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DR1769, a Protein with N-Terminal Beta Propeller Repeats and a Low-Complexity Hydrophilic Tail, Plays a Role in Desiccation Tolerance of *Deinococcus radiodurans*

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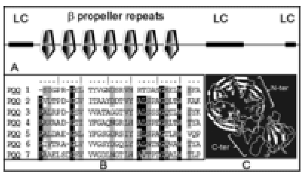

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FIG 1

Functional domain analysis of DR1769. (A) Primary sequence of DR1769, searched for the presence of functional motifs showing 7 beta propeller repeats making PQQ-interacting motifs (PQQ) in the N-terminal domain and low complexity (LC) region, largely confined to the C-terminal domain; (B) beta propeller repeats (PQQ1 through PQQ7) showing the typical signature of the WD motif, starting with consensus G and ending with W; (C) modeled structure of DR1769 showing typical arrangement of 7 beta propeller repeats of the N-terminal domain (N-Ter) and disorganized C-terminal domain (C-Ter).

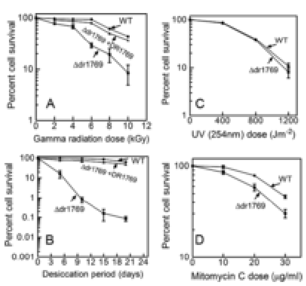

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FIG 2

DNA damage response of the Δ*dr1769* mutant. *D. radiodurans* cells (■), Δ*dr1769* mutant cells (●), and Δ*dr1769* cells expressing wild-type DR1769 (▲) were treated with different doses of gamma radiation (A), desiccation (B), UV (254 nm) (C), and mitomycin C (D). Different dilutions of these cells were plated on LB agar plates supplemented with the required antibiotics, numbers of CFU per milliliter were computed, and the percent cell survival was calculated by considering the percentage of CFU of untreated samples as 100%.

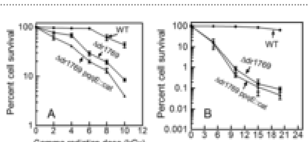

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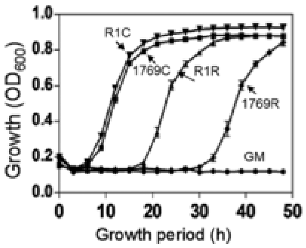
FIG 3

Genetic interaction of *pqqE* and *dr1769* loci in DNA damage response of *D. radiodurans. D. radiodurans* (■), Δ*dr1769* mutant (●), and Δ*dr1769pqqE*::*cat* (▲) cells were treated with different doses of gamma radiation (A) and desiccation (B), the number of CFU per milliliter was computed, and percent cell survival was calculated by considering the percentage of CFU of untreated samples as 100%.

FIG 4



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Effect of *dr1769* deletion on normal growth and recovery from desiccation. *D. radiodurans* R1 (▼, ▲) and Δ*dr1769* mutant (■,♦) cells were grown in rich medium under normal conditions (▼, ■) and stressed at 5% humidity for 20 h and then allowed to recover (▲, ♦). Optical density at 600 nm was measured continuously on a microtiter-based density reader. Growth medium (●, GM) was taken as the blank control.

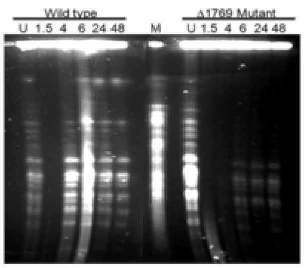

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FIG 5

DSB repair kinetics during postirradiation recovery in *D. radiodurans. D. radiodurans* R1 (wild type) and Δ*dr1769* mutant (Δdr1769 mutant) cells were treated with 6.5 kGy gamma radiation and allowed to grow at different time periods (1.5, 4, 6, 24, and 48 h), and PFGE was carried out along with unirradiated (U) respective controls.

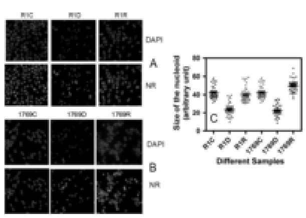

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FIG 6

Effect of *dr1769* deletion on nucleoid compactness under desiccation. *D. radiodurans* R1 (A) and Δ*dr1769* mutant (B) untreated cells (R1C, 1769C) were incubated at 5% humidity for 24 h (R1D, 1769D) and allowed to recover in rich medium under normal growth conditions (R1R, 1769R). These cells were stained for nucleoid (DAPI) and with Nile red (NR), and images were taken with a fluorescence microscope. (C) The false blue areas of DAPI-stained images were measured using Image J software and plotted in GraphPad Prism software. Data shown here are representative of a reproducible experiment repeated three times independently. For a color version of this figure, you may refer to Fig. S2 in the supplemental material.

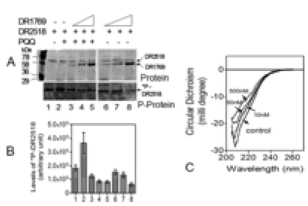

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FIG 7

DR1769 effect on DR2518 autophosphorylation and DR1769 interaction with pyrroloquinoline quinone (PQQ). Purified recombinant DR2518 was mixed with increasing molar ratios of 1:1 (lanes 3 and 6), 1:5 (lanes 4 and 7), and 1:10 (lanes 5 and 8) of purified recombinant DR1769 in the presence (lanes 2 to 5) and absence (lanes 1, 6, 7, 8) of 10 nM PQQ and 1μCi [^{32}P]ATP for 1 h. (A) Proteins were separated on an SDS-PAGE gel, the gel was stained with Coomassie blue (Protein) and dried, and autoradiogram (P-protein) was developed. (B) The intensity of phosphoprotein bands of each lane (1 to 8) were computed and plotted. (C) Interaction of PQQ with recombinant purified DR1769 was checked by circular dichroism spectroscopy in the absence (control) and presence of increasing concentrations (10 nM, 50 nM, and 500 nM) of PQQ.