gamma and UV radiation[8]. *Deinococcus* strains are also is isolated from air samples such as *Deinococcus aerius*[9], *Deinococcus aetherius*[10], *Deinococcus cellulosilyticus*[11], *Deinococcus aerolatus*[12], and *Deinococcus aerophilus*[12] which reported to show high levels of radiation resistance.

The irradiation damages the cellular integrality by producing reactive oxygen species (ROS), which leads to cell death[13,14], but the radiation resistant bacterial species belonged to *Rufibacter*, *Deinococcus*, *Spirosoma*[15] and *Hymenobacter*, contain the genomic features to survive in such a tough conditions. There are numerous genomic features are reported from the radiation resistant species belonged to the genus *Deinococcus*, *Rufibacter*, and *Hymenobacter*[16]. Those resistant bacteria contain enzymes involved in the nucleotide excision repair (NER) pathway that restores the damaged DNA after irradiation. The genes that effectively remove the single-strand breaks (SSB) and double-strand breaks (DSB) caused by the irradiation was also characterized[17]. To understand the genomic insights of radiation resistance and survival strategies after gamma

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irradiation, we undertook the whole genome sequencing of strain *Spirosoma pulveris* JSH 5-14^T isolated from the gamma ray-irradiated dust sample collected at Nonsan, Chungnam province, South Korea and report the complete genome sequence. *Spirosoma pulveris* JSH 5-14^T comprises several vital proteins tangled in DNA repair system, e.g., nucleotide excision repair (NER) pathway, which helps to restore the damaged DNA.

Results

Analysis of radiation resistance

Spirosoma pulveris JSH 5-14^T from a gamma ray-irradiated dust sample collected at Nonsan (GPS; N36°18′71″ E127°9′87″), Chungnam province, South Korea[8]. Strain JSH 5-14^T, is a Gram-negative, strictly aerobic, non-motile, a curved bacterium with comma-forming, and a curved rod-shaped bacterium (Figure 1). Stain JSH 5-14^T showed moderate survival characteristics for gamma and UVC radiation compares with that of *Deinococus radiodurance* R1[8]. The D_{10} value of strain JSH 5-14^T is 8 kGy and 400 J/m^2, respectively for gamma and UCV radiation, meanwhile *Deinococus radiodurance* showed over 12 kGy and 700 J/m^2.

Genomic properties

The complete genome of JSH 5-14^T consists of a circular chromosome of 7,188,680 bp with the GC content of 50.50%. The complete sequence analysis showed 5,948 genes (5,896 protein-coding genes and 52 RNA genes) and 8 rRNA operons. 4,315 genes were annotated to contain putative functions and remaining genes are classified as hypothetical or converted hypothetical proteins. Also, we assorted 3,154 genes into 25 COGs (Cluster of Orthologues Groups)[18] as shown in Table 1 and Figure 2.

DNA repair pathways

The NER pathway involves a protein complex excinuclease UvrABC having subunits A, and C were found in *Spirosoma pulveris* JSH 5-14^T. 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[19]. The genome of strain JSH 5-14^T also confirmed the presence of UV damage repair endonuclease (UvdE) coding gene that has 86% amino acid sequence similarity to the UVDE protein of *D. radiodurans* R1[17], which proivde the full UV resistance. The genome analysis also revels the key gens for DNA recombination repair

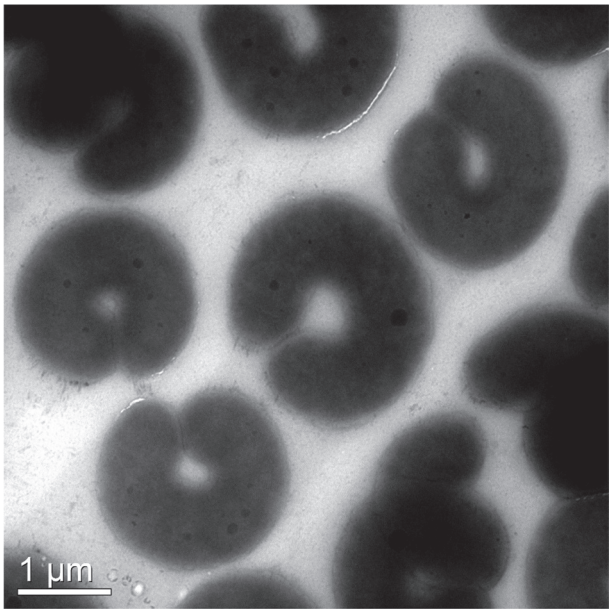

Figure 1. Transmission electron micrograph of *Spirosoma pulveris* JSH 5-14^T. The cells were grown on R2A agar for 3 days at 25°C. Bar = 1 μm.

pathways (i.e., *recA*, *recF*, *recJ*, *recN*, *recO*, *recQ*, *recR*, *ssb ruvA*, *ruvB*, and *ruvC*) that play a central role in nucleic acid metabolism.) and DNA mismatch repair proteins (i.e., MutS, MutS2, and MutL) present in the strain. The similar pathway was reported in *D. radiodurans* that play a substantial role in the reconstruction of the *D. radiodurans* genome and DNA repairing[20].

Conclusion

Here we report the genomic features of UV and gamma radiation resistant bacterium *Spirosoma pulveris* JSH 5-14^T isolated from the gamma ray-irradiated dust sample collected at Nonsan, Chungnam province, South Korea[8]. Similar with other radiation resistant bacteria belong to the genus *Deinococcus*, strain JSH 5-14^T contains key proteins involved in DNA repair processes including excinuclease UvrABC that repairs the damaged DNA caused by the ionizing radiation[21]. In the current study, we have reported the key enzymes involved in the NER pathways and the complete genome of the resistant strain. The 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 dust samples traveled in the air currents in higher attitude contain several bacterial population[22], and the cell viability is highly

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

Code	Value	%	Description
J	201	5.75	Translation
A	–	–	RNA processing and modification
K	232	6.64	Transcription
L	101	2.89	Replication, recombination, and repair
B	1	0.03	Chromatin structure and dynamics
D	27	0.77	Cell cycle control, mitosis, and meiosis
Y	–	–	Nuclear structure
V	148	4.23	Defense mechanisms
T	186	5.32	Signal transduction mechanisms
M	317	9.07	Cell wall/membrane biogenesis
N	22	0.63	Cell motility
Z	1	0.03	Cytoskeleton
W	5	0.14	Extracellular structures
U	19	0.54	Intracellular trafficking and secretion
O	149	4.26	Posttranslational modification, protein turnover, chaperones
C	177	5.06	Energy production and conversion
G	307	8.78	Carbohydrate transport and metabolism
E	237	6.78	Amino acid transport and metabolism
F	74	2.12	Nucleotide transport and metabolism
H	167	4.78	Coenzyme transport and metabolism
I	185	5.29	Lipid transport and metabolism
P	211	6.04	Inorganic ion transport and metabolism
Q	106	3.03	Secondary metabolites biosynthesis, transport and catabolism
R	438	12.53	General function prediction only
S	167	4.78	Function unknown
–	2794	46.97	Not in COGs

*COG: Cluster of Orthologue Genes

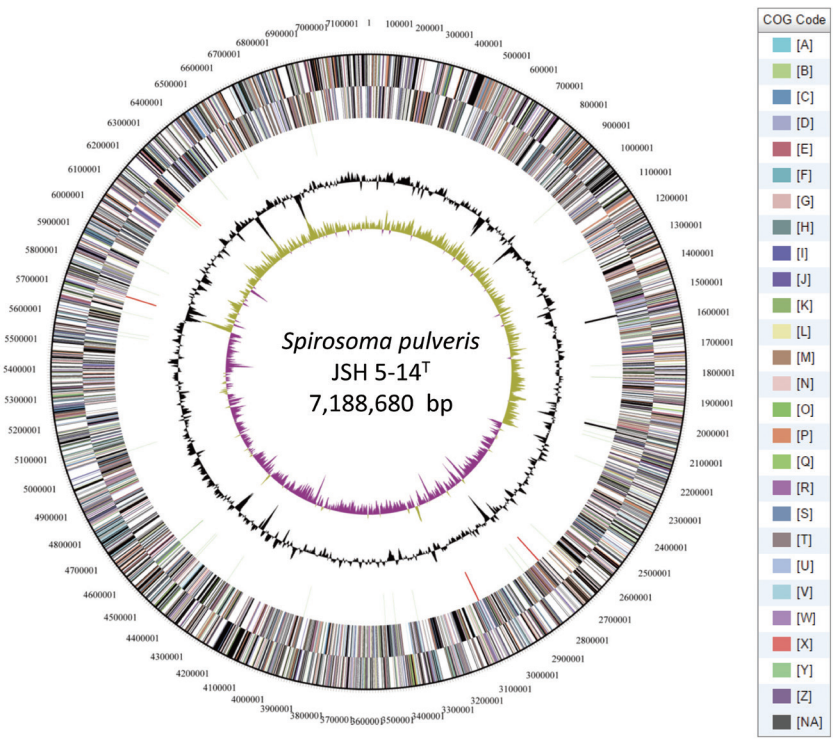

Figure 2. Graphical circular map of *Spirosoma pulveris* JSH 5-14^T. From external of map to the interior side: color by COG categories and RNAs on forward strand, genes on forward strand, genes on reverse strand, color by COG categories and RNAs on opposite strand, GC content, GC skew.

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	105×
MIGS-30	Assemblers	PacBio SMRT Analysis 2.3.0
	NCBI accession	CP014960
	GOLD ID	Ga0188103
	NCBI bioproject ID	PRJNA318150
MIGS-13	Source material identifier	JSH5-14

*MIGS: Minimum Information about a Genome Sequence

affected by serval factors including irradiation during longer traveling time. The genome of the *Spirosoma pulveris* JSH 5-14^T consists of several key genes, for radiation resistance which protects the cells from the hard survival conditions. Investigation of the functional genes of these proteins will allow us a better understanding of the mechanism of resistance employed by these radiation-resistant bacteria, which may be useful for environmental bioengineering to degrade the xenobiotic chemicals near the nuclear power stations and other radiation-affected areas.

Materials and Methods

Analysis of gamma and UV radiation resistance

The survival rate after exposure to gamma and UVC radiations was measurement using the early stationary phase (~10^9 CFU/mL) of bacteria cultured in TGY broth (1% tryptone, 0.1% glucose, 0.5% yeast extract Difco Laboratories, Detroit, Mich, USA) was used. Cells 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. *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].

Genome project history

The genomic DNA was extracted using a genomic DNA extraction kit (Solgent, Korea) according to the standard protocol. The genome sequence of *Spirosoma pulveris* JSH 5-14^T was deposited at DDBJ/EMBL/GenBank under the accession number CP014960. The genome project for JSH 5-14^T is listed in the Genome OnLine Database (GOLD) as project Ga0188103. Genome sequencing and annotation were carried out using Pacific Biosciences RS II platform. The genome sequence project information was shown in Table 3 with the association with MIGS (ver. 2.0) identifiers[27].

Table 3. Genome Statistics.

Attribute	Value	% of total[a]
Genome size (bp)	7,188,680	100.00
DNA coding region (bp)	6,380,567	88.76
DNA G+C content (bp)	3,629,961	50.50
No. of contigs	1	
Total genes	5,948	100.00
RNA genes	52	0.87
rRNA operons	8	0.13
Protein-coding genes	5,896	99.13
Pseudo genes	0	0.0
Genes with function prediction	4,315	72.55
Genes assigned to COGs	3,154	53.03
Genes assigned Pfam domains	4,542	76.36
Genes with signal peptides	955	16.06
Genes with transmembrane helices	1,390	23.37

[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.

Genome sequencing and assembly

A library was constructed according to Pacific Biosciences RS II sequencing method manual. The 147,557 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 7,188,680 (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[28]. The tRNAScan-SE tool[29] was used to find tRNA genes. Ribosomal RNA genes and

ncRNA were predicted using RNAmmer_ENREF_39[30] and Infernal[31]. 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 data-bases implemented in the IMG systems.

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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. Min Ji Kim declares that he has no conflict of interest. Ju Yeon Lee declares that he has no conflict of interest. Chang-Gyeom Kim 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.

Authors' contribution Sathiyaraj Srinivasan and Myung Kyum Kim contributed equally.

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