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Pyrroloquinoline quinone and a quinoprotein kinase support γ-radiation resistance in *Deinococcus radiodurans* and regulate gene expression

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Deinococcus radiodurans is known for its extraordinary resistance to various DNA damaging agents including γ-radiation and desiccation. The *pqqE:cat* and Δ*dr2518* mutants making these cells devoid of pyrroloquinoline quinone (PQQ) and a PQQ inducible Ser/Thr protein kinase, respectively, became sensitive to γ-radiation. Transcriptome analysis of these mutants showed differential expression of the genes including those play roles in oxidative stress tolerance and (DSB) repair in *D. radiodurans* and in genome maintenance and stress response in other bacteria. *Escherichia coli* cells expressing DR2518 and PQQ showed improved resistance to γ-radiation, which increased further when both DR2518 and PQQ were present together. Although, profiles of genes getting affected in these mutants were different, there were still a few common genes showing similar expression trends in both the mutants and some others as reported earlier in *oxyR* and *pprI* mutant of this bacterium. These results suggested that PQQ and DR2518 have independent roles in γ-radiation resistance of *D. radiodurans* but their co-existence improves radioresistance further, possibly by regulating differential expression of the genes important for bacterial response to oxidative stress and DNA damage.

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Introduction

Pyrroloquinoline quinone (PQQ) was first identified as a coenzyme for methanol dehydrogenase [1], which was subsequently shown to interact covalently with other enzymes [2]. It acts as a redox cofactor for glucose dehydrogenase and catalyses the direct oxidation of glucose to gluconic acid [3]. This process helps micro-organisms in solubilizing the insoluble phosphates into inorganic phosphorous [4, 5]. Recently, the roles of PQQ as an antioxidant [6] and in oxidative stress tolerance of both bacteria [7] and mammalian cells [8] have been demonstrated. Further, it is found that PQQ could stimulate the activity of protein kinases from both prokaryotes and eukaryotes [9–11]. Bacteria producing PQQ promote plant growth and provide bio-control against both fungal as well as bacterial plant patho-gens [12, 13]. The molecular basis of PQQ functions as a growth promoter, biocontrol agent, and in DNA repair and radiation resistance is not known except that its role in transcriptional regulation of gene expression has been shown in mammalian system [14].

Deinococcus radiodurans R1 is characterized for its extraordinary radiation resistance. Amongst different mechanisms, the efficient DNA double strand break (DSB) repair [15, 16] and strong oxidative stress tolerance mechanisms are primarily attributed to the extreme phenotypes of this bacterium [17, 18]. Although, the effect of γ-radiation induced DNA damage on gene expression [19, 20] and on protein turnover [21, 22] have been reported in *D. radiodurans*, the molecular basis of DNA damage response leading to these effects is not fully known. Furthermore, the existence of classical SOS response mechanism has been functionally ruled in this bacterium [23, 24] but PprI (IrrE), [25, 26] and OxyR [27]

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proteins have been characterized as the global response regulators of radiation and oxidative stress in *D. radiodurans*. Deletion mutants of these genes also showed differential expression of a large number of genes in response to γ-radiation and oxidative stresses. The genome of this bacterium also encodes a large number of uncharacterized stress response regulator and sensor kinases [28, 29]. Recently some of these protein kinases and response regulators have been functionally characterized and their roles in radiation resistance and DSB repair [30–32] have been demonstrated. *D. radiodurans* cells lacking PQQ (*pqqE:nptII*) and DR2518 (Δ*dr2518*) a Ser/Thr type protein kinase, show a significant loss of wild type tolerance to γ-radiation [11, 31]. Although, it suggests the role of PQQ in extraordinary radioresistance of this bacterium, the molecular mechanisms underlying the roles of PQQ in radioresistance and DSB repair still remained unknown and would be worth investigating.

Here we report that the *pqqE:nptII* and Δ*dr2518* mutants of *D. radiodurans* which had lost wild type resistance to γ-radiation, also showed differential expression of genes encoding the proteins known for their roles in intermediary metabolism, stress response, and bacterial growth under stressed conditions. The Δ*dr2518pqqE:cat* double mutant, which does not increase the γ-sensitivity of Δ*dr2518* single mutant, also did not show additional effects on expression of though, a limited number of representative genes checked. However, the Δ*dr2518pqqE:cat* double mutant, which was more sensitive to γ-radiation than *pqqE* single mutant, also showed a different expression pattern of these selected genes. These results indicated that mutations in both *pqqE* (PQQ minus) and *dr2518* (a quinoprotein kinase minus) genes could affect gene expression in *D. radiodurans*. Although, the profiles of genes affected in both mutants independently were different, there are however, certain common and functionally important genes which are differentially expressed in both mutants suggesting that PQQ and DR2518 might have different direct or indirect regulatory roles in regulation of gene expression. This might account the different levels of γ-radiation resistance in these mutants and the earlier findings on the epistatic effect(s) of these genes in radiation resistance of *D. radiodurans*.

Materials and methods

Bacterial strains and materials

D. radiodurans (ATCC13239) was a generous gift from Dr. M. Schaefer, Germany [33]. Wild type *D. radiodurans* and its mutants were grown aerobically in TGY (0.5% bacto tryptone, 0.3% bacto yeast extract, 0.1% glucose) broth or agar as required, at 32 °C. Enzymes and other molecular biology grade chemicals were obtained from Roche Biochemicals (Germany), Sigma–Aldrich Chemical Company (USA), New England Biolabs (USA), and Bangalore Genei (India).

Isolation of deletion mutants and cell survival studies

The *pqqE:nptII* disruption mutant used in this study was isolated and reported earlier [11]. The detailed studies on Δ*dr2518* and Δ*dr2518pqqE:cat* mutants have been reported earlier [31]. Cell survival studies were carried out as described earlier [34]. In brief, the exponentially growing cells of *D. radiodurans* and mutants were harvested and suspended in 1/5th volume of normal sterile saline. The cells were exposed with different doses of γ-radiation on ice as described earlier [35] at a dose rate 5.86 kGy h^{-1} in Gamma chamber (GC 5000, ^{60}Co, Board of Radiation and Isotopes Technology, India). Appropriate dilutions were spread on TGY agar plates with appropriate antibiotics and the numbers of CFU/ml were recorded after 48 h of incubation at 32 °C. Similarly, the *Escherichia coli* BL21 cells harboring pET28a+ and expressing DR2518 kinase on pET2518, were grown in LB medium supplemented with 200 μM IPTG and treated with different doses or a fixed dose of 60 Gy γ-radiation at 4 °C. These cells were plated on LB agar plate supplemented with 10 μM PQQ and cell survival was monitored at 37 °C.

Transcriptome studies

Details of microarray preparation, quality control, and the procedures followed in microarray hybridization and data analysis are as described in Ref. [30]. In brief, the total RNA was prepared from three replicates of wild type and mutants. The 14 μg total RNA was annealed with 10 μg random hexamer primers in a total volume of 20 μl at 70 °C for 10 min followed by 2 min incubation on ice. The cDNA synthesis was carried out at 42 °C overnight in 31 μl reaction mixture using SuperScript III Reverse Transcriptase (Invitrogen) with 0.5 mmoles L^{-1} dNTP mix containing amino allyl-dUTP (GE, Piscataway, NJ). The reaction was terminated by adding 20 μl of 0.5 moles L^{-1} EDTA followed by 20 μl of 1 mole L^{-1} NaOH and heating at 65 °C for 20 min.

The reaction mixture was neutralized with 50 μl of 1 mole L^{-1} HEPES buffer pH 7.0 and unincorporated free amino allyl-dUTPs were removed by ultra filtration with YM30 columns (Millipore, Inc.). The cDNAs were coupled to 1 pmol Cy3 or Cy5 dyes (GE Healthcare, Inc.) in 0.1 mole L^{-1} sodium carbonate buffer for 2 h at room temperature and free Cy3 or Cy5 were removed. The labeled cDNA pools of wild type and mutant were mixed and hybridized

simultaneously with the DNA chips in a solution containing 3× saline sodium citrate (SSC), 0.3% SDS, and 24 μg of unlabeled herring sperm DNA and slides were washed as described in Ref. [36]. Measurement of spot intensity and normalization were carried out as described in Ref. [37]. Normalization and statistical analysis were carried out in the R computing environment (2.12, Raqua on the Windows) using the linear models for microarray data package (Limma) [38]. Within Limma, prior to channel normalization, microarray outputs were filtered to remove spots of poor signal quality by excluding those data points with mean intensity less than two standard deviations above background in both channels. Then, global LOESS normalization was used to normalize all data, and the 3-replicate spots per gene in each array were used to maximize the robustness of differential expression measurement of each gene via the "lmFit" function [39]. Design of microarray and identity of samples employed on microarray along with transcriptome data obtained from both mutants, have been submitted to Gene Expression Omnibus Repository (GEO Accession numbers GPL8947 for microarray platform, *pqqE* mutant series GSE17722 with transcriptome data GSM442567 and GSM442568 and *dr2518* mutant series GSE17724 with transcriptome data GSM442540 and GSM442538.

Real-time PCR analysis of selected genes

Quantitative real-time PCR (RT-PCR) was performed as described previously [35] with the RNA isolated from wild type and mutants and cDNA prepared as described above in transcriptome analysis. RT-PCR was carried out on PCR machine (Corbett rotor gene 3000). Real-time detection of RT-PCR product was done using SyBr green 2× master mix kit (Sigma), as per manufacturer's instructions. RT-PCR of selected genes was carried out using gene specific internal primers as detailed in Table 1 and the levels of transcripts were estimated and normalized with the levels of DR1343 (glyceraldehyde 3-phosphate dehydrogenase (GAP) transcript as an internal reference control. Fold change in mutants were calculated with respect to these genes expression levels in wild type cells.

All the experiments were repeated at least three times and results were reproducible. Data presented without statistical analysis were from a typical experiment.

Results

Both PQQ and quinoprotein kinase mutants were sensitive to γ-radiation

Earlier it is shown that both synthesis and activity of DR2518, a protein having multiple PQQ binding motifs and a eukaryotic type Ser/Thr protein kinase domain, are enhanced in response to γ-radiation [31]. The *pqqE:nptII* (hereafter referred as *pqqE* mutant) and Δ*dr2518* cells showed 3 and 5 log cycles decrease in wild type tolerance of γ-radiation at 10 kGy (Fig. 1A). Accordingly, the D10 for *pqqE* mutant (cells devoid of PQQ) and Δ*dr2518* mutants were decreased to ∼8 and ∼2 kGy, respectively, as compared to 12 kGy, the D10 of wild type bacterium. Less resistance of these mutants to γ-radiation shows a good correlation with their DSB repair efficiency as reported earlier [11, 31]. While wild type cells reassemble its genome in 4 h, the mutant cells took 24 h. Both *pqqE* and *dr2518* genes are epistatically linked, as the concomitant deletions of both genes showed same effect on radiation resistance as that of *dr2518* deletion. These are also metabolically linked where *pqqE* (PQQ synthase) is required for the synthesis of PQQ while *dr2518* (Ser/Thr protein kinase) requires PQQ for its activity. Since, PQQ could influence gene expression in mammalian cells [14] although working through the involvement of tyrosine kinase, the possibility of PQQ also working through the Ser/Thr quinoprotein kinases cannot be ruled out. Involvement of STPKs in regulation of gene expression in response to abiotic stress has been suggested [40]. Therefore, the possibility of mutants devoid of PQQ and a Ser/Thr quinoprotein kinase affecting gene expression in *D. radiodurans* could be envisaged and monitored in cells grown under normal and γ-radiation stressed conditions.

The *pqqE* mutant showed differential gene expression in *D. radiodurans*

Transcriptome analysis of *pqqE* mutant showed repression of 98 genes and induced expression of 171 genes by 1.5 or higher folds (GEO accession numbers GSM442567 and GSM442568) in response to γ-radiation. The number of genes showing differential expression in unirradiated cells was significantly less as compared to cells treated with γ-radiation (Fig. 1B). Real-time PCR validation of selected genes from microarray data confirmed the trend in gene expression in *pqqE* mutant but the fold change comparison between RT-PCR and microarray showed slight variation (Fig. 2). From the total number of genes, nearly 20% induced and 14% repressed genes in *pqqE* mutant were hypothetical proteins. Those repressed in *pqqE* mutant belonged to proteins involved in protein synthesis, RNA metabolism, and stress related two-component system and DNA repair. Amongst these, the most notable ones were Mn^{2+} dependent superoxide dismutase (DR1279) putative singlet oxygen resistance protein (DR1367), putative pyrroline-5-carboxylate dehydrogenase (DRA0030) (Table 2) and a serine threonine protein kinase (DRA0334) showing between 1.5 and 2.0-

Table 1. List of primers used for real-time PCR studies in *dr2518* mutant and wild type.

Sl no.	Names	Nucleotide sequence RT-PCR primers for *dr2518* mutant	Purpose
1	DR0099F	5′-CAGGCGAAGACCGAGTGA-3′	DR0099
2	DR0099R	5′-ATGCGGAGGGCTTTGACG-3′	DR0099
3	DR1289F	5′-ACGCCTTCCTCGTTCAGA-3′	DR1289
4	DR1289R	5′-CCGGCCCAACATTCAGTA-3′	DR1289
5	DR2325F	5′-CCGCAGCGTTTGCAGGTT-3′	DR2325
6	DR2325R	5′-TGCCGGGCAGTACATCGTG-3′	DR2325
7	DR2040F	5′-GATGGTCGGAATCAGGTAGTGG-3′	DR2040
8	DR2040R	5′-GCCCGAAAGTGCCCAGTA-3′	DR2040
9	DR1998F	5′-CCCGTTCACGACAACCAGA-3′	DR1998
10	DR1998R	5′-CGCGTCACGGATGAAGAAG-3′	DR1998
11	DR1913F	5′-CGGGCAGTGGTGGAACA-3′	DR1913
12	DR1913R	5′-CGAGGTCGGCCAGGATT-3′	DR1913
13	DR0070F	5′-GGACCAGCGGCGAGATT-3′	DR0070
14	DR0070R	5′-TGGCGAGGCTGACGTATT-3′	DR0070
15	DRC0034F	5′-CATTAGCGTGGATGGTT-3′	DRC0034
16	DRC0034R	5′-CATTAGCGTGGATGGTT-3′	DRC0034
17	DRC0018F	5′-GTTCCCAGCACCCTTCA-3′	DRC0018
18	DRC0018R	5′-GCCCTGTTTCTTGAGCC-3′	DRC0018
19	DR1685F	5′-CTGGGCTTTCCGGTGCT-3′	DR1685
20	DR1685R	5′-CGGCGGCTTTCTGGTAGA-3′	DR1685
21	DR2273F	5′-CGACCTGTACTGGAACGC-3′	DR2273
22	DR2273R	5′-CAGTGCTGTGCCAATGTTT-3′	DR2273
23	DR0058F	5′-CGGCACTTCCAGATTGACC-3′	DR0058
24	DR0058R	5′-GCTGACCCGGCTTACCAT-3′	DR0058
25	DR1367F	5′-TCCCAAGATGATCCGGGA-3′	DR1367
26	DR1367R	5′-GTCATCAGTTCCTCGGC-3′	DR1367
27	DRA0030F	5′-TGAGGCAAGCGTGTACGA-3′	DRA0030
28	DRA0030R	5′-ACCAGTGCATTTGCGATTGA- 3′	DRA0030
29	DR2113F	5′-CGACGAGCACGTGACCA-3′	DR2113
30	DR2113R	5′-CGTTCGTAGATGCGGCA-3′	DR2113
31	DRA0145F	5′-CGCAAGACTCGCTCGACA-3′	DRA0145
32	DRA0145R	5′-GAATGGTTTGATGCCGCT-3′	DRA0145
33	DR1884F	5′-TCCCACCATTCAGGATGT-3′	DR1884
34	DR1884R	5′-GCAGCGTGGTCAGGTTGT-3′	DR1884
35	DR1279F	5′-ATGGCTTACACTCTTCCCCA-3′	DR1279
36	DR1279R	5′-TGCCGAAGGCGCCTGTTGA-3′	DR1279
37	DR2444F	5′-ATGACCGACCTGCCATCTCT-3′	DR2444
38	DR2444R	5′-TCAGGCTCCAGCAGCCACA-3′	DR2444
39	DRA0290F	5′-GTCTGGTACACTCAACTTAT-3′	DRA0290
40	DRA0290R	5′-TCATCCCGAACAGCAAGATGA-3′	DRA0290
41	DR2050F	5′-ATGGCAAAAGGAACGTTC-3′	DR2050
42	DR2050R	5′-TGATCATGTTCTTGACGT-3′	DR2050
43	DR0167F	5′-AAGCTGAAGCCTCCA-3′	DR0167
44	DR0167R	5′-AATCGCGTGCCGATTTCGT-3′	DR0167
45	DR0615F	5′-AACCTCGAAGAAGAAGT-3′	DR0615
46	DR0615R	5′-CCGCTTCCTGCTCGCCA-3′	DR0615

fold repression. The roles of Mn^{2+} dependent superoxide dismutase and catalase (repressed by 1.5-fold in *pqqE* mutant) are shown in oxidative stress tolerance. Roles of Mn^{2+} [38] and PQQ [7] in bacterial response to oxidative stress have also been demonstrated. It was shown earlier that *E. coli* cells expressing PQQ had induced levels of catalase and SOD activities [7]. These results might suggest that PQQ modulates the oxidative stress tolerance in this bacterium, by controlling the levels of antioxidant enzymes but also functions beyond its role as antioxidant. Earlier a peroxide sensor and transcriptional regulator OxyR, has been reported from *D. radiodurans*. Deletion mutant of *oxyR* gene also showed repression of catalase (DR1998) and SOD (1279) and DRA0334 (putative STPK) of this bacterium [27]. PprI has been found to be a global regulator of radiation response and its deletion mutant showed the repression of several genes including DR2128 (DNA directed RNA polymerase), DRA0244 (LexA)

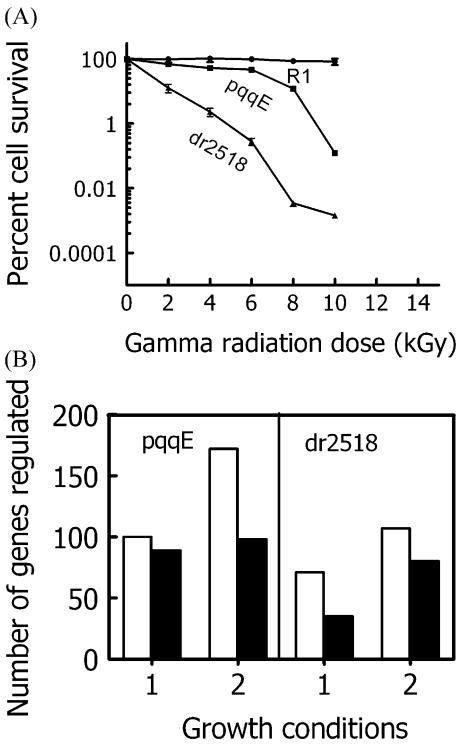

Figure 1. Effect of γ-radiation on cell survival and genes expression in quinomutants. (A) Wild type (wild type) and deletion mutant of *D. radiodurans* for DR2518 (Δ*dr2518*) and *pqqE* encoding PQQ synthase (*pqqE*) were grown till late logarithmic phase and treated with different doses of γ-radiation. Cell survival was monitored as described in Materials and Methods Section. (B) Both PQQ minus (pqqE) and DR2518 (dr2518) mutants cells were grown under non-irradiated (1) and γ-irradiated (2) conditions and transcriptome analysis was carried out. Total number of genes showing 1.5 or higher folds upregulation (open boxes) and downregulation (gray boxes) in both mutants were estimated and presented.

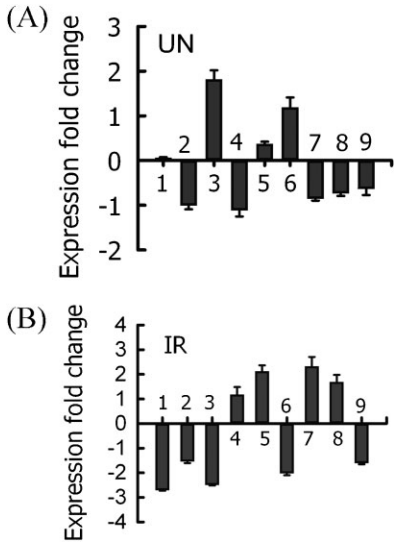

Figure 2. Real-time PCR analysis of selected genes in *pqqE:cat* mutant. Selected genes such as *dr1367* (1), *drA0030* (2), *dr2113* (3), *drA0145* (4), *dr1884* (5), *dr1279* (6), *dr2444* (7), *drA0290* (8), *dr2050* (9), showing two or more than twofold change in microarray analysis were taken and RT-PCR analysis was carried out using gene specific internal primers. Changes in wild type was subtracted from mutant's data and normalized against the levels of reference gene *dr1343*, encoding glyceraldehydes 3-phosphate.

and DRA0346 (PprA) [26], which were repressed by 1–5- to 2.0- folds in *pqqE* mutant (accession No. GSM442568). Induced expression of certain genes was also observed in *pqqE* mutant grown in both normal as well as γ-stressed conditions (Table 3). The important genes that showed induction in *pqqE* mutant were those encoding ABC transporters, electron transport chain components, oxidative stress response genes, iron transport genes, chromosome maintenance, DNA metabolism, and a large number of transferases, etc. This indicated that PQQ mutant with several physiological defects adjust its cellular homeostasis by increasing the synthesis of defense proteins involved in protection of biomolecules. There were four genes (DR0157, DR0548, DR0751, and DRA0192) in *oxyR* mutant [27] and one gene DR2373, in *pprI* mutant [26] showing more than 2.0-fold induction, showed induced expression in *pqqE* mutant. These results suggested the possibility of PQQ directly or indirectly, controlling the expression of genes involved in oxidative stress tolerance in bacteria. A large number of genes that differentially expressed in *pqqE* mutant were not found in transcriptome of *oxyR* and *pprI* mutant cells indicating that the PQQ possibly functions independent of the mechanisms underlying the OxyR and PprI regulation of γ-radiation response and gene expression.

The *dr2518* deletion affected gene expression in *D. radiodurans*

In order to understand the functional interaction of PQQ and DR2518 *in vivo* and the role of PQQ inducible STPK type protein kinase (DR2518) on gene expression, the transcriptome analysis of Δ*dr2518* mutant was carried out under both normal and γ stressed conditions. Interestingly, the microarray analysis showed the differential expression of a large number of genes encoding the components of intermediary metabolism, a reasonably large percentage of hypothetical proteins and proteins implicated in stress response and signal transduction mechanisms. Like *pqqE* mutant, the number of genes affected in Δ*dr2518* mutant was also more in γ-irradiated cells than unirradiated (Fig. 1B). Microarray analysis of *dr2518* mutant transcripts showed a good agreement with RT-PCR validation of selected representative genes from microarray data (Fig. 3). The Δ*dr2518*

Table 2. The 27 highly repressed genes in *pqqE* mutant cells in response to γ-radiation (source: GEO accession no. GSM442568).

ORF Id	Putative functions	Fold change	*p*-Value
DRA0039	Mannosyltransferase (mtfB)	13.52504152	1.68E−06
DRA0040	First mannosyl transferase	7.095319267	0.000338074
DRA0043	Lipopolysaccharide biosynthesis protein, putative	6.560691815	4.93E−05
DRA0038	Rhamnosyltransferase, putative	6.355281064	2.32E−05
DRA0304	Conserved hypothetical protein	4.740326488	0.000279817
DRA0036	Hypothetical protein	4.137201441	0.003831985
DRA0037	Glycosyltransferase	3.891154081	0.013987175
DRA0028	Hypothetical protein	3.102091854	0.000227757
DR1367	Singlet oxygen resistance protein, putative	2.983300251	0.001837882
DRA0042	Glucose-1-phosphate thymidylyltransferase (rfbA)	2.971325838	0.000705602
DR1552	Hypothetical protein	2.964734938	0.053770762
DRA0027	Conserved hypothetical protein	2.742575589	0.004003547
DR2371	Hypothetical protein	2.553765967	0.020559608
DR1366	Amidotransferase HisH, putative	2.498413258	0.012839498
DR2252	Hypothetical protein	2.497414568	0.01339147
DR2524	Ribosomal protein L28 (rpmB)	2.241564758	0.000613265
DR2050	Elongation factor TU (tuf-2)	2.225956512	0.000518791
DR2044	Ribosomal protein L10 (rplJ)	2.224026654	2.04E−05
DR2126	Ribosomal protein S11 (rpsK)	2.214119547	3.81E−05
DR0219	Hypothetical protein	2.155392886	0.001698249
DR0224	Conserved hypothetical protein	2.10390055	0.015100161
DR2114	Ribosomal protein L30 (rpmD)	2.025453603	0.002452057
DR1279	Superoxide dismutase (sodA), Mn family	2.00349391	0.005850005
DRA0030	1-Pyrroline-5-carboxylate dehydrogenase, putative	1.97925788	0.003960807
DR2113	Ribosomal protein S5 (rpsE)	1.978605061	8.77E−05
DRA0368	Conserved hypothetical protein	1.977863344	0.000896329
DR0311	Ribosomal protein L3 (rplC)	1.942213217	2.89E−06

cells exposed to γ-radiation showed both upregulation and downregulation of a large number of genes (GEO accession numbers GSM442540 and GSM442538). A large number of genes encoding hypothetical proteins were induced in *dr2518* mutant exposed to γ-radiation (Table 4). Amongst the known proteins, the notable ones were Ser/Thr protein kinase (DR0058), transposases (DR1453, DRB0120, DR2028) with authentic frameshift, DNA topology modulating protein (DR2273), HRDC family protein (DR2444) and a sensor histidine kinase (DRB0090). Interestingly, DR0095, DR1263, and DR2409 which were repressed in *oxyR* mutant by 11.2-, ∼21-, and 6.3-folds, were induced by ∼2.29-, 1.543-, and 2.25-fold, respectively, in *dr2518* mutant. DRA0211 encoding a transcriptional regulator was induced in *dr2518* mutant and also in *oxyR* mutant. DR2370, DR0167 (PprI), and DR0074 (LexA), which were induced in *pprI* mutant, these were also showed transcriptional activation in *dr2518* mutant. The *dr2518* mutant also showed repression of a number of genes (Table 5). Amongst these, the important ones were DR0099 (single stranded DNA binding protein), DR0572 (SpoIID-related protein), DR0856 (DNA polymerase III epsilon subunit), DR1629 (cyclic DiGMP phosphodiesterase A), DR1913 (DNA gyraseA), DR1998 (catalase), DR2040 (ParA family proteins), DR2325 (serine protease), DR2424 (transposase), DRC0018 (XerD), DRC0034 (PQQ synthase), and several transcriptional regulators. The roles of all these proteins in DNA metabolisms like recombination, segregation, and structure maintenance have been demonstrated in other system. Comparison of gene expression profiles in *oxyR* and *pprI* mutants with *dr2518* showed that DR1312 (nuclease component) and DR0307 (elongation factor G), which were induced in *pprI* and *oxyR* mutants, respectively and DR0099 (SSB) was repressed in *pprI* mutant, were repressed in *dr2518* mutant. Interestingly DR1998 (catalase, KatA) and DR0865 (DNA dependent ferric uptake protein) which were repressed in *oxyR* were also repressed in *dr2518* mutant. These results suggested the important role of DR2518 in transcriptional regulation of genes, which were differentially regulated in *oxyR* and *pprI* mutants and that could attribute to DR2518 roles in radiation resistance and DSB repair of this bacterium. From the large number of genes that are differentially expressed in *oxyR* and *pprI* mutants, a very small fraction were appeared in the list of differentially expressed genes in *dr2518* mutant. This may suggest that the mechanisms underlying DR2518 regulation of γ-resistance and gene expression appear to be different from the one being employed by OxyR and PprI regulators in *D. radiodurans*.

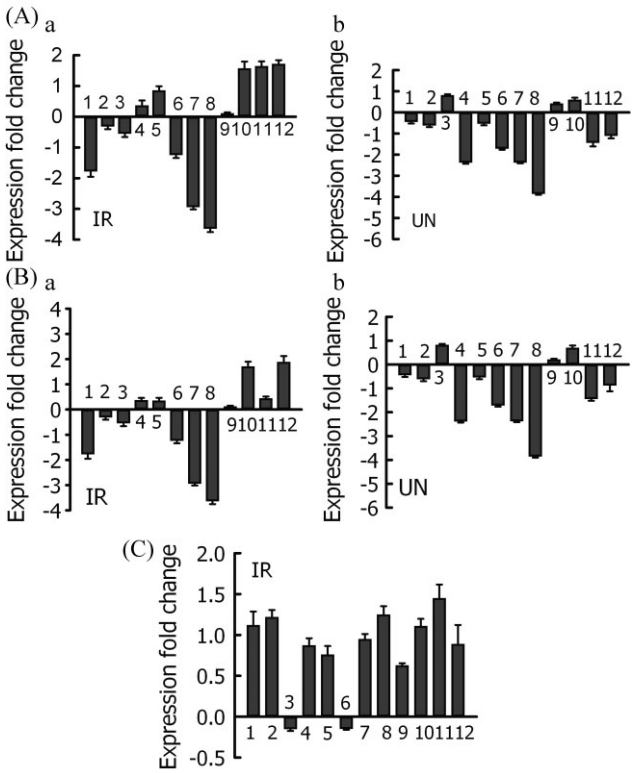

Figure 3. Validation of microarray data and comparison of real-time PCR analysis of selected genes in *pqqE:cat*, Δ*dr2518* and Δ*dr2518 pqqE:cat* double mutant. Selected genes such as *dr0099* (1), *dr1289* (2), *dr2325* (3), *dr2040* (4), *dr1998* (5), *dr1913* (6), *dr0070* (7), *drC0034* (8), *drC0018* (9), *dr1685* (10), *dr2273* (11), and *dr0058* (12) showing 1.5 or higher fold changes in the levels of transcript in microarray analysis of Δ*dr2518* mutant were taken and the levels of expression in Δ*dr2518* (A) and Δ*dr2518pqqE:cat* double (B) mutants grown under γ-radiation stressed (IR) and normal (UN) conditions were checked by RT-PCR. Similarly, the expression of these genes was checked with irradiated (IR) samples of *pqqE* mutant (C). Changes in wild type was subtracted from mutant's data and normalized against the levels of reference gene *dr1343*, encoding glyceraldehyde 3-phosphate.

Common genes showing differential expression in *pqqE* and *dr2518* mutants

DR2518 shows both functional and physical interaction with PQQ *in vitro* [31] and both PQQ and DR2518 play important roles in γ-radiation resistance of this bacterium. Although, *pqqE* and *dr2518* mutants showed differential expression of a different set of genes, there were still a large number of genes showing similar trends of expression in both these mutants (Table 6). The other genes that were repressed and could be implicated in radiation resistance and DNA damage tolerance were HTH transcriptional regulator (DR_1422), cobalamin (vitamin B12) biosynthesis protein (DR_A0012), serine threonine protein kinase (DR_A0335), peroxidase (DR_1538), molybdenum cofactor synthesis protein (DR_1292), hypothetical cytosolic protein (DR_2173), a Nudix family phosphohydrolase (DR_0784), hypothetical proteins (DR_1043, DR_1547, DR_C0039), GCN5-related *n*-acetyltransferase (DR_1137), phage HK97 GP10 family (DR_A0103), copper amine oxidase domain protein (DR_2489), methyltransferase type II (DR_2562) and GCN5-related *N*-acetyltransferase (DR_1716). Those showed induced expression were *n*-carbamyl putrescine amidase (DR_2535), hypothetical proteins (DR_B0039, DR_2229, DR_1842), ATP-dependent DNA helicase (DR_2444), a metal dependent phosphohydrolase (DR_2462), the ribosomal proteins (DR_0320, DR_2129) and glyoxylase bleomycin resistance protein (DR_2208). Amongst these, the expression of DR0237 was induced in *pprI* mutant but repressed in both *pqqE* and *dr2518* mutants while DR1708, DR2240, and DR1317 of *oxyR* mutant and DRA0018 of *pprI* mutant were also induced in *dr2518* mutant also. This indicated that although both PQQ and DR2518 regulate different set of genes under both normal and γ-stressed conditions, there are important proteins being regulated by both factors independently. Comparison of transcriptome data of *oxyR*, *pprI* mutants with *pqqE* and *dr2518* mutants revealed a very limited number of genes having similar pattern of gene expression. Majority of the genes expressing in all these cases were different, suggesting the independent pathway of gene expression controlled by these proteins. Although, PQQ and DR2518 are functionally linked, the possibility of both these functioning independently in gene expression may be likely.

PQQ could improve γ-radiation resistance in *E. coli* cells expressing DR2518

E. coli cells expressing DR2518 showed nearly 2.3 ± 3.5-fold improved tolerance at 100 Gy dose of γ-radiation as compared to untransformed control (Fig. 4A). These cells were treated with 100 Gy of a single dose γ-radiation and plated on LB with and without 10 μM PQQ. The cells expressing DR2518 showed nearly fivefold increased resistance to γ-radiation in presence of PQQ as compared to cells grown in absence of PQQ. PQQ effect on cells harboring vector control was nearly 1.5-fold (Fig. 4B). These results suggested that although the PQQ and DR2518 independently could improve γ-radiation resistance in *E. coli*, the presence of both PQQ and DR2518 together further significantly enhanced γ-radiation resistance by nearly twofold over the DR2518 expressing cells control and nearly fivefold over untreated/untransformed controls.

Table 3. The 38 significantly induced genes in *pqqE* mutant cells in response to γ-radiation (source: GEO accession no. GSM442568).

Locus	Annotation	Fold change	*p*-Value
DR1441	Conserved hypothetical protein	12.75619	0.003501699
DR0469	Conserved hypothetical protein	11.17167162	0.003853526
DR0157	Hypothetical protein	8.321797103	0.004863397
DR0827	Hydrolase, CbbY-CbbZ-GpH-YieH family	5.6708265	0.006947352
DRB0094	Hypothetical protein	4.682433335	7.54E−05
DR2462	Conserved hypothetical protein	4.508248252	5.81E−05
DR0839	Hypothetical protein	4.292144847	0.057787549
DR0852	Hypothetical protein	3.99276757	0.008727625
DRA0362	Hypothetical protein	3.62156628	0.011828896
DRA0141	Hypothetical protein	3.444511791	0.031880043
DR0809	Conserved hypothetical protein	3.266387511	0.001042149
DR0449	Hypothetical protein	3.260990235	0.013753842
DR2413	Chromate transport Protein	3.208288714	0.001749646
DR2375	Transcription elongation factor (greA-3)	3.030784243	0.001253869
DR2444	Nucleic acid-binding protein, HRDC family	2.647458557	0.000115363
DR1842	Hypothetical protein	2.642368994	0.000950779
DR2341	birA bifunctional protein	2.637316225	0.003745894
DR1716	Conserved hypothetical protein	2.615260022	0.045018616
DR0616	Conserved hypothetical protein	2.556110788	0.011654085
DR1739	Conserved hypothetical protein	2.506187419	0.00348072
DRA0145	Peroxidase, putative	2.4979162	0.0061937816
DR0279	Conserved hypothetical protein	2.458971076	0.012514865
DR0709	snoG protein, putative	2.413157799	0.001420911
DR1962	Hypothetical protein	2.274436948	1.88E−05
DR0858	Hypothetical protein	2.272618158	0.025364429
DRA0290	Cell division protein FtsH (ftsH-3)	2.20122582	0.000166338
DR2270	Conserved hypothetical protein	2.179146879	0.041024827
DR1902	Exodeoxyribonuclease V, subunit RecD,	2.178728356	0.038599831
DRB0042	Oligopeptide transport periplasmic protein	2.178382258	0.030323056
DRA0256	Phenylacetyl-CoA ligase	2.168646703	0.000693909
DRB0107	Ribonucleotide reductase, NrdI family	2.039108702	0.001535386
DR1884	Transcriptional regulator	2.038200041	0.038843911
DR1960	Acetyl-CoA acetyltransferase (atoB-3)	2.035155485	0.008958612
DR0693	Ammonium transporter (amtB)	2.031838947	0.021897421
DR1264	Hypothetical protein	2.03125767	4.74E−05
DR2309	Hypothetical protein	2.030023693	5.73E−06
DRA0277	Malate synthase (aceB)	2.011368476	8.24E−05

Discussion

D. radiodurans shows extraordinary resistance to several abiotic stresses including radiation and desiccation [18]. Transcriptome analysis of cells exposed to γ-radiation was carried out to understand the molecular basis of the extreme phenotypes. These cells show differential expression of genes including those involved in intermediary metabolism, signal transduction, DNA recombination, and repair functions as shown in other prokaryotes [19, 20]. The classical SOS response, a best characterized DNA damage response mechanism in bacteria, is found missing in *D. radiodurans*. This makes it an interesting system to study the molecular basis of γ-radiation inducible gene expression. γ-Radiation exposure leads to oxidative damage of biomolecules and produces DNA strand breaks [18], and therefore, various regulatory proteins having roles in both oxidative stress and γ-response have been studied for their possible roles in gene expression. It has been shown that the deletion of a response regulator (DrRRA) [41], PprI [26], and OxyR a novel sensor and regulator of oxidative stress [27], could affect gene expression. However, the profiles of genes showing differential expression in each of these cases were different. This might indicate that these proteins are possibly regulating certain genes required tightly for respective phenotypes but also affect other genes that are functionally redundant in normal metabolism of this bacterium. Further, we have shown that *E. coli* expressing *pqqE* of *D. radiodurans*, conferring improved tolerance to oxidative stress [7] and radiation induced DNA damage [10]. On the other hand, the *D. radiodurans* cells having the disrupted copy of *pqqE* (*pqqE:nptII*) in genome, fails to make PQQ [11] and such cells become sensitive to γ-radiation and hydrogen peroxide, as compared to wild type. This confirmed the role of PQQ in both oxidative

Table 4. The 36 highly induced genes in *dr2518* mutant cells in response to γ-radiation (source: GEO accession no. GSM442540).

Locus	Annotation	Fold change	*p*-Value
DR2462	Conserved hypothetical protein	14.88673543	1.77E−08
DR0808	UDP-*N*-acetylglucosamine pyrophosphorylase	8.150054816	0.001827
DR2229	Hypothetical protein	6.902455363	2.41E−07
DR2535	Hydrolase, putative	6.099025879	7.64E−08
DR1941	3-Oxoacyl-acyl carrier protein synthase II (fabF)	5.810254562	6.37E−07
DR2444	Nucleic acid-binding protein, HRDC family	5.079680915	8.14E−09
DR1842	Hypothetical protein	5.054097387	1.92E−05
DR2100	Conserved hypothetical protein	3.933331478	4.04E−05
DR1129	Hypothetical protein	3.556312924	3.06E−07
DR0502	Alanyl-tRNA synthetase-related protein	3.314546819	0.000275
DR0809	Conserved hypothetical protein	3.24061023	7.20E−06
DR1588	Aminotransferase, class I, putative	3.077893004	0.006549
DR2273	DNA topology modulation protein FlaR family	2.956529653	0.000254
DR2028	Transposase, authentic frameshift	2.943704859	0.00012
DR1761	Conserved hypothetical protein	2.89018275	0.000741
DR1601	Nodulin 21-related protein	2.726388044	0.001383
DR1877	Conserved hypothetical protein	2.615388858	0.002193
DR1453	Transposase, putative, authentic frameshift	2.587103101	5.49E−06
DR0023	Phosphoribosylaminoimidazole carboxylase (purE)	2.583775292	0.000312
DR2370	Pyruvate dehydrogenase complex, E3	2.512158204	0.000173
DRA0369	Hypothetical protein	2.370463894	0.011166
DR1953	Hypothetical protein	2.337813613	0.015099
DR0095	ABC transporter, ATP-binding protein	2.294565816	0.004137
DR2172	Hypothetical protein	2.280425824	5.51E−05
DR0929	Acylphosphatase, putative	2.274271685	0.001701
DR2487	Cytochrome C4, putative	2.265967762	0.005797
DR1263	Conserved hypothetical protein	2.254297145	0.000379
DRB0090	Sensor histidine kinase, copper metabolism	2.211732698	0.000242
DRB0107	Ribonucleotide reductase, NrdI family	2.10379356	0.049325
DR1181	Hypothetical protein	2.088038137	0.000716
DRA0195	Oxidoreductase	2.08540336	0.015241
DR1825	Hypothetical protein	2.063385741	2.08E−05
DRA0024	Hypothetical protein	2.043299676	0.001558
DR0269	Hypothetical protein	2.034339323	0.015931
DRB0120	Transposase, authentic frameshift	2.029873458	0.01283
DR0058	Serine-threonine protein kinase	2.020950291	0.000731

stress as well as DNA strand break tolerance in this bacterium. Further, it is shown that PQQ is an inducer of eukaryotic type Ser/Thr protein kinase DR2518, which has a role in radiation resistance and DSB repair. Expression of this kinase in *E. coli* could help these cells in higher tolerance of γ-radiation, which improved further in presence of PQQ as compared to untransformed cells and only DR2515/PQQ controls (Fig. 4). Earlier, it has been shown that transgenic *E. coli* making PQQ acquired higher resistance to radiation and this effect of PQQ was shown to be again through a periplasmic protein kinase, YfgL [10]. *E. coli* expressing DR2518 another protein kinase from *D. radiodurans* also showed improved γ-radiation resistance in presence of exogenously supplied PQQ. The possibility of PQQ contributing in radiation resistance of *E. coli* through DR2518 and YfgL protein kinases involving the phosphorylation of DNA metabolic proteins may be speculated. Profiling of phosphoproteins from *E. coli* cells making PQQ and DR2518 separately and together, would be worth doing independently to check if both these kinases function through a common pathway in *E. coli*. These finding suggested that DR2518 alone although provides, higher radiation resistance in bacteria, its function gets improved further in presence of PQQ. Genetic interaction of *dr2518* and *pqqE* shown earlier has also supported this observation. In this case the *dr2518* mutant showed higher sensitivity to γ-radiation as compared to *pqqE* mutant (Fig. 1) but double mutant (Δ*dr2518 pqqE:cat*) did not add further to Δ*dr2518* mutant sensitivity [31]. This suggested that although the interaction of both PQQ and DR2518 helps *D. radiodurans* in its higher radiation resistance, these components could also function independently.

Microarray analysis of transcriptomes of Δ*dr2518* and *pqqE:cat* mutants suggested that both PQQ and DR2518

Table 5. The 62 significantly repressed genes in *dr2518* mutant cells in response to γ-radiation (source GEO accession no. GSM442540).

Locus	Annotation	Fold change	*p*-Value
DR1494	NADH dehydrogenase I, L subunit (nuoL)	7.617453912	0.001954
DR2325	Serine protease, subtilase family	2.582522378	0.096051
DR2040	ParA family ATPase	2.301816175	0.090571
DR1137	Hypothetical protein	2.243525161	0.007734
DR2388	Lipoprotein signal peptidase (lspA)	2.232964075	0.011043
DR0070	Hypothetical protein	2.105601651	0.050294
DRA0037	Glycosyltransferase	2.085883992	0.000201
DRA0186	Putative cytochrome P450	2.068305089	0.01294
DR1629	c-Di-GMP phosphodiesterase A	1.978885199	0.000282
DR1824	Glucose-1-phosphate adenylyltransferase	1.976620357	0.009027
DR2604	Conserved hypothetical protein	1.902468613	0.002799
DR1178	Hypothetical protein	1.836008182	3.18E−05
DR0099	Single-stranded DNA-binding protein (ssb)	1.825159281	0.002066
DR1373	Metal binding protein, putative	1.806573788	1.70E−05
DR1919	Conserved hypothetical protein	1.802689945	7.59E−06
DR0955	Hypothetical protein	1.753231612	0.001776
DR1998	Catalase (katA)	1.728175486	0.0166656
DRC0009	Hypothetical protein	1.72189353	3.09E−05
DR0734	Hypothetical protein	1.721093494	0.000302
DRA0160	Phosphate transporter, ATP-binding protein	1.713936398	0.023858
DR2218	Hypothetical protein	1.713302486	0.010322
DR0572	Sporulation protein SpoIID-related protein	1.70653967	4.52E−05
DR2274	Conserved hypothetical protein	1.704857982	0.004143
DR2108	Molybdate metabolism regulator	1.701767928	5.29E−05
DR1257	L-Sorbosone dehydrogenase	1.679130464	0.000417
DR0043	Hypothetical protein	1.666510176	1.08E−05
DR1244	Conserved hypothetical protein	1.657724871	0.054154
DR1586	Glutamate racemase (murI)	1.641722968	0.00036
DR0775	Chorismate synthase (aroC)	1.637380523	0.004689
DR2501	Transcriptional regulator	1.635747132	0.002624
DRB0051	Hypothetical protein	1.630550023	0.001156
DR1076	Cell wall synthesis protein, putative	1.63001575	0.075859
DR1183	mazG protein (mazG)	1.621139588	0.000586
DR1982	Thioredoxin reductase (trxB)	1.61470575	0.000169
DR0307	Elongation factor G (fus-1)	1.610101869	0.005755
DR1913	DNA gyrase, subunit A (gyrA)	1.60451301	0.0190602
DR2414	Hypothetical protein	1.602202408	0.030934
DR2413	Chromate transport protein	1.595715723	2.22E−05
DRA0041	RfbB	1.587818704	0.04455
DRA0119	Conserved hypothetical protein	1.578316651	0.001147
DR2430	Hypothetical protein	1.577847021	0.0098391
DR2246	Hypothetical protein	1.57596021	0.000564
DR2252	Hypothetical protein	1.569134437	0.034691
DR2336	Potassium channel, putative	1.56024713	0.000485
DRC0039	Hypothetical protein	1.55406783	0.000646
DR2056	Hypothetical protein	1.551375466	2.03E−05
DR0056	Benzoate membrane transport protein	1.551225912	0.047442
DR0219	Hypothetical protein	1.546215279	0.009865
DR1807	Conserved hypothetical protein	1.545654112	2.03E−05
DR2617	Cytochrome AA3-controlling protein CtaA	1.545309641	0.005643
DR0856	DNA polymerase III, epsilon subunit	1.544159849	0.014474
DRC0034	Coenzyme PQQ synthesis protein, putative	1.54088651	0.007334
DRA0073	Cation-transporting P-type ATPase	1.540885851	0.030964
DR0640	S-adenosylmethionine synthase (metK)	1.53925069	0.014956
DR1131	Ferrochelatase (hemH)	1.520241578	0.000115
DRC0018	Integrase-recombinase XerD, putative	1.520040403	0.002578
DR0331	Hypothetical protein	1.506498759	0.040598
DR2331	Conserved hypothetical protein	1.506232205	0.000616
DR1241	Hypothetical protein	1.505683824	0.005482

(*Continued*)

Table 5. (*Continued*)

Locus	Annotation	Fold change	*p*-Value
DR2574	Transcriptional regulator, HTH_3 family	1.503828471	0.015887
DRA0071	Transcriptional repressor SmtB (smtB)	1.503660419	0.009057
DR1772	Hypothetical protein	1.502621947	0.01618
DR2021	Conserved hypothetical protein	1.500006498	0.003582

work independently as well as in tandem for conferring bacterial resistance to γ-radiation. A large number of genes showing differential expression in each of these mutants were found to be different (Tables 2–5). However, there are common genes which showed identical pattern of expression in both of these mutants (Table 6). The roles of common genes in γ-resistance would be worth investigating independently. Further, the representative genes showing differential expression in *dr2518* mutant also showed similar trends in double mutant (Δ*dr258pqqE:cat*; compare Fig. 3A and B) but these were differentially regulated in *pqqE* single mutant (Fig. 3C). This might indicate that all genes under the control of PQQ and DR2518 were not same and that could account to their differential effects in γ-radiation. Earlier, the transcriptome analyses of different radiation sensitive mutants have also shown the different profiles of gene expression in response to γ-radiation [20, 26, 27, 41]. In addition, there were certain common genes exhibiting similar patterns of expression were found amongst those mutants. A number of genes that showed differential expression in PprI [26] and OxyR [27] mutants were also affected in *pqqE* and *dr2518* mutants under this study. These include the catalase (DR1998) and SOD (1279) and DRA0334 (putative STPK) in *oxyR* mutant and DR2128 (DNA directed RNA polymerase), DRA0244 (LexA) and DRA0346 (PprA) in case of *pprI* mutant, when compared with *pqqE* mutant. Similarly, there were two proteins DR1312 (nuclease component) and DR0307 (elongation factor G), upregulated in *pprI* mutant and DR1998 (catalase, KatA) and DR0865 (DNA dependent ferric uptake protein) upregulated in *oxyR* mutant while the DR0099 (SSB) was downregulated in *pprI* mutant. Interestingly, all these genes were downregulated in

Table 6. List of common genes showing downregulation and upregulation in response to γ-radiation in quinomutant (*pqqE*) and *dr2518* (source: GEO accession no. GSM442540 and GSM442568).

Downregulation		Upregulation	
ORFs id	**Known or putative functions**	**ORFs Id**	**Known or putative functions**
DR_A0037–DR_A0038	Glycosyl transferase family 2	DR_A0304	Hypothetical protein
DR_0219	Hypothetical protein	DR_1367	Pyridoxine biosynthesis protein
DR_2114	50s ribosomal protein L30	DR_C0024	Hypothetical protein
DR_A0368	UDP-galactopyranose mutase	DR_C0008	Hypothetical protein
DR_2046	Ribosomal protein L11	DR_1438	Extracellular solute-binding protein family 1
DR_A0006	Cyclase dehydrase	DR_A0353	Chemotaxis sensory transducer
DR_1247	Succinyl-beta subunit manganese ion binding	DR_0718	Aspartate 1-decarboxylase
DR_C0003	Water dikinase, kinase activity	DR_0577	Histidine kinase
DR_A0016	Sulfate adenylyltransferase	DR_1708	Conserved repeat domain protein
DR_1749	Nlp p60 protein	DR_2240	hypothetical protein
DR_0307	Elongation factorG	DR_A0018	Putative 5′-nucleotidase
DR_1298	Hypothetical protein	DR_0167	IrrE, regulatory protein
DR_0888	Putative bacterial cell wall degrading enzyme	DR_0605	Diguanylate cyclase
DR_A0231	Oxidoreductase	DR_2526	Dihydrolipoamid dehydrogenase
DR_2103	Translation elongation factor g	DR_1544	Putative acyl-dehydrogenase
DR_0424	Imidazoleglycerol-phosphate dehydratase	DR_1897	Hypothetical protein
DR_0237	Peptidylprolyl isomerase	DR_2344	Hypothetical protein
		DR_1106	Multifunctional complex protein
		DR_1768	Tetratricopeptide TPR2 repeat protein
		DR_1801	Hypothetical protein
		DR_1737	Signal peptidase 1
		DR_1317	Hypothetical protein
		DR_1948	Trigger factor protein folding
		DR_B0126	Transcriptional family protein
		DR_2186	Acetylhomoserine-acetylserine sulfhydrylase

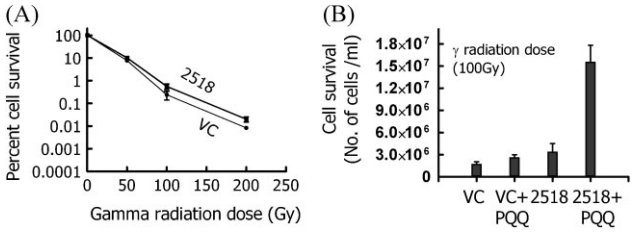

Figure 4. Functional interaction of PQQ and DR2518 in *E. coli* cells exposed to γ-radiation. *E. coli* cells expressing DR2518 and harboring vector was treated with different doses of γ-radiation and survival was measured (A). The cells harboring vector (VC) and DR2518 (2518) were treated with 100 Gy γ-radiation and survival was measure in presence (+PQQ) and absence PQQ (10 μM; B).

dr2518 mutant but not in *pqqE* mutant. This indicated that the spectra of genes repressed in *dr2518* mutant including SSB and DRB0099, were potentially more important for radiation resistance, DSB repair, and recombination as compared to the genes repressed in *pqqE* mutant. Downregulation of important genes like SSB, *drB0099*, etc., in *dr2518* mutant, but not in *pqqE* mutant, may be accounted to the higher γ-radiation sensitivity in *dr2518* mutant as compared to *pqqE* mutant. Bacterial SSB has been shown to be an important DNA recombination and DSB repair protein [42]. Recently, the SSB of *D. radiodurans* [43–45] and DRB0099, a histidine kinase of two component system (TCS) [32], have been characterized for both *in vitro* activities and *in vivo* roles in radioresistance. Earlier it was demonstrated that the catalase and superoxide dismutase mutants of *D. radiodurans* are sensitive to γ-radiation as compared to wild type cells [46]. Independent studies have demonstrated the roles of PprI, OxyR, PQQ, and DR2518 in γ-radiation resistance. A large number of genes that are getting affected in these mutants were different. Although, there are few genes showing similar trends of expression amongst these mutants, further studies on these complements might reveal the molecular basis of these regulators roles in γ-radiation resistance. Nevertheless, this suggested that both oxidative stress and DSB response in *D. radiodurans* are regulated through a much more complex network of genes yet to be discovered. The expression levels of *pprI* and *oxyR* in *pqqE* and *dr2518* mutant showed interesting results. The levels of *pprI* were induced by 3.52 ± 0.213-fold in *dr2518* mutant while no change was observed in *pqqE* mutant. The *oxyR* expression was reduced only marginally by 1.781 ± 0.231-fold, in *pqqE*, which was decreased by 4.512 ± 0.127-fold in *dr2518* mutants. Results shown here together supported the roles of PQQ and DR2518 in direct or indirect regulation of gene expression in this bacterium. Role of PQQ in the regulation of gene expression has been shown in mammalian system, albeit through the involvement of protein kinases including tyrosine kinase [9, 14]. Understanding on the role of PQQ in regulation of gene expression and post-translational regulation of protein functions might provide the most plausible basis to the versatility of PQQ in regulation of ubiquitous functions in living organisms.

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Conflict of interest

We coauthors have no conflict of interest.

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