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ORIGINAL PAPER

Complete genome sequence of *Hymenobacter* sp. DG25A, a gamma radiation-resistant bacterium isolated from soil

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Abstract A gram-negative, rod-shaped, non-motile, gamma and UV-tolerant bacterium, *Hymenobacter* sp. DG25A, was isolated from a soil sample collected in South Korea. The isolated strain demonstrated high level of resistance against gamma irradiation, with a D10 value of 6 kGy. The complete genome of *Hymenobacter* sp. DG25A was found to consist of a single chromosome (3,777,136 bp). The bacterium was isolated from a gamma ray-irradiated soil sample and was found to contain enzymes involved in the accumulation of manganese, which protects proteins from oxidation by reactive oxygen species. The genome also encodes enzymes for the nucleotide excision repair pathway, which leads to the efficient removal of double-strand breaks caused by ionizing radiation such as gamma and UV rays. An understanding of these genomic features can potentially be used in several biotechnological applications such as for the degradation of harmful contaminants or radioactive waste.

Keywords: *Hymenobacter*, Radiation toxicity, Gamma ray, Genome sequence

The bacterial family *Cytophagaceae* belongs to the phylum *Bacteroides* and consists of 30 valid bacterial genera, including *Hymenobacter*[1]. The genus *Hymenobacter* contains several species reported to be gamma and UV radiation resistant[2-9]. In this study, we report a complete genome sequence and the mechanism of radiation resistance of a novel *Hymenobacter* strain isolated from a gamma ray-irradiated soil sample collected in Seoul city (GPS; N 37°33′30″ E127°00′01″), South Korea. Strain DG25A was characterized based on polyphasic taxonomic techniques, and the phylogenetic position of this strain was confirmed by 16S rRNA gene sequence analysis.

Ionizing radiation disrupts cell function by producing reactive oxygen species (ROS)[12,13], and thereby can induce cell death. The ROS generated in response to radiation cause severe damage to intracellular proteins and lipids. *Deinococcus radiodurans* and *D. geothermalis* are well-known radiation-resistant bacteria containing high quantities of manganese ions that protect proteins from oxidation after exposure to ionizing radiation[21]. These radiation-resistant bacteria produce enzymes involved in the nucleotide excision repair (NER) pathway, which protects against DNA damage, and their genomes are reported to contain other genes involved in the efficient removal of double-strand breaks (DSB) caused by the ionizing radiation[10,11].

Hymenobacter sp. DG25A showed a high level of resistance against gamma radiation, with a D10 value of 6 kGy. Therefore, to gain insight into its mechanism of radiation resistance and to understand bacterial strategies for survival upon exposure to ionizing radiation, we carried out whole genome sequencing of this bacterium. Genome annotation confirmed the presence of key enzymes involved in the NER pathway that limit DNA damage from gamma and UV radiation.

Morphology and physiology

Hymenobacter sp. DG25A was isolated from a soil sample collected in Seoul, Korea. The cells were gram-negative, aerobic, non-spore-forming rods, 0.3-0.8 μm

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in diameter and 1.5-2.4 μm in length (Figure 1). Colonies grown on R2A agar were red pigmented and mucoid. Growth occurred at pH 6-7. Cells grew weakly on NA but not on LB and TSA.

Radiation resistant analysis

Hymenobacter sp. DG25A showed the characteristic survival curve for gamma-radiation resistance, similar to that of *D. radiodurance* $R1^T$ (Figure 2a). *Hymenobacter* sp. DG25A showed lower levels of resistance (52.2% and 81.0% survival) to 3 kGy of gamma radiation than that (88% survival) of *D. radiodurance* $R1^T$.

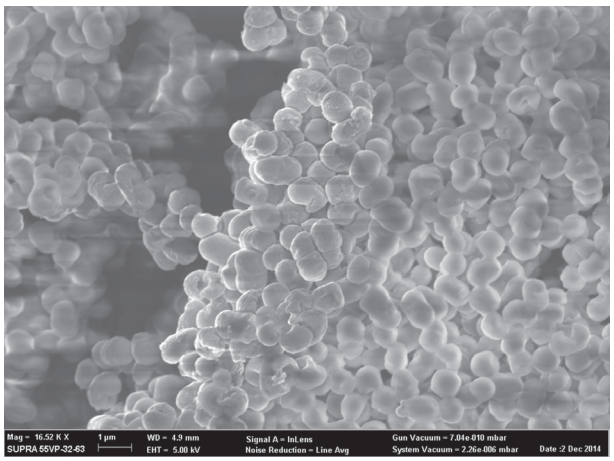

Figure 1. Scanning electron micrograph of *Hymenobacter* sp. DG25A. The cells were grown on R2A agar for 3 days at 25°C. Bar, 1 μm.

Similarly, *Hymenobacter* sp. DG25A showed less resistance to UVC radiation (Figure 2b).

16S rRNA gene analysis

The three copies of the 16S rRNA gene found in the DG25A genome were identical (1,498 bp) and the sequence was similar to those of closely related taxa obtained from GenBank[14]. *Hymenobacter* sp. DG25A was found to belong to the genus *Hymenobacter*, with the closest relative being *H. tibetensis* NRRL B-51271^T (96.5%). A phylogenic tree based on the neighbor-joining method clearly placed strain DG25A in this genus, with high bootstrap support (Figure 3).

Genome properties

Hymenobacter sp. DG25A was found to consist of a 3,777,136-bp circular chromosome with a GC content of 57.29%. A total of 3,293 genes were predicted. Among these, 3,241 genes were annotated as protein-coding genes, 52 were considered RNA genes, and 2,376 genes were assigned putative functions. The remaining genes were annotated as hypothetical or converted hypothetical proteins. A total of 1,874 genes were categorized into Clusters of Orthologous Groups (COGs)[15]. These are presented in Table 1 and Figure 4.

Predicted pathway of DNA recovery

The complete genome of *Hymenobacter* sp. DG25A was annotated, and its features revealed key enzymes involved in DNA recovery after exposure to ionizing radiation, as well as the presence of manganese trans-

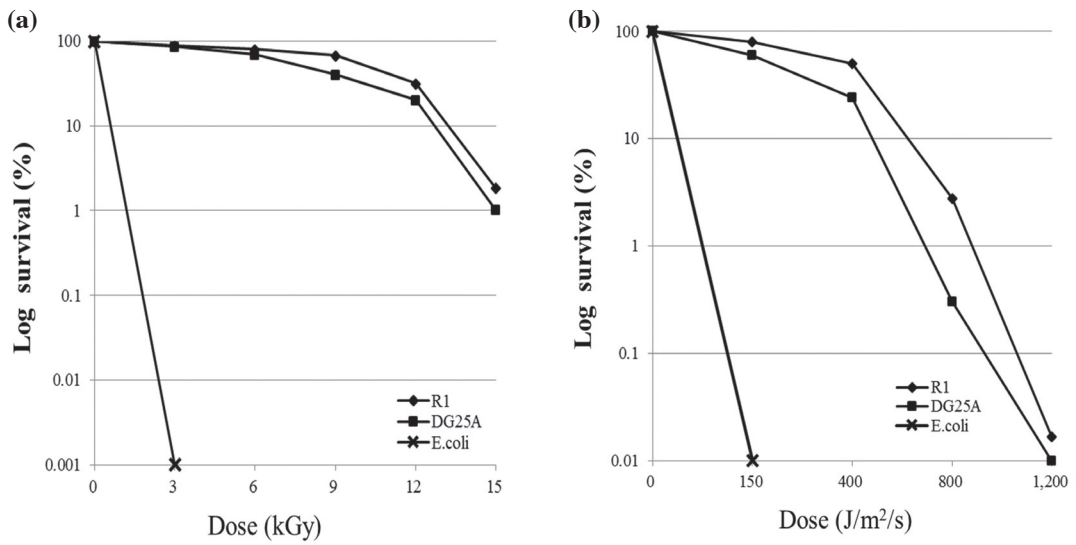

Figure 2. Representative survival curves for strain DG25A cells (■), *Deinococcus radiodurans* R1 (◆), and *Escherichia coli* K12 (X) following exposure to (a) gamma radiation (b) or UVC.

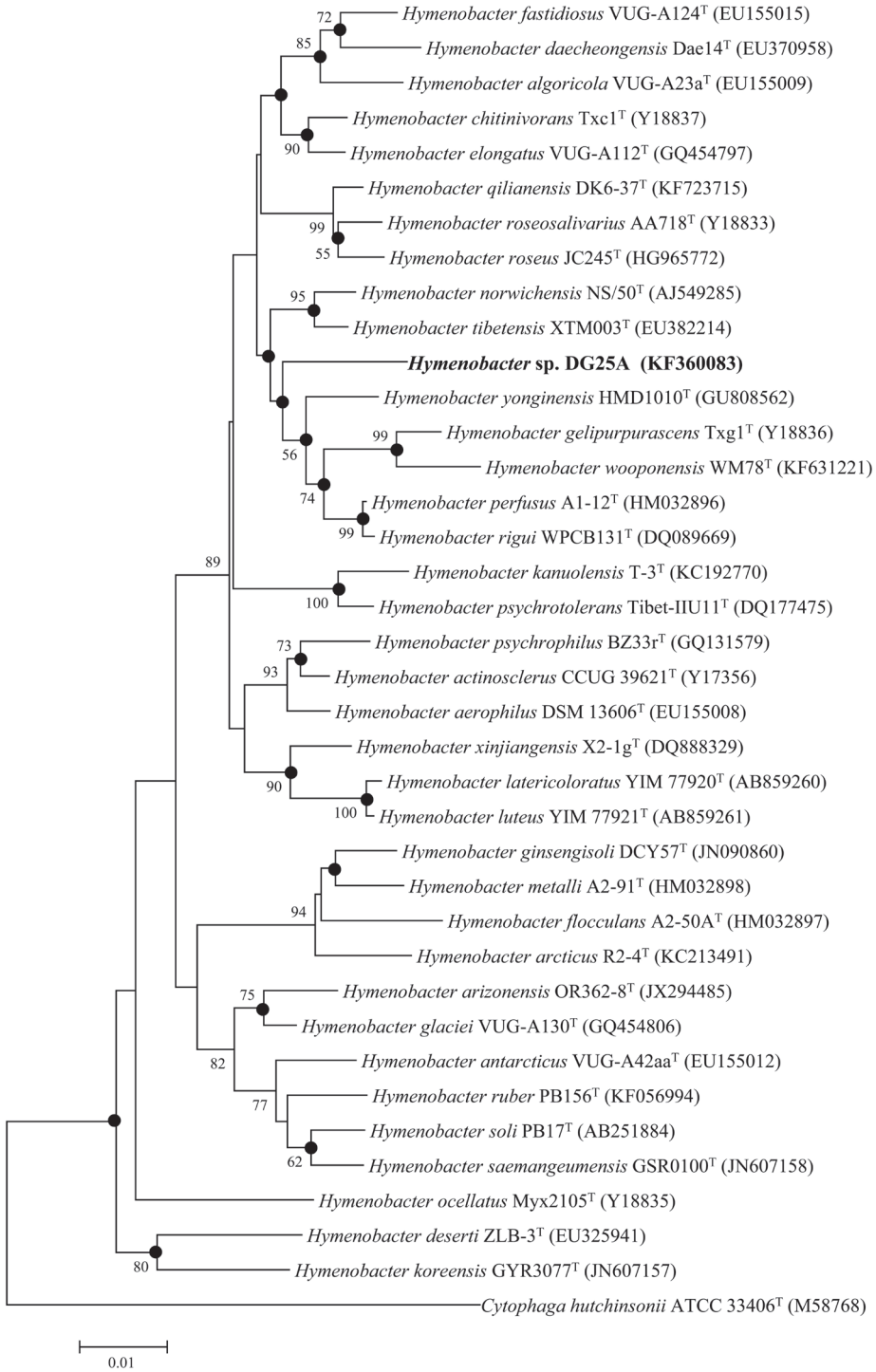

Figure 3. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences of *Hymenobacter* sp. DG25A and related taxa. Numbers at branch nodes represent bootstrap values (>50%) obtained from 1,000 replicates. Closed circles indicate the corresponding nodes recovered using the maximum parsimony method.

port genes. Damaged DNA is repaired through the UvrABC and RecA- and MutS-mediated pathways. Clusters of genes involved in NER[16-18] are present in this genome. These include two copies of excinuclease ABC subunit A, excinuclease ABC subunit A, and excinuclease ABC subunit C[19]. Similarly, RecA- and

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

Code	Value	Percentage	Description
J	193	9.34	Translation
A	–	–	RNA processing and modification
K	97	4.7	Transcription
L	83	4.02	Replication, recombination, and repair
B	1	0.05	Chromatin structure and dynamics
D	28	1.36	Cell cycle control, mitosis, and meiosis
Y	–	–	Nuclear structure
V	56	2.71	Defense mechanisms
T	78	3.78	Signal transduction mechanisms
M	199	9.63	Cell wall/membrane biogenesis
N	15	0.73	Cell motility
Z	1	0.05	Cytoskeleton
W	1	0.05	Extracellular structures
U	16	0.77	Intracellular trafficking and secretion
O	115	5.57	Posttranslational modification, protein turnover, chaperones
C	136	6.58	Energy production and conversion
G	100	4.84	Carbohydrate transport and metabolism
E	172	8.33	Amino acid transport and metabolism
F	64	3.1	Nucleotide transport and metabolism
H	129	6.24	Coenzyme transport and metabolism
I	111	5.37	Lipid transport and metabolism
P	95	4.6	Inorganic ion transport and metabolism
Q	55	2.66	Secondary metabolites biosynthesis, transport and catabolism
R	224	10.84	General function prediction only
S	96	4.65	Function unknown
–	1,419	43.09	Not in COGs

COG, Cluster of Orthologous Groups

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	136×
MIGS-30	Assemblers	PacBio SMRT Analysis 2.3.0
	INSDC ID	CP012623
	GOLD ID	Ga0097687
	NCBI project ID	32293
MIGS-13	Source material identifier	DG25A

MIGS, minimum information about a genome sequence

Muts-mediated pathway genes such as those encoding DNA mismatch repair protein (MutS), RecA protein, and other proteins implicated in DNA repair functions were found in the genome.

Conclusions

The molecular mechanism underlying DNA repair and the key enzymes involved in the repair process were presented in this report. Toxic ionizing radiation induces the rapid production of ROS, which damage proteins, lipids, and nucleic acids of cells, and leads to cell death. *Hymenobacter* sp. DG25A showed high levels of resistance against gamma radiation[20], with a D10 value of more than 6 kGy. Radiation-resistant bacteria have repair mechanisms mediated by the UvrABC pathway, which prevent DNA damage after exposure to ionizing radiation[21]. The mechanisms of radiation toxicity resistance and key enzymes involved may be

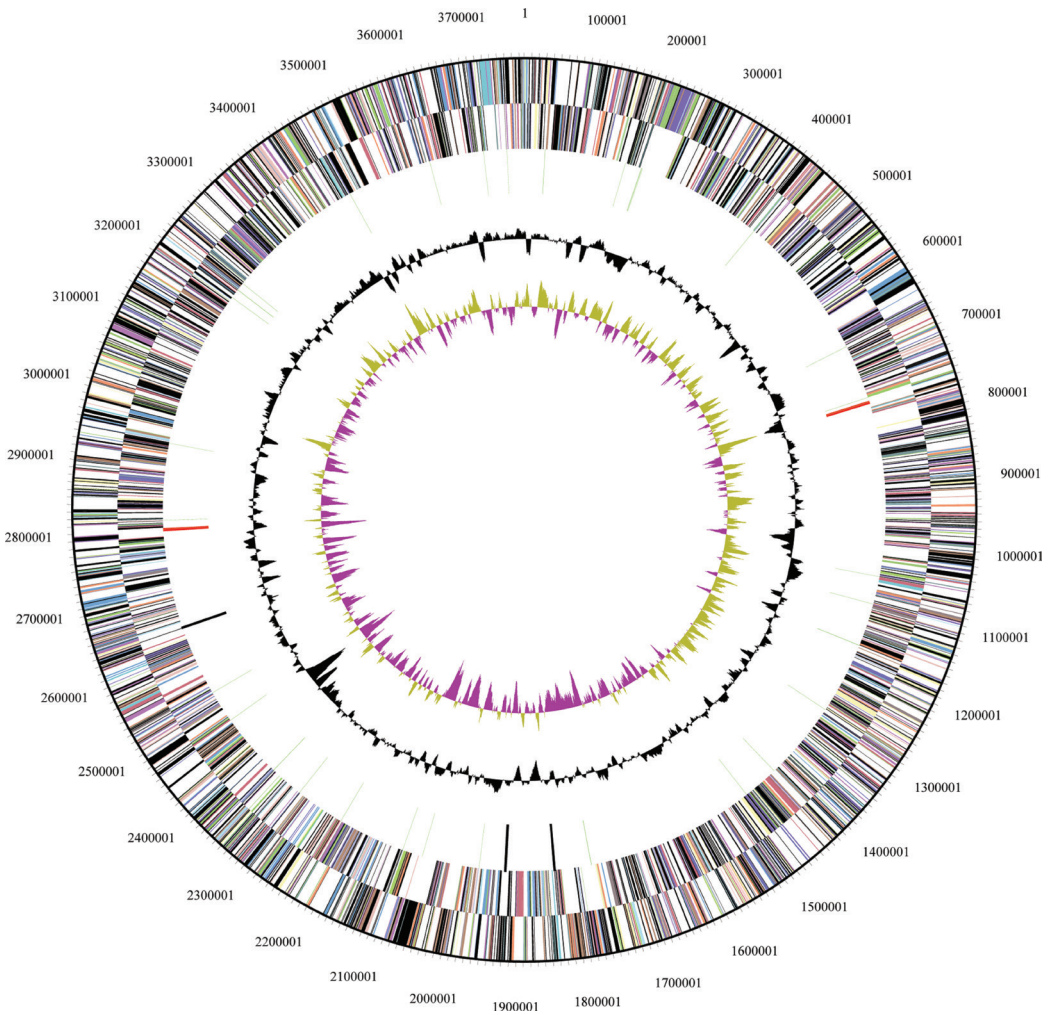

Figure 4. Graphical circular map of the *Hymenobacter* sp. DG25A genome. From the outside to the center: colors based on COG categories, RNAs on the forward strand, genes on the forward strand, genes on the reverse strand; colors based on COG categories, RNAs on the reverse strand, GC content, and GC skew.

used in environmental bioengineering to degrade xenobiotic chemicals near nuclear power stations and other radiation-affected areas.

Materials & Methods

Isolation and growth conditions

Hymenobacter sp. DG25A was isolated from gamma ray-irradiated soil. One gram of soil was irradiated with 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. An aliquot (100 μL) of each dilution was spread onto ten-fold diluted R2A agar plates (Difco, USA) and incubated at 25°C for one week. Single colonies were selected and transferred to new R2A plates that were incubated for an additional 3 days. Two purified strains were tentatively identified using the EzTaxon-e server[22] (http://eztaxon-e.ezbiocloud.net) after 16S rRNA gene sequencing. Cells were preserved in R2A medium supplemented with 20% (v/v) glycerol at −80°C.

Gamma and UV radiation resistance analysis

To determine 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, MI, USA) was used. The bac-

terial cultures were irradiated using a cobalt-60 based gamma irradiator, with a source strength of approximately 100 kCi (3.7 PBq) and a dose rate of 70 Gy/min. For comparison of resistance levels with those of 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, plated in triplicate on TGY agar plates, and incubated[23-26]. Similarly, for the UVC resistance 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 an additional 3 days prior to enumeration of colonies[27,29].

DNA isolation and phylogenic analysis

Genomic DNA was extracted using a genomic DNA purification kit (Promega) and cleaned using a PowerClean DNA Clean-Up Kit (MO BIO Laboratories) according to the standard protocols recommended by the manufacturers. The 16S rRNA gene sequences were edited using BioEdit[29]. A multiple alignment was performed using ClustalX[30], after which evolutionary distances between sequences were calculated using the Kimura two-parameter model[31]. A phylogenetic tree was constructed using the neighbor-joining method[32] in MEGA5[33], with bootstrap values based on 1,000 replications[34]. A maximum parsimony method[35] with a min-min heuristic algorithm was also used to construct a phylogenetic tree.

Genome project history

Genome sequencing and annotation were carried out using a Pacific Biosciences RS II platform. The genome sequence of strain DG31D was deposited in the DDBJ/EMBL/GenBank database under the accession number CP010777. The genome project for DG31D is listed in the Genome OnLine Database (GOLD) as project Gp0109415. Genome sequence project information is shown in Table 2 with the associated MIGS (ver. 2.0) identifiers[36].

Genome sequencing and annotation

A library was constructed according to the Pacific Biosciences RS II sequencing manual. A total of 87,887 sequencing reads were obtained and assembled using PacBio SMRT Analysis (version 2.3.0) with default options. The final assembly resulted in one contig with a corresponding genome size of 4,820,793 bp (Table 3). Functional annotation and gene prediction was performed with the Integrated Microbial Genomes-Expert Review (IMG-ER) platform and Prodigal and JGI GenePRIMP pipelines[37]. The tRNAScan-SE tool[38] was used to find tRNA genes. Ribosomal RNA genes and non-coding RNA were predicted using RNAmmer_ENREF_39[39] and Infernal[40]. Protein coding genes were identified using Prodigal, followed by a round of manual curation using the JGI GenePRIMP pipeline. The predicted coding DNA sequence was compared with those in the databases TIGR-fam, Pfam, and COG, implemented in the IMG system platform.

Table 3. Genome statistics.

Attribute	Value	Percent of total[a]
Genome size (bp)	3,777,136	100.00%
DNA coding region (bp)	3,327,401	88.09%
DNA G+C content (bp)	2,164,083	57.29%
No. of contigs	1	
Total genes	3,293	100.00%
RNA genes	52	1.58%
rRNA operons	0	0%
Protein-coding genes	3,241	98.42%
Pseudo genes	0	0%
Genes with function prediction	2,376	72.15%
Genes assigned to COGs	1,874	56.91%
Genes with signal peptides	542	16.46%
Genes with transmembrane helices	734	22.29%

[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.
bp, base pair; DNA, deoxyribonucleic acid; RNA, ribonucleic acid

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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.

Conflict of Interest Sathiyaraj Srinivasan declares that he has no conflict of interest. Seung-Yeol Lee declares that he has no conflict of interest. Myung Kyum Kim declares that he has no conflict of interest. Hee-Young Jung declares that he has no conflict of interest.

Author contributions statement All authors contributed extensively to the work presented in this paper. S. Srinivasan, M. K. Kim and H-Y Jung designed the experiment and wrote the manuscript. Whole-experiment was conducted by S. Srinivasan and S-Y Lee arranged manu-

script. Rate of contribution: S. Srinivasan, M. K. Kim and H-Y Jung 30% each and S-Y Lee 10% each.

References

1. Abaydulla, G. *et al*. *Rufibacter tibetensis* gen. nov., sp. nov., a novel member of the family *Cytophagaceae* isolated from soil. *Antonie Van Leeuwenhoek* **101**:725-731 (2012).
2. Srinivasan, S. *et al*. *Hymenobacter terrae* sp. nov., a bacterium isolated from soil. *Curr Microbiol* **70**:643-650 (2015).
3. Collins, M. D., Hutson, R. A., Grant, I. R. & Patterson, M. F. Phylogenetic characterization of a novel radiation-resistant bacterium from irradiated pork: description of *Hymenobacter actinosclerus* sp. nov. *Int J Syst Evol Microbiol* **50**:731-734 (2000).
4. Zhang, Q. *et al*. *Hymenobacter xinjiangensis* sp. nov., a radiation-resistant bacterium isolated from the desert of Xinjiang, China. *Int J Syst Evol Microbiol* **57**:1752-1756 (2007).
5. Dai, J. *et al*. *Hymenobacter tibetensis* sp. nov., a UV-resistant bacterium isolated from Qinghai-Tibet plateau. *Syst Appl Microbiol* **32**:543-548 (2009).
6. Chung, A. P., Lopes, A., Nobre, M. F. & Morais, P. V. *Hymenobacter perfusus* sp. nov., *Hymenobacter flocculans* sp. nov. and *Hymenobacter metalli* sp. nov. three new species isolated from an uranium mine waste water treatment system. *Syst Appl Microbiol* **33**:436-43 (2010).
7. Lee, J. J. *et al*. *Hymenobacter swuensis* sp. nov., a gamma-radiation-resistant bacteria isolated from mountain soil. *Curr Microbiol* **68**:305-310 (2014).
8. Jung, J. H. *et al*. Complete genome sequence of *Hymenobacter swuensis*, an ionizing-radiation resistant bacterium isolated from mountain soil. *J Biotechnol* **178**:65-66 (2014).
9. Srinivasan, S., Joo, E. S., Lee, J. J. & Kim, M. K. *Hymenobacter humi* sp. nov., a bacterium isolated from soil. *Antonie Van Leeuwenhoek* **107**:1411-1419 (2015).
10. Srinivasan, S. *et al*. Complete genome sequence of rufibacter sp. DG31D, a bacterium resistant to gamma and uv radiation toxicity. *Mol Cell Toxicol* **11**:415-421 (2015).
11. Kim, M. K. *et al*. Complete genome sequence of Deinococcus swuensis, a bacterium resistant to radiation toxicity. *Mol Cell Toxicol* **11**:315-321 (2015).
12. 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* **2012**:605905 (2012).
13. 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).
14. Benson, D. A. *et al*. GenBank. *Nucleic Acids Res* **43**: D30-35 (2015).
15. Tatusov, R. L. *et al*. The COG database: an updated version includes eukaryotes. *BMC Bioinformatics* **4**:41 (2003).
16. Kawanishi, M. *et al*. Molecular evidence of the involvement of the nucleotide excision repair (NER) system in the repair of the mono (ADP-ribosyl)ated DNA adduct produced by pierisin-1, an apoptosis-inducing protein from the cabbage butterfly. *Chem Res Toxicol* **20**:694-700 (2007).
17. Earl, A. M., Mohundro, M. M., Mian, I. S. & Battista, J. R. The IrrE protein of *Deinococcus radiodurans* R1 is a novel regulator of *rec*A expression. *J Bacteriol* **184**: 6216-6224 (2002).
18. Cai, Y., Geacintov, N. E. & Broyde, S. Ribonucleotides as nucleotide excision repair substrates. *DNA Repair* (*Amst*) **13**:55-60 (2014).
19. Ferrezuelo, F., Prieto-Alamo, M. J., Jurado, J. & Pueyo, C. Role of DNA repair by (A)BC excinuclease and Ogt alkyltransferase in the final distribution of LacI-d mutations induced by N-butyl-N-nitrosourea in *Escherichia coli*. *Mutagenesis* **13**:507-514 (1998).
20. Lee, J. J. *et al*. *Deinococcus swuensis* sp. nov., a gamma-radiation-resistant bacterium isolated from soil. *J Microbiol* **51**:305-311 (2013).
21. Daly, M. J. A new perspective on radiation resistance based on *Deinococcus radiodurans*. *Nat Rev Microbiol* **7**:237-245 (2009).
22. Kim, O. S. *et al*. Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* **62**:716-721 (2012).
23. 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).
24. 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).
25. 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).
26. 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).
27. Im, S. *et al*. Comparative survival analysis of 12 histidine kinase mutants of *Deinococcus radiodurans* after exposure to DNA-damaging agents. *Bioprocess Biosyst Eng* **36**:781-789 (2013).
28. Selvam, K., Duncan, J. R., Tanaka, M. & Battista, J. R. DdrA, DdrD, and PprA: components of UV and mitomycin C resistance in *Deinococcus radiodurans* R1. *PLoS One* **8**:e69007 (2013).
29. Hall, T. A. In Nucleic acids symposium series 95-98 (1999).
30. Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. The CLUSTAL_X win-

dows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**:4876-4882 (1997).
31. Kimura, M. The neutral theory of molecular evolution. *Sci Am* **241**:98-100, 102, 108 passim (1979).
32. Saitou, N. & Nei, M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**:406-425 (1987).
33. Tamura, K. *et al*. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* **28**: 2731-2739 (2011).
34. Felsenstein, J. Confidence Limits on Phylogenies: An approach using the bootstrap. *Evolution* **39**:783-791 (1985).
35. Fitch, W. M. Toward defining the course of evolution: Minimum change for a specific tree topology. *Syst Zool* **20**:406-416 (1971).
36. Field, D. *et al*. The minimum information about a genome sequence (MIGS) specification. *Nat Biotechnol* **26**:541-547 (2008).
37. Markowitz, V. M. *et al*. IMG ER: a system for microbial genome annotation expert review and curation. *Bioinformatics* **25**:2271-2278 (2009).
38. 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).
39. Lagesen, K. *et al*. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Res* **35**:3100-3108 (2007).
40. Nawrocki, E. P., Kolbe, D. L. & Eddy, S. R. Infernal 1.0: inference of RNA alignments. *Bioinformatics* **25**:1335-1337 (2009).