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Comparison of the Genomes of Deinococcal Species Using Oligonucleotide Microarrays

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The bacterium *Deinococcus radiodurans* is one of the most resistant organisms to ionizing radiation and other DNA-damaging agents. Although, at present, 30 *Deinococcus* species have been identified, the whole-genome sequences of most species remain unknown, with the exception of *D. radiodurans* (DRD), *D. geothermalis*, and *D. deserti*. In this study, comparative genomic hybridization (CGH) microarray analysis of three *Deinococcus* species, *D. radiopugnans* (DRP), *D. proteolyticus* (DPL), and *D. radiophilus* (DRPH), was performed using oligonucleotide arrays based on DRD. Approximately 28%, 14%, and 15% of 3,128 open reading frames (ORFs) of DRD were absent in the genomes of DRP, DPL, and DRPH, respectively. In addition, 162 DRD ORFs were absent in all three species. The absence of 17 randomly selected ORFs was confirmed by a Southern blot. Functional classification showed that the absent genes spanned a variety of functional categories: some genes involved in amino acid biosynthesis, cell envelope, cellular processes, central intermediary metabolism, and DNA metabolism were not present in any of the three deinococcal species tested. Finally, comparative genomic data showed that 120 genes were *Deinococcus*-specific, not the 230 reported previously. Specifically, *ddrD*, *ddrO*, and *ddrH* genes, previously identified as *Deinococcus*-specific, were not present in DRP, DPL, or DRPH, suggesting that only a portion of *ddr* genes are shared by all members of the genus *Deinococcus*.

Keywords: *Deinococcus*, comparative genomic hybridization (CGH) microarray, *ddr*

The genus *Deinococcus*, and in particular the species *D. radiodurans*, shows a high level of resistance to the lethal effects of DNA-damaging agents, including ionizing radiation (IR) and ultraviolet radiation, diverse genotoxic chemicals, and desiccation [1, 15, 31]. For example, the radiation dose yielding 10% survival (D_{10}) of *D. radiodurans* is ~16 kGy, whereas that of *Escherichia coli* is ~0.7 kGy [23]. The extraordinary survival, without mutagenesis, of *Deinococcus* bacteria following irradiation has given rise to a question: how is radiation-damaged DNA repaired in this microorganism? First of all, the radioresistance of *D. radiodurans* cannot be related to prevention of DNA damage, because *D. radiodurans* DNA is as susceptible to IR-induced DNA double-strand breaks (DSBs) as that of radiosensitive bacteria such as *E. coli* [4]. Indeed, the *D. radiodurans* genome sustains hundreds of DNA DSBs after exposure to 10 kGy of IR, whereas only a few such DSBs can kill an *E. coli* cell [3].

After the complete genome sequence of *D. radiodurans* was made available, it was widely expected that novel proteins critical for IR resistance and involved in reassembly of the shattered chromosome post-irradiation would be discovered. However, comparative analyses demonstrated that the *D. radiodurans* genome encodes almost all of the typical prokaryotic DNA repair genes and pathways. Furthermore, its DNA repair systems appear to be less complex and diverse than those of radiation-sensitive bacteria [10, 18, 23]. This suggests that this species uses a relatively conventional DNA repair system, but with greater efficiency than other microorganisms. The possibility that *D. radiodurans* possesses a novel DNA repair mechanism remains, because its genome contains 1,002 unique ORFs of unknown function [32]. A series of studies into the DNA repair system revealed the mechanism by which *D. radiodurans* reconstructs a functional genome from radiation-induced chromosomal fragments; namely, extended synthesis-dependent strand annealing (ESDSA) [28, 35]. This process depends on not only a functional RecF pathway, an auxiliary pathway for recombinational DNA

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repair in cells devoid of RecBCD proteins, but also on a number of *Deinococcus*-specific proteins such as Ddr (DNA damage response) A and B, which are induced by irradiation [2, 29].

Of about 30 *Deinococcus* species, the genomes of two, *D. geothermalis* and *D. deserti*, have been sequenced and compared with that of *D. radiodurans* [11, 19]. These studies have reduced the confirmed number of novel genes that may be involved in recovery from IR. Because the distinctive characteristics of *Deinococcus* bacteria are likely in part determined by genes that are unique to this genus, whole-genome comparison is a useful tool for identifying *Deinococcus*-specific proteins that are involved in radiation resistance. However, whole-genome sequencing is an expensive and laborious technique. Recently, comparative genomic hybridization (CGH) has been used to compare unsequenced bacterial genomes and understand the role of genes unique to a particular species [26].

We used CGH to compare the genome of *D. radiodurans* to those of three other *Deinococcus* species (*D. radiopugnans*, *D. proteolyticus*, and *D. radiophilus*), using CombiMatrix oligonucleotide arrays based on *D. radiodurans* sequences. Some *Deinococcus*-specific genes, detected previously in *D. radiodurans*, *D. geothermalis*, and *D. deserti*, were absent in *D. radiopugnans*, *D. proteolyticus*, and *D. radiophilus*.

Materials and Methods

Bacterial Strains and Growth Conditions

D. radiodurans R1 (ATCC 13939), *D. radiopugnans* (ATCC 19172), *D. radiophilus* (ATCC 27602), and *D. proteolyticus* (ATCC 35074) were obtained from the American Type Culture Collection (ATCC). Strains were cultured to the 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.

Genomic DNA Isolation

Genomic DNA extraction was performed as described previously [30]. 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 TE buffer (10 mM Tris-HCl, 0.1 mM EDTA; pH 8.0) containing 2 mg/ml lysozyme (Sigma Chemical, St. Louis, Mo, USA). This mixture was incubated at 37°C for 30 min. Proteinase K/SDS solution [20 ml; 10 μg/ml Proteinase K, 10% (w/v) SDS; Sigma Chemical Co.] was added and the 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). The 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 of nuclease-free water, and stored at −20°C.

Microarray Experiments

The CombiMatrix 12K CustomArray DNA chip (CombiMatrix Corp., Mukilteo, WA, USA) was prepared based on putative ORF sequences of *D. radiodurans* R1. A total of 3,128 oligonucleotide probes for 3,128 of the 3,187 annotated ORFs of *D. radiodurans* R1 were created by the CombiMatrix Automated Probe-Design Suite. Probes were from 35 to 40 nucleotides in length. Probe design files for array synthesis were generated with Layout Designer (CombiMatrix Corp.). Each oligonucleotide probe was spotted in triplicate. Array-based CGH experiments were performed using the two-color approach according to the manufacturer's instructions (Agilent Genomic DNA Labeling Kit PLUS protocol). Briefly, genomic DNA (2.5 μl) was labeled using random primers (5 μl) for the exo-Klenow fragment (40 U) and 1 mmol Cy5-dUTP (sample) or Cy3-dUTP (reference) at 37°C for 2 h. Uncoupled fluorescent nucleotides were removed using a Microcon YM-30 filter in a 1.5-ml microfuge tube, and purified for hybridization as described in the manufacturer's instructions. The hybridization procedure is summarized below. Prehybridization buffer [50 μl; 6× SSPE, 0.05% (v/v) Tween 20, 20 mM EDTA, 5× Denhardt's solution, 100 ng salmon sperm DNA per μl, and 0.05% (w/v) SDS] was added to the microarray through the solution portals and prehybridization was performed at 65°C for 15 min. Labeled DNA (5 μl) was added to the hybridization buffer [6× SSPE, 0.05% (v/v) Tween 20, 0.05% (w/v) SDS] to a final volume of 50 μl and denatured by heating to 95°C for 4 min. Prehybridized slides were washed with 6× SSPET [6× SSPE, 0.05% (v/v) Tween 20], dried, and incubated in hybridization buffer at 50°C for 16 h. Slides were washed at 50°C with 6× SSPET for 5 min and then at RT with 3× SSPET for 1 min and again at RT with 0.5× SSPET for 1 min and finally at RT with PBST [2× PBS, 0.1% (v/v) Tween 20] for 1 min. Slides were dried immediately and scanned for fluorescent intensity using an Axon GenePix 4000B microarray scanner (Molecular Devices, Sunnyvale, CA, USA). Data were recorded as 16-bit multi-image TIFF files.

Microarray Data Analysis

The signal intensity of each spot in the microarray was quantified using the GenePix Pro 6.0 software (Molecular Devices). Poor features were excluded from further analysis if they contained abnormalization or were within regions of high fluorescent background. Signal intensities were corrected by subtracting the local background value [26]. Data normalization was performed using the global normalization method [35]. Briefly, global normalization was examined using the following equation: $N_i=(R_i-B_i)/M$, where N is the normalized signal intensity, R is the fluorescence signal intensity, *i* is the gene index, B is the background signal, and M is the median of total signal intensity. Statistical significance of the data was determined by one-way ANOVA, and *p* values less than 0.05 were taken as statistically significant. Sample/reference (*D. radiodurans*) ratios of signal intensities were calculated and transformed to logarithm base 2. ORFs were regarded as absent if the final ratios of signal intensities were less than −1 on the $\log_2$ scale. We used the Institute for Genomic Research (TIGR) database (http://cmr.jcvi.org/cgi-bin/cmr/shared/genome.cgi) to check the functions of *D. radiodurans* ORFs.

Southern Blot Analysis

Southern blot was used to validate the array results, using the probes employed to generate each ORF present in the *D. radiodurans* R1 genome (Table S1). Southern blot hybridization was carried out using a digoxigenin DNA labeling and detection kit as per the manufacturer's instructions (Roche Applied Science). Probe DNA was adjusted to 25 ng/ml with autoclaved double-distilled water to a final volume of 15 µl. DNA was denatured in a boiling water bath for 10 min and then quickly chilled in an ice bucket. DNA samples were then mixed with labeling solution that contained 10× concentrated hexanucleotides, 10× dNTP labeling mix (1 mM dATP, 1 mM dCTP, 1 mM dGTP, 0.65 mM dTTP, and 0.35 mM DIG-11-dUTP, pH 7.5), and 2U Klenow enzyme. The mixtures were then incubated overnight at 37°C. Prior to adding the probes, the DNA-labeled mixtures were denatured by boiling for 5 min and rapidly cooled in ice. The denatured DIG-labeled DNA probe was added to preheated DIG hybridization buffer (3.5 ml/100 cm^2 nylon membrane) containing 0.5 M Na_2HPO_4 (pH 7.2), 1% (w/v) BSA, 1 mM EDTA, and 7% (w/v) SDS. The probe and hybridization mixtures were added to genomic DNA on nylon membranes and incubated for 6–12 h, with gentle agitation at the appropriate hybridization temperature [calculated from both the GC content and homology of the probes to their target sequences according to the following equation: T_m=49.82+0.41 (% G+C)–(600/I), where I=length of hybrid in base pair; $T_{opt.}=T_m-20$ ~25°C). After hybridization, the membranes were rinsed briefly (5 min) in washing buffer containing 0.1 M maleic acid, 0.15 M NaCl (pH 7.5), and 0.3% (v/v) Tween 20. Membranes were then incubated for 30 min in 100 ml of blocking solution (supplied by the manufacturer). Antibody solution was prepared as follows: anti-Digoxigenin-AP was diluted in 20 ml of blocking solution (150 mU/ml). The diluted anti-Digoxigenin-AP was added to the membranes and incubated for 30 min, followed by washing twice for 15 min each in 100 ml of washing buffer. For color development, 0.2 ml of NBT/BCIP (supplied by the manufacturer) was added to detection buffer (10 ml; 0.1 M Tris-HCl, 0.1 M NaCl, pH 7.5) and the membranes were incubated in 10 ml of freshly prepared detection buffer overnight in the dark.

Survival Curves

Exponential-phase *Deinococcus* spp. cultures (2×10^7 CFU/ml) were irradiated at room temperature using a ^{60}Co gamma irradiator (IR-79 point source; AECL, Ottawa, ON, Canada) in the radiation dose range from 0 to 20 kGy for 1 h, and then evaluated for their ability to survive IR. Irradiated cultures were diluted, plated in triplicate on TGY agar plates, and incubated for 3 days at 30°C, and then the CFU was determined.

RESULTS AND DISCUSSION

CGH Microarray Analysis

The genomic contents of three *Deinococcus* species, *D. radiopugnans* (DRP), *D. proteolyticus* (DPL), and *D. radiophilus* (DRPH), were analyzed by CGH using custom oligonucleotide arrays composed of *D. radiodurans* (DRD) ORF-specific probes. Of all ORFs showing a statistically significant expression ratio ($p<0.05$), we defined a gene as absent when the $\log_2$ sample/reference intensity ratio was less than −1 [24]. Absent ORFs in DRP, DPL, and DRPH are shown in Supplementary Table S2 to S4. DRP, DPL, and DRPH did not possess 871, 444, and 464 ORFs of those detected in DRD, respectively (Fig. 1). Each species shared a proportion of absent ORFs: 267 ORFs between DRP and DPL, 250 between DPL and DRPH, and 292 between DRP and DRPH. Consequently, 162 DRD ORFs were not detected in any of the three *Deinococcus* spp. tested (Fig. 1).

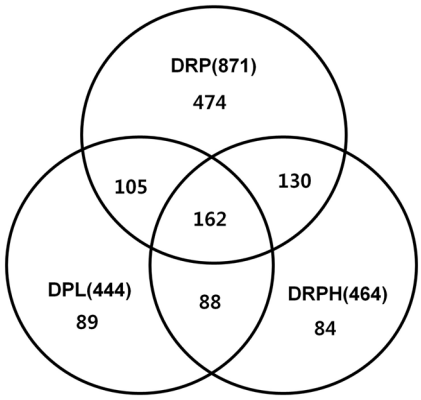

Fig. 1. Venn diagram showing the distribution of absent genes of test strains in CGH microarray.
The number of total absent genes in each strain is represented in a parenthesis.

The proportion of total absent genes in each functional category is shown in Table 1. When the proportion of total absent ORFs was compared with that of each functional category, absent ORFs were found to be widespread throughout the genome, regardless of the deinococcal species tested. The unusually low proportion of "mobile and extra chromosomal element function" genes may be attributable to the low number of probes spotted onto arrays: 16 probes of 67 ORFs (Table 1). DRD possesses a number of genes that encode mechanisms of protein degradation and amino acid (AA) catabolism to compensate for incomplete AA biosynthetic pathways [23, 31]. We found that the rate of absent genes involved in AA biosynthesis was half of the average (Table 1), suggesting that these genes are essential and necessary for AA assimilation. Because the genome content of DRD and DRP was markedly different, especially in those genes involved in cellular processes, it may be that DRP has evolved a different lifestyle. Therefore, investigation of the characteristics of DRP may result in novel applications for this interesting and apparently unique bacterium.

Genes Absent in DRP, DPL, and DRPH

A total of 162 ORFs were absent in all three deinococcal species (Fig. 1 and Table S5). Southern blot hybridization confirmed the absence of 16/17 of these genes, the exception being DRA0135 (Fig. 2). This was mostly

Table 1. Distribution of absent ORFs for each test strain by functional categories.

Functional category[a]	Number of ORFs		Number of ORFs absent			Absent ORFs (%)		
	DRD genome	array	DRP	DPL	DRPH	DRP	DPL	DRPH
Amino acid biosynthesis	82	82	12	6	8	14.6	7.3	9.8
Biosynthesis of cofactors, prosthetic groups, and carriers	65	65	16	8	9	24.6	12.3	13.8
Cell envelope	102	102	30	22	18	29.4	21.6	17.6
Cellular process	90	90	38	12	16	42.2	13.3	17.8
Central intermediary metabolism	157	157	41	22	23	26.1	14.0	14.6
DNA metabolism	84	83	29	13	15	34.9	15.7	18.1
Energy metabolism	206	206	43	21	19	20.9	10.2	9.2
Fatty acid and phospholipid metabolism	55	55	12	6	8	21.8	10.9	14.5
Hypothetical proteins	1,482	1,475	429	226	220	29.1	15.3	14.9
Mobile and extrachromosomal element function	67	16	3	1	1	18.8	6.3	6.3
Protein fate	88	88	25	15	18	28.4	17.0	20.5
Protein synthesis	119	119	34	15	22	28.6	12.6	18.5
Purines, pyrimidines, nucleosides, and nucleotides	54	54	13	7	9	24.1	13.0	16.7
Regulatory functions	130	130	36	12	18	27.7	9.2	13.8
Transcription	29	29	7	4	6	24.1	13.8	20.7
Transport and binding proteins	198	198	54	25	29	27.3	12.6	14.6
Unknown function	179	179	49	29	25	27.4	16.2	14.0
Total	3,187	3,128	871	444	464	27.8	14.19	14.83

[a]Functional categories of *D. radiodurans* ORFs were based on information from The Institute for genome Research Web site (http://cmr.jcvi.org/tigr-scripts/CMR/shared/genomes.cgi).

consistent with the CGH data. It seems likely that a section of DRA0135, which hybridizes to the probe on the chip, has suffered from genetic variations. Excluding ORFs that encode hypothetical proteins or those with unknown function, 77 ORFs were functionally classified using the TIGR database (Table 2).

Two (*trpF* and *trpG*) of the seven *trp* genes for tryptophan biosynthesis were absent in all three *Deinococcus* spp. (Table 2). The classical structure of the *trp* operon is *trpE-G-D-C-F-B-A* [12]. However, in DRD, this structure was split into one (*trpF* [DR0123], *trpC* [DR1426]) or two *trp* genes (*trpB* [DR0941]–*trpA* [DR0942], *trpG* [DR1766]–*trpD* [DR1767]) enclosed between non-*trp* genes. In addition, DRD had an extra copy of the *trpE* gene (*trpE-1* [DR0196], *trpE-2* [DR1791]). In this context, it is not unusual that some *trp* genes were not detected in the other *Deinococcus* species. It was previously reported that the primary biosynthetic pathways of some AAs (*e.g.*, cysteine, lysine, and serine) were incomplete in DRD [31], suggesting the existence of secondary biosynthesis pathways for these AAs [20, 31]. Therefore, it is possible that DRP, DPL, and DRPH may have a unique mechanism for tryptophan biosynthesis.

The cell envelope of DRD, which consists of at least six layers, is unusual in terms of its structure and composition. The outermost layer is made up of regularly packed hexagonal protein subunits (the S-layer, or hexagonally packed intermediate layer) [18]. DRD encoded a number of S-layer proteins (DR0383, DR1115, DR1185, DR2508, DR2577) that may provide environmental protection (*e.g.*, resist desiccation or thermal stresses) [14], whereas DR0383 and DR2508 were not detected in DRP, DPL, or DRPH (Table 2), suggesting that one of the characteristic features of DRD, resistance to desiccation, may not appear in these three *Deinococcus* species.

The *gabD* gene, which was absent in DRP, DPL, and DRPH (Table 2), encodes succinate semialdehyde dehydrogenase (SSADH), which metabolizes the succinate semialdehyde released from γ-aminobutyrate. This cycle is carbon and nitrogen balanced, and the reductive part of the TCA cycle would generate additional energy [25]. However, because *yneI*-encoded SSADH can substitute for the *gabD*-encoded SSADH activity in *E. coli* [8], it is possible that a *yneI* ortholog exists in one or more of the three *Deinococcus* species investigated here. It is noteworthy that orthologs of both *gabD* and *yneI* have been detected in only 65/495 sequenced genomes [8]. The functional substitution of a gene with an extra gene with a similar function is more evident in the case of *ssb*. Single-stranded DNA-binding (SSB) protein is essential in all organisms and is involved in DNA replication, recombination, and repair [6]. However, *ssb* was not detected in DRP, DPL, or DRPH (Table 2).

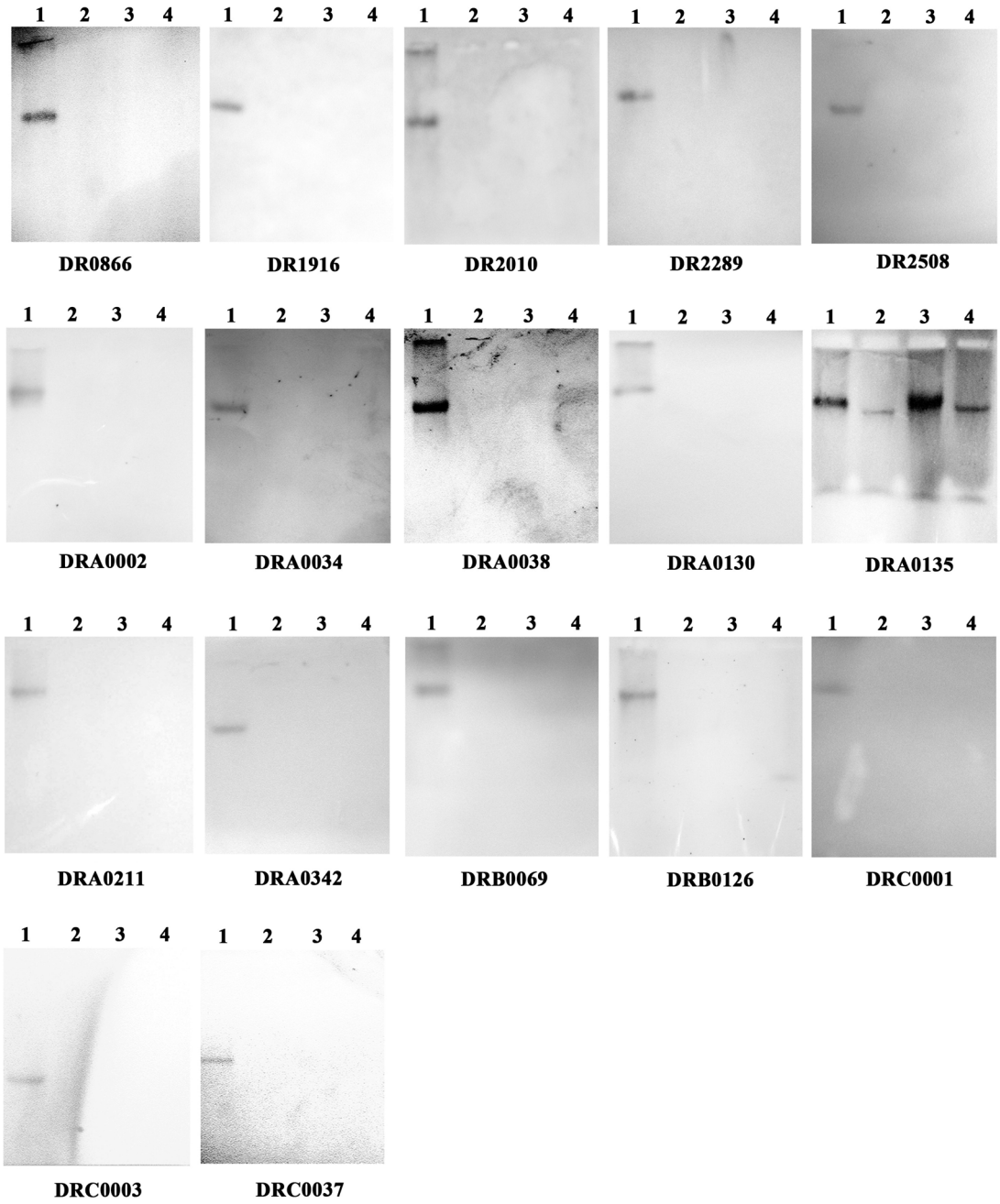

Fig. 2. CGH microarray data validation by using Southern blot analysis.
Lane 1, *D. radiodurans* (DRD); lane 2, *D. radiopugnans* (DRP); lane 3, *D. proteolyticus (DPL)*; lane 4, *D. radiophilus* (DRPH).

DRD has an additional SSB known as DdrB, which is the prototype of a new bacterial SSB family [22]. As expected, *ddrB* was present in all three *Deinococcus* species (Table S2 to S4). Expansion of the Nudix (MutT) hydrolase family is one of the most prominent features of the DRD genome. DRD encodes 23 Nudix family proteins that are likely involved in the decomposition of damage products under stress conditions such as desiccation and/or irradiation [17]. It seems reasonable to hypothesize that other *Deinococcus* species also possess a variety of Nudix hydrolases. Therefore, the loss of DR2356 (Table 2) can probably be compensated for by the presence of other Nudix hydrolases.

The *recG* gene is a structure-specific DNA helicase that is found in nearly all bacterial species [27]. RecG is involved in branch migration and resolution of Holliday junctions during DNA replication, recombination, and repair [16]. Deletion of *recG* resulted in an obvious growth

Table 2. Common absent genes.

ID	Name	Description
Amino acid biosynthesis		
DR0123	*trpF*	Phosphoribosyl anthranilate isomerase
DR1766	*trpG*	Anthranilate synthase component II
Biosynthesis of cofactors, prosthetic groups, and carriers		
DR0153	*ribD*	Riboflavin-specific deaminase
DR2405	*ubiE*	Ubiquinone/menaquinone biosynthesis methyltransferase
Cell envelope		
DR0383	DR0383	S-Layer-like array-related protein
DR1232	DR1232	Pilin; type IV; putative
DR1372	DR1372	Hypothetical protein
DR2102	DR2102	Plasmid stability protein; putative
DR2149	DR2149	Innermembrane protein; 60 kDa; putative
DR2508	*hpi*	Hexagonally packed intermediate-layer surface protein
DRA0034	*amsG*	UDP-galactose–lipid carrier transferase
DRA0035	DRA0035	O-Antigen transporter RfbX; putative
DRA0037	DRA0037	Glycosyltransferase
DRA0038	DRA0038	Rhamnosyltransferase; putative
DRA0041	*rfbB*	Thymidinediphosphoglucose 4,6-dehydratase
DRA0042	*rfbA*	Glucose-1-phosphatethymidylyl transferase
DRC0013	DRC0013	*N*-Acetylmuramoyl-L-alanine amidase; putative
Cellular process		
DR0433	DR0433	Beta-lactamase; putative
DR1538	*osmC*	Osmotically inducible protein C
DR1756	DR1756	Periplasmic serine protease Do; putative
DR2242	DR2242	Thiol-specific antioxidant protein; putative
DR2257	DR2257	Erythromycin esterase; putative
DRA0002	DRA0002	Chromosome partitioning protein; ParB family
DRA0255	*aac*	Aculeacin A acylase
DRB0129	DRB0129	Hemolysin; putative
Central intermediary metabolism		
DR0249	DR0249	Oxidoreductase; putative
DR0604	DR0604	Acetyl transferase; putative
DR0973	DR0973	Gamma-carboxymuconolactone decarboxylase
DR2279	DR2279	Alcohol dehydrogenase; zinc-containing
DRA0003	*gabD*	Succinate-semialdehyde dehydrogenase
DRA0066	*nagA*	N-Acetylglucosamine-6-phosphate deacetylase
DRA0130	DRA0130	Methyltransferase; putative
DRA0319	*ureAB*	Urease; beta/gamma subunit
DRA0325	DRA0325	*N*-Glycosidase F; putative
DRB0036	DRB0036	Oxidoreductase
DRB0046	*phoB*	Alkaline phosphatase
DRC0036	DRC0036	Oxidative cyclase; putative
DR0026	DR0026	Methyltransferase; putative
DNA metabolism		
DR0100	*ssb*	Single-stranded DNA-binding protein
DR0438	*ddrH*	Hypothetical protein
DR1916	*recG*	DNA helicase RecG
DR2356	DR2356	MutT/nudix family protein
Energy metabolism		
DR1498	*nuoH*	NADH dehydrogenase I; H subunit
DR1503	*nuoD*	NADH dehydrogenase I; D subunit

Table 2. Continued.

ID	Name	Description
DR2353	*ansA*	L-Asparaginase
DRA0067	DRA0067	CoA transferase; subunit B
Fatty acid and phospholipid metabolism		
DRA0143	DRA0143	3-Hydroxybutyryl-CoA dehydrogenase
Protein fate		
DR0112	DR0112	Glutamine cyclotransferase
DR0606	*groES*	Chaperonin
DR1536	DR1536	Serine protease; subtilase family
DR1551	DR1551	Carboxyl-terminal protease; putative
DR1836	*ffh*	Signal recognition particle protein
DRB0069	DRB0069	Serine protease; subtilase family
Protein synthesis		
DR0119	*efp*	Elongation factor P
DR2124	*rpmJ*	Ribosomal protein L36
DR2555	*gatB*	Aspartyl/glutamyl-tRNA amidotransferase subunit B
DRB0094	*rnl*	RNA ligase
Purines, pyrimidines, nucleosides, and nucleotides		
DR2289	*gmk*	Guanylate kinase
Regulatory function		
DR0997	*ddrI*	Transcriptional regulator; FNR/CRP family
DR1042	*padR*	Pex-related protein
DRA0211	DRA0211	Transcriptional regulator; GntR family
DRA0214	DRA0214	Trp repressor-binding protein WrbA; putative
DRB0024	*rsbR*	Sigma-B regulator RsbR
DRB0126	DRB0126	Transcriptional regulator; TetR family
Transcription		
DR2010	*rimM*	16S rRNA processing protein RimM; putative
Transport and binding proteins		
DR0563	*malG*	Maltose ABC transporter; permease protein
DR0565	DR0565	Amino acid ABC transporter; permease protein
DR1302	*potA*	Spermidine/putrescine ABC transporter; ATP-binding protein
DR1665	DR1665	ABC transporter; periplasmic substrate-binding protein; putative
DR2118	*livF*	Branched-chain amino acid ABC transporter; ATP-binding protein
DR2277	DR2277	Amino acid ABC transporter; permease protein
DRA0135	DRA0135	ABC transporter; periplasmic substrate-binding protein; putative
DRA0246	DRA0246	Extracellular solute-binding protein; family 5

defect and a marked decrease in DRD resistance to gamma radiation [33]. Interestingly, we found that the radioresistances of DRP, DPL, and DRPH were lower than that of DRD (Fig. 3). So many genes and factors are surely involved in this phenotype of DRP, DPL, and DRPH, but these data suggest that RecG can be one of the essential factors for the radiation resistance of *Deinococcus* spp.

Deinococcus-Specific Genes

Groot *et al*. [11] identified 230 genes, mostly of unknown function, that are specifically conserved in the three sequenced *Deinococcus* genomes: DRD, *D. geothermalis*, and *D. deserti*. We ascertained the genes conserved in all six *Deinococcus* spp. using CGH. Of the 230 reported by Groot and colleagues, 120 were identified as *Deinococcus*-specific in this study (Table S6). Only 11 ORFs whose function could be predicted are listed in Table 3. Genes contributing to the radioresistance of *Deinococcus*, such as IrrE (also called PprI) [5] and PprA [20], were well conserved (Table 3). Studies using PprI deletion mutants have shown that this protein acts as a general switch controlling downstream DNA repair pathways [13]. Studies performed *in vitro* have suggested that PprA binds preferentially to double-stranded DNA carrying strand breaks, and that it stimulates the DNA end-joining reaction catalyzed by DNA ligases [20].

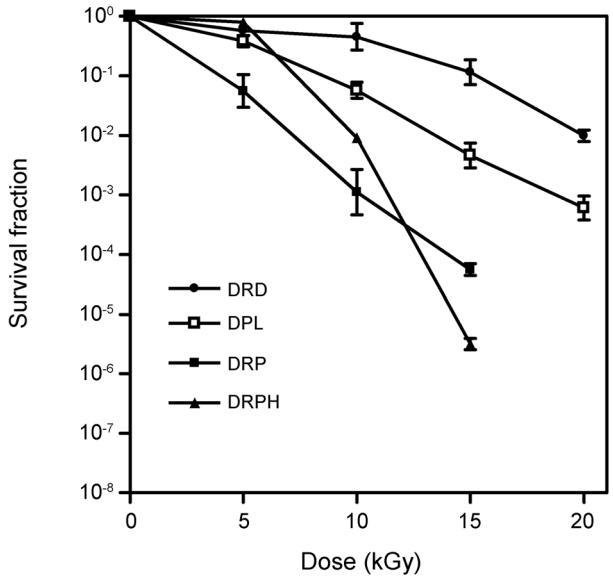

Fig. 3. Representative survival curves for deinococcal species DRD (closed circles), DPL (open squares), DRP (closed squares), and DRPH (closed triangles) following exposure to gamma radiation.
Values are the means ± standard deviation of triplicate experiments (n=6).

DRD genes that encode proteins of unknown function that are induced in response to IR and desiccation were assigned the designation *ddr* (DNA damage response). A total of 16 such genes were differentiated by the letters A–P [29]. Among them, five (*ddrB*, *ddrC*, *ddrD*, *ddrH*, and *ddrO*) have been previously identified as *Deinococcus*-specific [11]. However, our data suggest that three of these (*ddrD*, *ddrH*, and *ddrO*) are not *Deinococcus*-specific (Table S4). Conservation of *ddrB* is entirely explicable because (i) DdrB plays a pivotal role in DNA repair, (ii) deletion of *ddrB* increases the sensitivity of wild type [29], and (iii) this gene encodes a novel bacterial SSB family [22]. However, although both *ddrC* and *ddrD* mutants did not exhibit any increased susceptibility relative to the wild type [29], *ddrC* was conserved in six *Deinococcus* spp. but *ddrD* was not (Table 3 and S6). In addition, DdrO, proposed as the global regulator of the radiation/desiccation response regulon [19], was also not conserved in the *Deinococcus* lineage (Table S6). Although some Ddr DNA repair functions are shared by other members of the genus *Deinococcus*, others may be unique to particular *Deinococcus* spp. Further research is required to define in detail the role(s) of Ddr proteins.

We investigated the genomic content of DRP, DPL, and DRPH using oligonucleotide arrays prepared based on putative ORF sequences of DRD. We were able to reduce the confirmed number of *Deinococcus*-specific genes from 230 to 120 (Table S6). This suggests that CGH microarray analysis is a rapid and powerful method for finding candidate strain-specific genes. Although these were mostly hypothetical proteins, our data are useful for screening genes and can provide clues to the extraordinarily high resistance of *Deinococcus* to IR. The CGH microarray method is also useful for genomic comparison of related strains, including unsequenced strains, but this technique is

Table 3. *Deinococcus*-specific genes.

DRD	*D. geothermalis*	*D. deserti*	DRP	DPL	DRPH	Name	Descriptions
Cellular process							
DR1985	Dgeo1291	Deide12030	+	+	+	DR1985	Beta-lactamase; putative
Central intermediary metabolism							
DR1673	Dgeo1457	Deide09250	+	+	+	DR1673	Oxidoreductase; putative
DNA metabolism							
DR0003	Dgeo0047	Deide23280	+	+	+	*ddrC*	DNA damage response
DR0070	Dgeo0295	Deide02990	+	+	+	*ddrB*	DNA damage response
DR0167	Dgeo0395	Deide03030	+	+	+	*IrrE*	*recA* regulator *IrrE*
DRA0346	Dgeo2628	Deide2p01380	+	+	+	*pprA*	DNA damage repair protein
Energy metabolism							
DR0343	Dgeo1250	Deide08270	+	+	+	DR0343	Cytochrome C family protein
DR0993	Dgeo1020	Deide12490	+	+	+	DR0993	Alpha-amylase
Regulatory function							
DRA0252	Dgeo2407	Deide2p02110	+	+	+	DRA0252	Transcriptional regulator; MerR family
Unknown function							
DR1480	Dgeo1193	Deide12320	+	+	+	DR1480	AlgP-related protein
DR2161	Dgeo0883	Deide12461	+	+	+	DR2161	Protein–tyrosine phosphatase-related protein

limited in that it cannot detect genes that are not present in the microarray [8]. In this study, we were unable to provide information on 59 DRD ORFs (Table 1). The comparative approach is limited by the detection threshold that can be applied at the genomic level. This is partly attributable to genetic variation, such as single nucleotide polymorphisms (SNPs), that can affect the hybridization behavior between the probe and target, which cannot be assessed by the methodology used in this study [7]. We confirmed the absence of a set of genes selected randomly by a Southern blotting assay (Fig. 2), but functional genomics techniques such as transcriptomics and proteomics will have to be applied to gain a full understanding of the meaning of these data.

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