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Distribution of *ddr* (DNA damage response) Genes among Species of *Deinococcus*

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Abstract - The bacterium *Deinococcus radiodurans* is one of the most resistant organisms to the effects of ionizing radiation and other DNA-damaging agents. In this study, distributions of 10 *ddr* (DNA damage response) genes were investigated in 8 species of *Deinococcus* by polymerase chain reaction (PCR). We have compared the sequences of *ddr* genes of *D. radiodurans*, *D. geothermalis* and *D. deserti*, and selected primers which are suitable for the detection of *ddr* in different species of *Deinococcus*. A sequence homology search and PCR assay showed that *ddrO*, which encodes a global regulator of the radiation-desiccation response, was most well conserved in the *Deinococcus* lineage.

Key words : *Deinococcus*, DNA damage response (*ddr*), Polymerase chain reaction (PCR)

INTRODUCTION

Deinococcus radiodurans R1, a small, red-pigmented, and tetrad-forming Gram-positive soil bacterium, is one of the most radiation-resistant organisms known (Cox and Batistta 2005). Although the extraordinary resistance of *D. radiodurans* to ionizing radiation (IR) has been extensively investigated from the first isolation in canned meat that had been irradiated to achieve sterility (Anderson *et al.* 1956) to the publication of the complete genome sequence of *D. radiodurans* in 1999 (White *et al.* 1999), the molecular mechanisms underlying this phenotype have not been fully revealed. The genome of *D. radiodurans* R1 is composed of two chromosomes (2.6 and 0.4 Mbp), a megaplasmid (0.2 Mbp), and a small plasmid (45 kbp), containing 3,187 open reading frames (ORFs) (White *et al.* 1999). Genome sequence analyses showed the specific expansion of the Nudix hydrolase and uncharacterized DinB (DNA damage inducible) protein families (Makarova *et al.* 2000) and the existence of typically eukaryotic genes in this bacterium (Makarova *et al.* 2001). These likely contribute to the extreme radiation resistance of *D. radiodurans*.

It is generally accepted that the DNA represents the most critical target of IR (Diehl 1995) and IR produces a wide spectrum of DNA lesions such as base modification, abasic sites and strand breaks damage (Symons 1994). Thus, it was expected that *D. radiodurans* had novel DNA repair proteins critical for IR resistance. However, DNA repair systems identified in *D. radiodurans* appear less complex and diverse than those reported for *Shewanella oneidensis*, which encode relatively complex DNA repair systems, are killed (over 90%) at the 70 Gy dose of irradiation that cause relatively little DNA damage (Daly *et al.* 2004; Ghosal *et al.* 2005). The genome of *Thermus thermophilus*, a radiosensitive thermophile from the same phylum, was compared to the genome of *D. radiodurans*. Although *Deinococcus* seems to have acquired numerous genes related to stress response systems from various bacteria, comparative analysis of the well-characterized genetic systems for replication, repair and

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recombination, and related functions in *T. thermophilus* and *D. radiodurans* shows that fractions of these genes in the respective genomes are very similar (Omelchenko *et al.* 2005).

However, a conclusion that there are no unique components of the repair systems might be premature since 1,002 *D. radiodurans* ORFs have no database matches (White *et al.* 1999). Tanaka *et al.* (2004) reported that some novel proteins named Ddr (DNA damage response) might be responsible for the extreme radioresistance of *D. radiodurans* on the basis of up-regulation of *ddr* genes following irradiation and increased susceptibility to IR of *ddr* mutants. Direct experiments show that DdrA binds to the 3-ends of single DNA strands to protect those ends from nuclease degradation (Harris *et al.* 2004) and DdrB is the prototype of a new bacterial single-stranded DNA binding protein family (Norais *et al.* 2009). Recently, these two proteins were proposed to be involved in an extended synthesis-dependent strand annealing (ESDSA) process, which is a distinctive DNA repair system in *D. radiodurans* (Bentchikou *et al.* 2010). A total of 16 such genes were differentiated by the letters A-P (Tanaka *et al.* 2004). Among them, five (*ddrB, ddrC, ddrD, ddrH*, and *ddrO*) have been previously identified as *Deinococcus*-specific (De Groot *et al.* 2009).

In this study, we surveyed distribution of *ddr* genes among *Deinococcus* species using PCR (polymerase chain reaction) assay.

MATERIALS AND METHODS

1. Bacterial strains and growth conditions

D. radiodurans R1 (DRD; ATCC 13939), *D. geothermalis* (DGT; KCTC12208), *D. deserti* (DDI; KCTC11782), *D. radiopugnans* (DRP; ATCC 19172), *D. proteolyticus* (DPL; ATCC 35074), *D. radiophilus* (DRPH; ATCC 27602), *D. murrayi* (DMI; KACC12209), and *D. indicus* (DIS; KACC-1772) were obtained from the American Type Culture Collection (ATCC), Korean Collection for Type Culture (KCTC), and Korean Agricultural Culture Collection (KACC). Strains were cultured to stationary phase at 30°C in liquid TGY medium (0.5% tryptone, 0.1% glucose, 0.3% yeast extract) or TGY plates supplemented with 1.5% Bacto-agar.

2. Genomic DNA isolation

Genomic DNA extraction was performed as described previously (Udupa *et al.* 1994). Cultures (200 ml) were harvested by centrifugation at 4°C and 6,000 × g for 15 min. Pellets were resuspended in 95% ethanol (20 ml) and incubated at room temperature (RT) for 10 min to remove outer membranes. Cells were then collected by centrifugation at 4°C and 6,000 × g for 15 min and the pellet was gently resuspended in a TE buffer (10 mM Tris-HCl, 0.1 mM EDTA; pH 8.0) containing 2 mg ml^{-1} lysozyme (Sigma Chemical, St. Louis, Mo.). This mixture was incubated at 37°C for 30 min. Proteinase K/SDS solution (20 ml; 10 μg ml^{-1} Proteinase K, 10% (w/v) SDS; Sigma Chemical Co.) was added and solutions were incubated for at least 3 h at 50°C. Lyzed cells were transferred to a centrifuge tube and extracted once with an equal volume of phenol-chloroform-isoamylalcohol (24 : 23 : 1). DNA was precipitated from the extracted material using sodium acetate (100 μl; 3 M; pH 7.0) and ice-cold 100% (v/v) ethanol (2 ml). DNA was collected by centrifugation at 4°C and 6,000 × g for 15 min and washed twice with 75% (v/v) ethanol. DNA was then air-dried, dissolved in 1 ml nuclease free water, and stored at −20°C.

3. PCR assay

PCR amplification was performed in a 20-μl reaction mixture of 10× reaction buffer (Bioneer, Daejeon, Korea), 10 pmol of each primer, 0.3 mM each of the four dNTPs, 1 U of HF DNA polymerase and 1 μl of template DNA (100 ng). Amplification was carried out using a Primus 96 thermal cycler (MWG Biotech Inc., High Point, NC, USA) with denaturation at 94°C for 30 s, annealing at 55 ~ 63°C for 30 s, and primer extension at 72°C for 30 s. Following amplification, 5 μl of each sample was visualized by standard gel electrophoresis in 1% agarose gel stained with ethidium bromide. DNA markers (1 kb DNA ladder; Fermentas, USA) were used to characterize the molecular size of the amplified products.

RESULTS AND DISCUSSION

1. Nucleotide sequence comparison of *ddr* genes

Of about 30 *Deinococcus* species, the genomes of three, DRD, DGT and DDI, have been sequenced up to now (White *et al.* 1999; Makarova *et al.* 2007; De Groot *et al.* 2009). We first checked whether sixteen *ddr* genes annotated in DRD (Tanaka *et al.* 2004) are present in each of the two

sequenced *Deinococcus* genomes. Homologues of *ddrF*, *ddrG*, *ddrJ*, *ddrK*, *ddrL* and *ddrP* were not found in both DGT and DDI strains (Table 1). Next, we compared nucleotide sequences of the remaining 10 *ddr* genes between DRD and DGT or DDI to measure the sequence similarity. First of all, the 10 *ddr* genes could be categorized into three groups based on the difference of nucleotide length among them (Fig. 1). Each length of *ddrA*, *E*, *N* and *O* genes in DRD was similar to that of homologues in DGT and DDI. On the contrary, the lengths of *ddrC*, *D*, *H* and *I* genes were shorter in DGT and DDI than DRD, but quite similar between DGT and DDI. Genes of *ddrB* and *M* showed different lengths in *Deinococcus* species tested.

A DNA sequence similarity score was calculated by a comparison between DRD and DGT or DRD and DDI. We showed only one region having similarity score exceeding 70% over at least 100 bp-aligned sequences (Fig. 1). The *ddrA*, *E*, *N* and *O* genes belonging to the first group showed similarity over their entire length between DRD and DGT/DDI, except *ddrN*: it was partially similar only between DRD and DDI. As expected, the DRD genes belonging to the second group, *ddrC*, *D*, *H* and *I*, exhibited DNA homology partially because these genes are shorter in DGT and DDI than DRD.

Table 1. Annotation of *ddr* genes in *D. radiodurans* (DRD), *D. geothermalis* (DGT) and *D. deserti* (DDI)

Common name	Gene ID			Temp (˚C)[a]
	DRD	DGT	DDI	
ddrA	DR0423	Dgeo0977	Deide09150	55
ddrB	DR0070	Dgeo0259	Deide02990	63
ddrC	DR0003	Dgeo0047	Deide23280	62
ddrD	DR0326	Dgeo2186	Deide01160	63
ddrE	DR0194	Dgeo1282	Deide11220	55
ddrF	DR0219	–	–	–
ddrG	DR0227	–	–	–
ddrH	DR0438	Dgeo0438	Deide20641	55
ddrI	DR0997	Dgeo0997	Deide12530	55
ddrJ	DR1263	–	–	–
ddrK	DR1264	–	–	–
ddrL	DR1439	–	–	–
ddrM	DR1440	Dgeo0089	Deide21810	56
ddrN	DR2441	Dgeo0078	Deide02930	55
ddrO	DR2574	Dgeo0336	Deide20570	55
ddrP	DRB0100	–	–	–

[a]annealing temperatures that were used in PCR assay

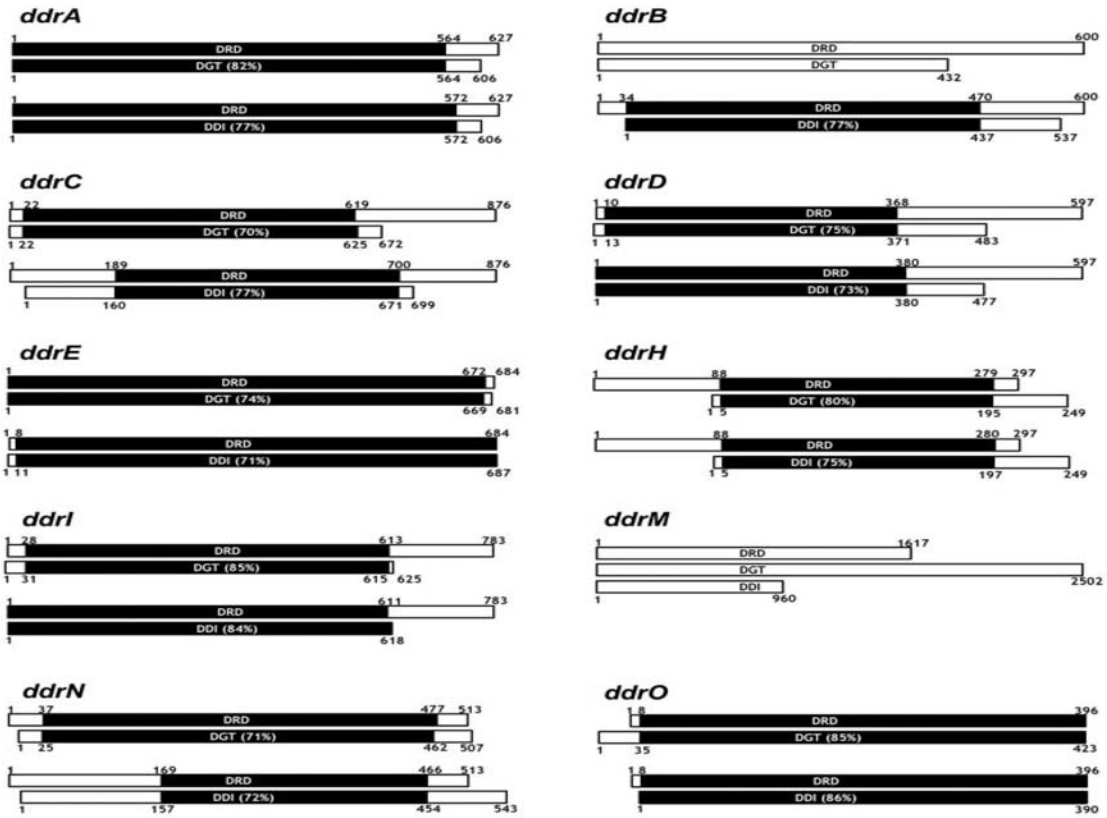

Fig. 1. Comparison of DNA sequence of the *ddr* genes. The whole region of *ddr* was indicated by white box, and the conserved region between *D. radiodurans* (DRD) and *D. geothermalis* (DGT) or DRD and *D. deserti* (DDI) is indicated by black box. The percent DNA similarity is given in parenthesis. The numbers refer to the position of nucleotide within each corresponding gene.

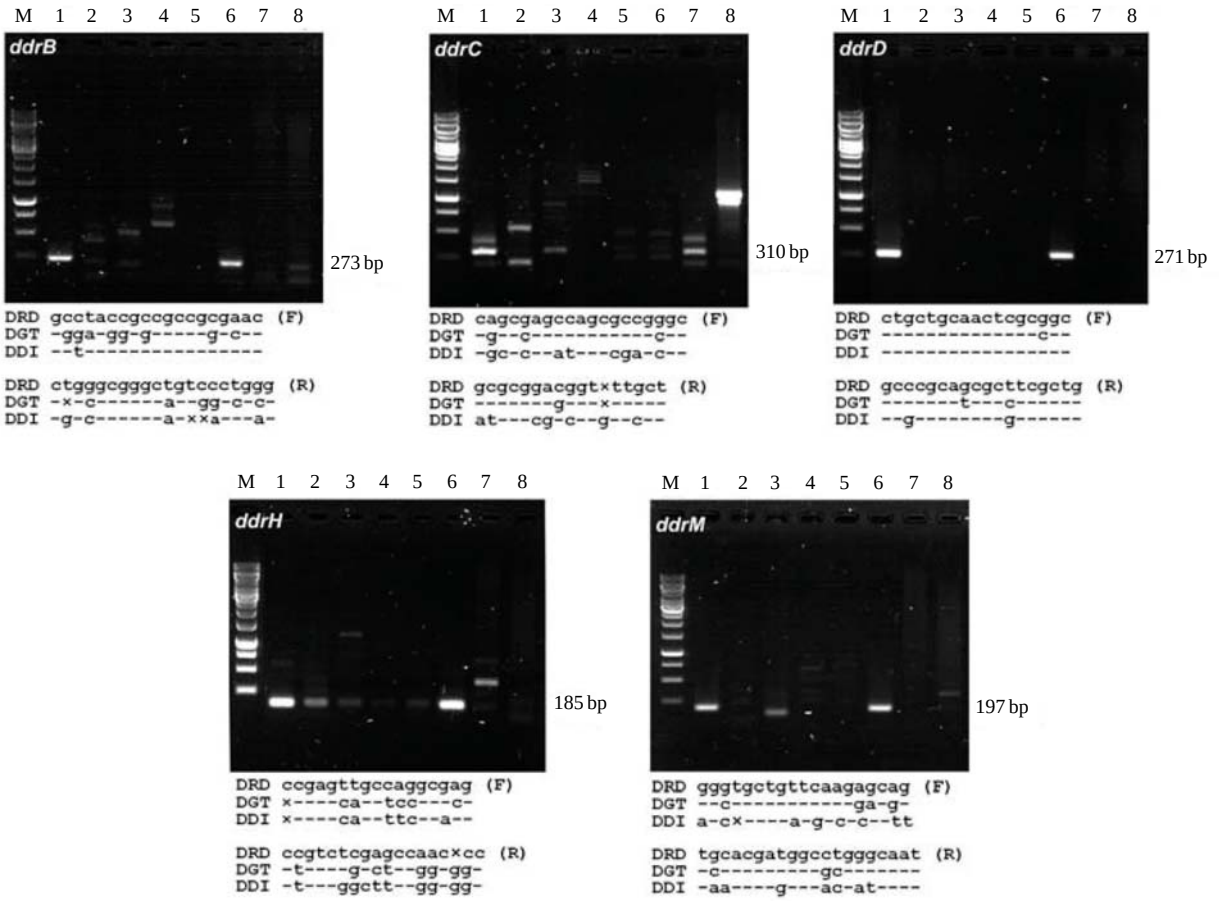

Fig. 2. PCR-based detection of the *ddr* genes having high genetic variation. DNA was isolated from different species of *Deinococcus*, followed by PCR analysis and agarose gel electrophoresis. Lanes: M, molecular size marker; 1-8, *D. radiodurans* (lane 1), *geothermalis* (2), *deserti* (3), *radiopugnans* (4), *proteolyticus* (5), *radiophilus* (6), *murrayi* (7) and *indicus* (8). The numbers on the right indicate the expected size of PCR product. The deviated nucleotide sequences within primers of *D. geothermalis* (DGT) or *D. deserti* (DDI) were aligned with corresponding sequences of *D. radiodurans* (DRD) below panels. PCR was performed with forward (F) and reward (R) primers which was designed based on the sequence of DRD.

However, the entire sequence of *ddrI* gene in DGT and DDI was similar to a partial region of DRD *ddrI*, whereas the other three genes were partially similar between DGT/DDI and DRD. As to the third group genes, *ddrB* had DNA homology only between DRD and DDI, and *ddrM* didn't share any sequences with DNA homology among three species. These diverse genetic variations of *ddr* genes suggest the possibility that the function of some Ddr proteins may not be conserved within *Deinococcus* sp. Especially, *ddrM* cannot be expected to play the same role in DRD, DGT and DDI.

2. PCR detection of *ddr* genes

PCR based assays were carried out for the detection of 10 *ddr* genes in DRD, DGT, DDI, DRP, DPL, DRPH, DMI and DIS to investigate the distribution of *ddr* genes in *Deinococcus* sp. We have compared the sequences of DRD *ddr* genes with corresponding sequences from DGT and DDI. Alignment of these sequences allowed the selection of a pair of oligonucleotides suitable as primers in PCR which have the least variability in the sequences. Primers were designed based on the sequences of DRD *ddr* genes (Figs. 2 and 3). Basically, PCR amplification was performed at annealing temperature of 55°C. When a predicted PCR product was not amplified from DRD genomic DNA, the annealing temperature was increased until getting the PCR product. The annealing temperature of each *ddr* gene was listed in Table 1.

Based on PCR results, *ddr* genes were categorized into two groups: (i) genes were not amplified from DGT and/or DDI genomic DNA; *ddrB*, *C*, *D*, *H* and *M* (Fig. 2), and (ii)

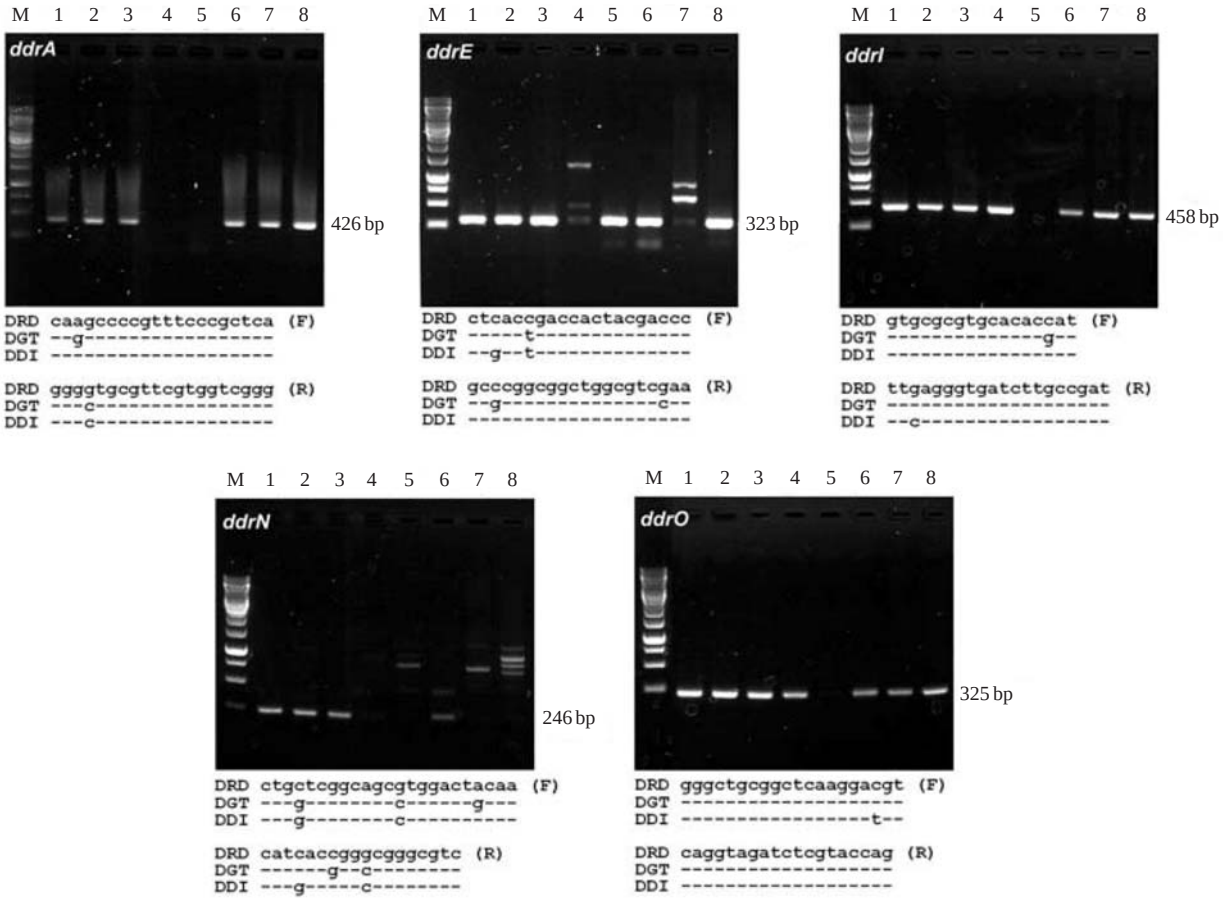

Fig. 3. PCR-based detection of the *ddr* genes which are well conserved among *Deinococcus* species. Refer to the legend of Fig. 2.

Table 2. Distribution of the *ddr* genes in different species of *Deinococcus*

Lane	Species	PCR detection of genes									
		ddrA	*ddrB*	*ddrC*	*ddrD*	*ddrE*	*ddrH*	*ddrI*	*ddrM*	*ddrN*	*ddrO*
1	*D. radiodurans*	+	+	+	+	+	+	+	+	+	+
2	*D. geothermalis*	+	−	−	−	+	+	+	−	+	+
3	*D. deserti*	+	−	−	−	+	−	+	−	+	+
4	*D. radiopugnans*	−	−	−	−	−	−	+	−	−	+
5	*D. proteolyticus*	−	−	−	−	+	+	−	−	−	−
6	*D. radiophilus*	+	+	−	+	+	+	+	+	+	+
7	*D. murrayi*	+	−	−	−	−	−	+	−	−	+
8	*D. indicus*	+	−	−	−	+	−	+	−	−	+

genes were amplified from both DGT and DDI genomic DNA; *ddrA*, *E*, *I*, *N* and *O* (Fig. 3). The predicted amplification product of *ddrB*, *D* and *M* was observed only in DRPH. The expected DNA fragment of *ddrH* was clearly visible in samples from DGT and DRPH, while a very weak band was produced with DPL. In the case of *ddrC*, nonspecific and multiple fragments were observed in DRD as well as other species of *Deinococcus* (Fig. 2). We can easily guess that mismatches in the sequences bound by primers resulted in the appearance of many nonspecific secondary products, because the sequences of DGT and DDI differed from those of DRD in many base pairs (Fig. 2).

On the contrary, group II genes was found to be well conserved among *Deinococcus* species compared to group I genes. The predicted amplification product of *ddrN* was seen only in DRD, DGT and DDI, but a 458 (*ddrI*) and a 325

(*ddrO*) bp fragment were specifically amplified from all species of *Deinococcus* tested in this study, except DPL (Fig. 3). This suggests that differences in only a few base pairs within priming binding sites are not a big problem when amplifying target genes. Although a second smaller band was also seen in conjunction with the 496-bp fragment of *ddrE* only in DRPH, the smaller fragment may have been the result of incomplete extension or an artifact of Taq polymerase. Interestingly, *ddrD* belonging to group I has a slight mismatch in primer binding site, but no product was produced in DGT and DDI despite repeated attempts. The PCR results are summarized in Table 2. Collectively, aside from DRD, DRPH reacted with most *ddr* primers except *ddrC*, whereas DPL reacted with only *ddrE* primer (Table 2).

Among 16 *ddr* genes, only four *ddr* genes, *ddrA*, *B*, *C* and *D* have been studied concerning its radiation resistance. DdrA (Harris *et al.* 2004) and *DdrB* (Norais *et al.* 2009) are single-stranded DNA binding proteins and contribute to the IR resistance of DRD (Tanaka *et al.* 2004; Bentchikou *et al.* 2010). However, the *ddrC* and *ddrD* single mutants do not increase sensitivity to IR (Tanaka *et al.* 2004). The entire sequence of *ddrA* is quite well conserved in DRD, DGT and DDI compared to *ddrB*, *C* and *D* (Fig. 1). In addition, the expected *ddrA* fragment was amplified from the genomic DNA of six *Deincoccus* species (Fig. 3), while the product of *ddrC* and *D* was seen only in DRD and DRPH. The product of *ddrB* was produced only in DRD. Taken together, this suggests that DdrA plays a more important role than DdrB, C and D in radiation resistance of not only DRD but also *Deinococcus* sp. The PCR fragment of *ddrO* and *ddrI* was observed in seven *Deinococcus* species (Fig. 3), but the length of *ddrI* was shorter in DGT and DDI than DRD (Fig. 1), suggesting that *ddrO* is the most well conserved gene of 16 *ddr* genes. DdrO was proposed as a global regulator of the radiation-desiccation response regulon in the *Deinococcus* lineage (Makarova *et al.* 2007). Therefore, further experiments are needed to investigate the role of DdrO in controlling radiation resistance of *Deinococcus*.

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REFERENCES

Anderson AW, Nordan HC, Cain RF, Parrish G and Duggan D. 1956. Studies on a radio-resistant micrococcus. I. Isolation, morphology, cultural characteristics, and resistance to gamma radiation. *Food Technol.* **10**:575-578.

Bentchkou E, Servant P, Coste G and Sommer S. 2010. A major role of the RecFOR pathway in DNA double-strand-break repair through ESDSA in *Deinococcus radiodurans*. *PLoS Genet.* **6**:e1000774.

Cox MM and Batistta JR. 2005. *Deinococcu radiodurans*-the consummate survivor. *Nature* **6**:882-892.

Daly MJ *et al*. 2004. Accumulation of Mn (II) in *Deinococcus radiodurans* facilitates gamma-radiation resistance. *Science* **306**:1025-1028.

De Groot AD *et al.* 2009. Alliance of proteomics and genomics to unravel the specificities of Sahara bacterium *Deinococcus deserti. PLoS Genet.* **5**:e1000434.

Diehl JF. 1995. Biological effects of ionizing radiation. pp. 89-132. *In*: Safety of Irradiated Foods. Marcel Dekker Inc., New York, NY.

Goshal D *et al.* 2005. How radiation kills cells: survival of *Denicoccus radiodurans* and *shewanella oneidensis* under oxidative stress. *FEMS Microbiol. Rev.* **29**:361-375.

Harris DR, Tanaka M, Saveliev SV, Jolivet E, Earl AM, Cox MM and Battista JR. 2004. Preserving genome integrity: The DdrA protein of *Deinococcus radiodurans* R1. *PLoS Biol.* **2**:e304.

Makarova KS, Aravind L, Daly MJ and Koonin EV. 2000. Specific expansion of protein families in the radioresistant bacterium *Deinococcus radiodurans. Genetica* **108**:25-34.

Makarova KS *et al.* 2007. *Deinococcus geothermalis*: The pool of extreme radiation resistance genes shrinks. *PLoS One* **9**:e955.

Norais C, Chitteni-Pattu S, Wood EA, Inman RB and Cox MM. 2009. DdrB protein, an alternative *Deinococcus radiodurans* SSB induced by ionizing radiation. *J. Biol. Chem.* **284**: 21402-21411.

Omelchenko MV, Wolf YI, Gaidamakova E, Matrosova VT, Vasilenko A, Zhai M, Daly MJ, Koonin EV and Makarova KS. 2005. Comparative genomics of *Thermus thermophilus* and *Deinococcus radiodurans*: divergent routes of adaptation to thermophily and radiation resistance. *BMC Evol. Biol.* **5**:57.

Symons MCR. 1994. Direct and indirect damage to DNA by ionizing radiation. *Radiat. Phys. Chem.* **43**:403-405.

Tanaka M, Earl AM, Howell HA, Park MJ, Eisen JA, Peterson SN and Battista JR. 2004. Analysis of *Deinococcus radiodurans*'s transcriptional response to ionizing radiation and

desiccation reveals novel proteins that contribute to extreme radioresistance. *Genetics* **168**:21-33.

Udupa KS, O'Cain PA, Mattimore V and Battista JR. 1994. Novel ionizing radiation-sensitive mutants of *Deinococcus radiodurans*. *J. Bacteriol.* **24**:7439-7446.

White O *et al*. 1999. Genome sequence of the radioresistant bacterium *Deinococcus radioduran* R1. *Science* **286**:1571-1577.

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