A ring-like nucleoid is not necessary for radioresistance in the Deinococcaceae
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Abstract

Background: Transmission electron microscopy images of Deinococcus radiodurans R1 suggest that the nucleoid of this species exists as a "ring-like" body, and have led to speculation that this structure contributes to the radioresistance of the species. Since extreme radioresistance is characteristic of six other species of Deinococcus, we have attempted to correlate nucleoid morphology and radioresistance by determining whether the genomic DNA of each of these species exhibit similar structures.

Results: The nucleoid morphologies of seven recognized species of Deinococcus, the radioresistant bacterium Rubrobacter radiotolerans, and the more radiosensitive deinococcal relative Thermus aquaticus were evaluated using epifluorescence and deconvolution techniques. Although the nucleoids of Deinococcus murrayi, Deinococcus proteolyticus, Deinococcus radiophilus, and Deinococcus grandis have structures similar to D. radiodurans, the majority of nucleoids found in Deinococcus radiopugnans and Deinococcus geothermalis lack any specific organization. The nucleoid of R. radiotolerans consists of multiple highly condensed spheres of DNA scattered throughout the cell. The genomic DNA of Thermus aquaticus is uniformly distributed throughout the cell.

Conclusion: There is no obvious relationship between the shape of a species' nucleoid and extreme radioresistance. However, the genomes of all extremely radioresistance species examined are highly condensed relative to more radiosensitive species. Whether DNA in this tightly packed configuration contributes to the radioresistance of these bacteria remains unknown, but this common structural feature appears to limit diffusion of fragments generated post-irradiation even in cells incapable of repairing strand breaks.

Background

The Deinococcaceae are a small family within the domain Bacteria that are distinguished by their ability to tolerate DNA double strand breaks [1,2]. There are eleven validly described species and seven of these tolerate what for most microorganisms is a sterilizing dose of ionizing radiation, exhibiting detectable survival after exposure to 10 kGy (1,000,000 Rad) γ radiation [3]. The ionizing radiation resistance of the four remaining species, Deinococcus indicus [4], Deinococcus frigens, Deinococcus saxicola, and Deinococcus marmoris [5] has not been reported. Ionizing radiation generates an array of DNA damage in the target cell, including many types of base damage, single, and double strand breaks [6]. Of these types of damage, DNA double strand breaks (DSBs) are considered the greatest threat to cell viability, and an excessive number of DSBs...
are expected to be lethal. *E. coli*, for example, cannot sur-
vive introduction of greater than one or two DSBs into its
genome [7]. In contrast, *D. radiodurans* survives doses of
ionizing radiation that generate greater than 100 DSBs per
genome without mutation or loss of viability [8-10]. It is
the ability to endure and accurately repair these lesions
that set the deinococci apart from other species.

The reasons for the deinococci's radioresistance are poorly
understood. Genetic and biochemical evidence obtained
from *D. radiodurans*, the best studied of these species,
argues that recovery requires RecA-mediated homologous
recombination [9,11,12]. *D. radiodurans* is multi-
genomic, and depending on the growth phase, cells con-
tain from 4 to 10 copies of their genome depending on
growth conditions [13,14]. It is believed that this
increased DNA content is protective in that it serves as a
reservoir of genetic information that can be used during
recombinatorial repair. In addition, it has been suggested
that there may be a pre-existing alignment of homologous
sequences between copies of the genome and that this
alignment accounts for the remarkable speed and fidelity
of DNA double strand break repair in this species [15].
However, the existence of such an alignment has not been
established.

Levin-Zaidman et al. [16] have reported that the genome
of *D. radiodurans* assumes a tightly packed ring-like struc-
ture that may represent an alternative mechanism for pro-
tecting *D. radiodurans* from DSBs. These authors suggest
this structure contributes to *D. radiodurans* radioresistance
by preventing fragments formed by DSBs from diffusing
apart during repair. Having the capacity to maintain the
linear continuity of its genome in the face of the extensive
fragmentation resulting from high dose ionizing radiation
would provide obvious advantages to *D. radiodurans*.
Assuming that DNA repair proteins can function within
the proposed structure, gene order is preserved and the
gaps generated by damage and subsequent DNA degrada-
tion could be bridged by homologous recombination
with redundant genetic information available in the
genome copies. Such an arrangement does not require a
pre-existing alignment between sequences, but if homol-
ogous genetic information is aligned prior to irradiation,
the time needed to effect repairs should be reduced.

In an attempt to gain insight into whether the structures
reported by Levin-Zaidman et al contribute to ionizing
radiation resistance, we have combined epifluorescence
and deconvolution microscopy to describe the structure
of a related but less radioresistant species, *Ther-
mus aquaticus*, and a phylogenetically distinct, but more
radioresistant [17] species *Rubrobacter radiotolerans* seek-
ing to correlate variations in nucleoid morphology with
radioresistance. We find little evidence to support the
assertion that a specific nucleoid structure is required for
bacterial ionizing radiation resistance, but note that the
 genomic DNA of the most radioresistant species exam-
ined is more condensed relative to more radiosensitive
species, suggesting that this generic feature is passively
facilitating repair processes.

**Results**

**The nucleoid of Deinococcus radiodurans R1**

Sequential digital images were collected at 100 nm incre-
ments from stationary phase cultures of *D. radiodurans* R1.
Cells were stained with the membrane dye FM4-64 and
the DNA specific dye DAPI (4’, 6-diamidino-2-phenylin-
dole), and the images obtained using each dye were
merged to form the sequence of images depicted in Fig. 1.
In agreement with previous descriptions of this species' nuculeoid, the genomic DNA of R1 exhibits a clear organized
pattern [16,18,19], distinct from that observed in *E. coli* (Fig. 2) where the DAPI-stained DNA appears
uniformly distributed throughout each cell. The DNA of R1
has a distinctive ring-like structure in each cell of this tet-
rad, suggesting that the genome of this species is spooled
around a sphere or cylinder that excludes the dye. Given

![Figure 1](http://www.biomedcentral.com/1471-2180/5/17)

**Figure 1**

Optical sections of a tetrad of *D. radiodurans* R1. The
series is in order from left to right of images within a row
and from top to bottom of rows within the figure. Images are
taken at 100 nm intervals. The DNA (blue) is stained with
DAPI and the lipid membrane (red) is stained with FM-4-64.
the size and organization of this structure we assume that the proposed core is primarily proteinaceous, but don’t exclude the possibility that other macromolecules, including DNA that is inaccessible to the dye, may be present in the core. Based on the images in Fig. 1, we believe that the DAPI-stained DNA exists as an equatorial band (or possibly a tight spiral) over the surface of this core. In each of the cells the band is tilted relative to the focal plane. For example, in the lower half of the tetrad, the bands are tilted slightly away from the viewer; toward the upper left in the cell on the left and toward the upper right in the cell on the right. Consistent with this interpretation, we find that the dye does not form a closed ring in every optical section and that the patterns vary from cell to cell. In many cells the DNA first appears in a crescent shape in one area of the cell. This crescent changes in sequential sections (Fig. 1), ultimately disappearing and giving way to a crescent shaped structure that mirrors where the dye first appeared. This pattern of changes is most simply explained by assuming that the DNA is on the surface of a larger structure capable of tilting about a central axis. When tilted, the ring of dye will cross the focal plane at an angle and produce a falcate shape.

The nucleoid of most, but not all, species of Deinococcus is similar to that of D. radiodurans R1

D. radiophilus and D. proteolyticus have a nucleoid reminiscent of that described for D. radiodurans (Fig. 1). In cross section, the DAPI accumulates in a halo surrounding a circular core that excludes the dye. However, the organization of the DNA in these species differs subtly relative to D. radiodurans; the DNA appears to completely surround the structure at the core of the nucleoid. This point is best illustrated in Fig. 3, which depicts 20 sequential sections (moving from upper left to the lower right panel) through the DAPI-stained nucleoids in a pair of D. proteolyticus cells. (The stained membrane is not included in this image.) The first image corresponds to a focal plane at the top of the nucleoid and with each successive image it becomes clear that the DNA forms a shell that follows the contour of an internal sphere that has not been stained. The nucleoid morphology of D. radiophilus was found to be identical to that of D. proteolyticus (data not shown).

The nucleoid of rod-shaped D. grandis is distinctive, but shares similarity to that of D. radiodurans. The DNA in most cells is highly condensed, localized to a specific area
of the cell (Fig 4). The sequential sections obtained from
D. grandis suggest that the DNA surrounds an unstained
core. A number of cells (~7%) exhibit additional
condensed spots of DAPI stain, as illustrated in the cell on
the right in Fig 4. The significance of these "spots" is not
known at present.

In D. murrayi, the ring of DAPI-stained DNA is remarkably
uniform (Fig. 5), varying little between cells. As with D.
radiodurans, the center of the ring is not stained, but the
number of sections containing DNA is more variable,
ranging between 20 and 30. This suggests that the nucle-
oid of D. murrayi may be more cylindrical than that of D.
radiodurans with the DNA forming a sleeve around the
core. Consistent with this interpretation, most of the cells
display a ring of DAPI in every optical section.

In contrast to the other species discussed thus far, only 2%
of D. radiopugnans and 10% of D. geothermalis cells exhibit
a structurally well-defined nucleoid (Figs. 6 and 7). In the
majority of these cells, the DNA is condensed in that it is
not uniformly spread throughout the cell as was observed
with E. coli (Fig 2), but it does not seem to be associated
with an intracellular structure. Instead the DNA appears to
be distributed chaotically through the cytosol. The
absence of a consistent pattern in DAPI staining within
most of the cells suggests that the DNA in these species
does not adopt a fixed shape.
The nucleoid of *Thermus aquaticus*

The genera *Thermus* and *Deinococcus* are part of the same phylum within the domain *Bacteria* [3]. Because of this relationship, we sought 1) to establish if *Thermus* species also exhibit increased tolerance to ionizing radiation, and 2) to determine if the nucleoid of this genus bore any resemblance to that of the deinococci. We assessed the ionizing radiation resistance of *Thermus aquaticus* YT-1, the type species for its genus, and compared it to *D. radiodurans* R1. As indicated in Fig. 8, YT-1 does not display extraordinary resistance relative to R1, but this strain is better able to survive γ radiation than the *E. coli* AB1157 control. After 1 kGy exposure, approximately 20% of the YT-1 population remained viable, whereas only 0.2% of the AB1157 culture survived.

Images of YT-1 (Fig. 9) failed to show evidence of sub-cellular organization or condensation similar to that observed in the deinococcal species. The pattern obtained with DAPI most closely resembles that observed in *E. coli* (Fig. 2).

The nucleoid of *Rubrobacter radiotolerans*

*Rubrobacter radiotolerans* is an extremely ionizing radiation resistant bacterium that is, based on 16S rDNA-based phyllogyen, unrelated to the *Deinococcaceae* [17]. This species is a member of a lineage within the Actinobacteria. The nucleoid of this species is quite different from that observed in the deinococci (Fig. 10). The DNA appears to form highly compact structures without a well-defined shape. Most cells contain two or three of these structures, and it is unclear whether each dye spot represents a unit length of genomic DNA. Based on differences in size and intensity, the spots do not appear to contain equivalent amounts of DNA, but these differences may be spurious. We suspect that the cell envelope of *R. radiotolerans* is less

![Figure 8](image1.png)

**Figure 8**
Representative survival curves for cultures of *Thermus aquaticus* YT-1, *E. coli* AB1157, and *Deinococcus radiodurans* R1. Values are the means +/- standard deviations of three independent experiments. *n* = 9.

![Figure 9](image2.png)

**Figure 9**
An epifluorescence image of *Thermus aquaticus*.

![Figure 10](image3.png)

**Figure 10**
Optical sections of a *Rubrobacter radiotolerans* cell. The series is in order from left to right of the images within a row and from top to bottom of the rows within the figure. Images are taken at 100 nm intervals.
permeable to DAPI than that of the other species examined in this study. Attempts to stain the DNA of the closely related *R. xylanophilus* [17] were unsuccessful; the dye being unable to penetrate the cell. Differing quantities of dye entering individual *R. radiotolerans* cells may account for the differences in dye intensity observed.

**The effect of ionizing radiation on nucleoid morphology**

As has been shown previously, when *D. radiodurans* cells are exposed to high dose γ radiation they exhibit substantial DNA damage [10]. The distinctive pattern formed on a pulsed field gel by the NotI digested genome (data not shown) is initially obliterated by the DNA double strand breaks introduced during a 5 kGy exposure [10,20], but after three hours recovery, the pattern is restored indicating that the majority of the breaks have been repaired. To establish whether the introduction of large numbers of DNA double-strand breaks affect the distribution of DNA within the irradiated cell, we examined nucleoid structure in exponential phase *D. radiodurans* two hours post-irradiation, comparing the distribution of DAPI stain to that of *E. coli* AB1157 treated identically (Fig. 11B). The differences in the images obtained are striking. While we are able to detect DAPI-stained material in some *E. coli* cells, the diffuse distribution, apparent in Fig. 2, is no longer evident; only infrequent spots of DAPI remain. Furthermore, images of *E. coli* cells captured three hours post-irradiation of 5 kGy show a complete loss of the DAPI-stained material (data not shown). One reason for this may be that the *E. coli* genome is being progressively degraded following irradiation [21]. In contrast, the nucleoids of *D. radiodurans* and *D. radiopugnans* appear intact and retain the ability to be stained (Fig. 11A and 11D). These images provide support for the hypothesis of

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**Figure 11**

The nucleoid of deinococcal strains and *E. coli* 2 hrs after exposure to 5000 Gy γ radiation. A1 – projection image of *D. radiodurans* R1 control cells; A2 – projection image of *D. radiodurans* R1 irradiated cells; B1 – 2D image of *E. coli* control cells; B2 – 2D image of *E. coli* irradiated cells; C1 – 2D image of Rec30 control cells; C2 – 2D image of Rec30 irradiated cells; D1 – one optical slice of a series of *D. radiopugnans* control cells; D2 – one optical slice of a series of *D. radiopugnans* irradiated cells.
Levin-Zaidman et al [16,19], which proposes that fragments generated by the double strand breaks may be in some manner held in place and protected from extensive degradation by cellular nucleases.

The notion that the release of fragments from the damaged genome of D. radiodurans is limited is also apparent in our analysis of the effects of ionizing radiation on the nucleoid of the recA strain, rec30. The rec30 strain, which lacks the ability to carry out homologous recombination [8,9], is incapable of reassembling its genome following irradiation. However, like R1, the nucleoid of rec30 remains a coherent structure (Fig. 11C) two hours post-irradiation, providing further evidence that fragments generated do not diffuse throughout the cytosol despite the presence of massive numbers of un-repaired DNA double strand breaks.

**Discussion**

Levin-Zaidman et al [16] have reported that genomic DNA of stationary phase cells of D. radiodurans is ordered as a tightly packed toroid, and it has been argued that this organization is in part responsible for D. radiodurans resistance to ionizing radiation [16,19]. These authors assume that the dense packaging characteristic of toroids restricts the diffusion of DNA fragments generated when cells are exposed to ionizing radiation, preventing a loss of genetic information that is needed for effective recovery from the insult. However, these authors also describe logarithmic phase cultures of D. radiodurans R1, and indicate that the nucleoids of these cells do not always exhibit a toroidal ring-like morphology even though they remain extremely radioresistant [2]. This observation seems to argue against a requirement for involvement of a specific nucleoid structure in ionizing radiation resistance. In addition, Daly et al [18] have demonstrated that growth in different media alters the organization of the nucleoid of D. radiodurans, and that the change does not correspond to changes in radioresistance. This group reports that ring-like nucleoids predominate in cultures growing in defined minimal medium, but that these cultures are more sensitive to ionizing radiation than cultures with fewer ring-like nucleoids growing in a rich medium.

We initiated this study in an attempt to correlate nucleoid structure with radioresistance, reasoning that if the inferences of Levin-Zaidman et al [16] are correct, it will be reflected in morphological differences between radioresistant and radiosensitive species. We have examined species known to be ionizing radiation resistant as well as strains that are more sensitive to γ irradiation, and in agreement with Daly et al [18] find no compelling evidence that specific structures contribute to radioresistance. We base this conclusion on our failure to identify a distinctive repetitive pattern in the DAPI-stained DNA associated with the majority of D. geothermalis and D. radiopugnans cells in stationary phase culture. Cultures of D. geothermalis [22] and D. radiopugnans [23] are as radioresistant as D. radiodurans, but their genomes are more fluid, arguing that a nucleoid need not maintain a well-defined shape to sustain ionizing radiation resistance.

Despite this conclusion, we note that among the extremely radioresistant species examined DAPI-stained DNA is condensed relative to what is observed in E. coli and T. aquaticus. The DAPI is localized within the more radioresistant cells, and not spread throughout the cytosol. This aggregation of DNA indicates that a basic tenet of the model of Levin-Zaidman et al [16] may be valid: specifically that the deinococci utilize mechanisms for protecting the fragments generated by strand breaks including a passive process that limits the diffusion of these fragments. This concept is most clearly demonstrated by the behavior of rec30 strain subsequent to irradiation. Despite the fact that this strain is incapable of restituting the majority of the DSBs generated, the rec30 nucleoid retains its shape.

However, it must be noted that under certain conditions E. coli cells can undergo changes in nucleoid morphology similar to those reported for the deinococci. E. coli nucleoids become condensed spheres with cores of unknown composition when protein synthesis is inhibited [24], and when the mukB locus is disrupted the resulting mutant maintains a ring-like nucleoid [25]. Clearly, since E. coli strains are much more sensitive to ionizing radiation relative to D. radiodurans, the presence of a condensed nucleoid alone is not a sufficient explanation for radioresistance. Instead, we assume the combination of a condensed nucleoid structure and unique protein-dependent DNA repair mechanisms is responsible for the radioresistant phenotype displayed by the deinococci.

The physical basis for the condensed nucleoid we observe is unknown. We suspect that this level of spatial organization is in large part mediated by proteins that either link copies of the genome together (assuming that a species contains more than one genome copy), or coordinates genome folding in a manner that generates the shapes we have described. For those species, such as D. radiodurans or D. murrayi, in which the nucleoid forms an obvious structure, we envision a protein lattice that acts as a scaffold that the genome is organized around. We predict these proteins are functionally analogous to the SMC (structural maintenance of chromosomes) proteins described in many eukaryotic and prokaryotic species [26-28]. It seems unlikely that genomic DNA is as condensed as the DNA-Dps assemblies described in some stationary phase bacteria [29,30]. These structures are tightly packed,
almost crystalline, and it is difficult to envision how an actively metabolizing cell could function under this circumstance; the DNA needs to remain accessible to proteins that catalyze essential housekeeping functions.

We also predict that the ionic composition of the cytosol of the deinococci examined favors the formation of the structures we observe. The Deinococcaceae exhibit unusually high intracellular levels of Mn\(^{2+}\) [18] and it is possible that accumulating this metal creates an environment that facilitates DNA condensation within this species in vivo through direct or indirect mechanisms. In vitro, the condensation of DNA can be achieved by adding a condensing agent, such as a multivalent cation, to an aqueous solution of DNA. The strong electrostatic interaction of the DNA and these cations neutralizes the repulsion of phosphate groups in the DNA backbone, and it has been shown that approximately 90% of the DNA charge must be neutralized for condensation to occur [31]. Intracellular Mn\(^{2+}\) may also indirectly facilitate nucleoid condensation by, for example, augmenting the function of deinococcal DNA-binding proteins. On the other hand, high Mn\(^{2+}\) content may have no affect on nucleoid morphology since, as described above, E. coli can have a condensed nucleoid even though this species has a much lower level of intracellular Mn\(^{2+}\) [18].

Years of experimental evidence irrefutably argue that DNA repair is essential for D. radiodurans recovery from high dose exposure to ionizing radiation, but this fact does not necessarily lead to the conclusion that efficient DNA repair is sufficient for extreme radioresistance. Although there has been a great deal of speculation concerning subtle differences in the properties of DNA repair proteins isolated from D. radiodurans, there has yet to be a convincing demonstration that this species’ DNA repair proteins are “better” relative to homologues found in more radiosensitive species. Given this, it remains a formal possibility that D. radiodurans DNA repair proteins function within a molecular environment that enhances their effectiveness. In other words, it seems likely that there are features of deinococcal physiology that augment DNA repair processes, allowing the conventional complement of DNA repair proteins to more efficiently deal with DNA damage. We believe that the images presented here suggest that the genomic DNA of the deinococci is more condensed relative to other species. We suggest that this aggregation is protective, and that it may significantly contribute to the radioresistance of these species by confining the fragments generated subsequent to irradiation and preserving the linear continuity of the damaged genome.

**Conclusion**

This work resulted in two key observations. First, all evidence obtained is consistent with the notion that the genomes of radioresistant species are more condensed than radiosensitive species. Second, irradiation does not seem to disturb the pattern of condensation observed in the deinococci, even when we examined that pattern in a recA defective strain incapable of repairing DNA double strand breaks. Therefore, in agreement with Levin-Zaidermen et al [16], we assume that the deinococci have the capacity to passively prevent the diffusion of DNA fragments post-irradiation, and that this ability may contribute to the radioresistance of these species.

**Materials and methods**

**Growth conditions**

Deinococcus radiodurans R1 (ATCC 13939), Deinococcus radiophilus (ATCC 27603), Deinococcus proteolyticus (ATCC 35074), Deinococcus radiopugnans (ATCC 19172), and Deinococcus grandis (ATCC 43672) were grown in TGY broth at 30°C as described elsewhere [3]. Deinococcus geotherma-lis (DSM 11300) and Deinococcus murrayi (DSM 11303) were grown in TGY broth at 50°C [17]. Rubrobacter radiotolerans (ATCC 51242) was grown at 30°C in a medium consisting of 1% tryptone, 0.5% yeast extract, 0.5% malt extract, 0.5% casamino acids, 0.2% meat extract, 0.2% glucose, 0.005% Tween 80, and 0.1% magnesium sulfate. Thermus aquaticus YT-1 (ATCC 25104) was grown at 70°C in Castenholz TYE medium (ATCC medium 723). E. coli AB1157 cultures were grown at 37°C in LB medium (1% tryptone, 0.5% yeast extract, and 1% NaCl).

Unless otherwise indicated, all cultures were grown to stationary phase prior to microscopic examination. Cultures were harvested with the following densities as measured by OD\(_{600}\): D. radiodurans, 1.2–1.8; D. radiophilus, 1.8–2.2; D. proteolyticus, 2.0; D. radiopugnans 1.3–2.0; D. grandis, 1.7; D. geotherma-lis, 1.8; D. murrayi, 1.3; R. radiotolerans, 1.9; T. aquaticus, 1.1. These values correspond to cultures with densities between 3 × 10\(^8\) and 1.3 × 10\(^9\) CFU/ml.

**Microscopy**

Cultured cells were stained with N-(3-triethylammoniumpropyl)-4-(6-(4-diethylamino)phenyl)hexatrienyl)pyridinium dibromide, (FM 4–64), and 4',6-Diamidino-2-phenylindole dihydrochloride, (DAPI), for the visualization of the lipid membrane and DNA, respectively. Ratios of the amounts of stationary phase culture cells to fluorescent dyes were a 3:1 ratio of culture cells to 0.0625 µg/µL of FM 4–64 solution to 3 µg/ml DAPI solution. The cells were then mounted on slides coated with 0.5% agarose.

Figures 1, 3, 4, 5, 6, 7, and 10 were created by capturing two-dimensional images at a series of points along the z-axis that were set at 100 nm apart. The N2.1 filter cube from Leica Microsystems was used for FM 4–64 detection, which includes a 580 nm longpass dichroic mirror, a 515–
560 nm bandpass excitation filter, and a 590 nm long pass emission filter. The A4 filter cube from Leica Microsystems was used for DAPI detection, which includes a 400 nm longpass dichroic mirror, a 360-40 nm bandpass excitation filter, and a 470-40 nm bandpass emission filter. The optical series was deconvolved with Slidebook 4.0 from Intelligent Imaging Innovations, Inc. (Denver, CO), using constrained iterative deconvolution and was then imported into Adobe Photoshop 7.0 as separate two-dimensional images. The two-dimensional images were then arranged in order as depicted.

The projection image in Fig. 11A was achieved with the Slidebook 4.0 software by compiling the stack of two-dimensional images into a single two-dimensional image.

All images were captured using a Leica DMRXA2 microscope and a Sensicam QE camera from The Cooke Corporation, (Auburn Hills, MI). The brightness and contrast of all images were enhanced using Adobe Photoshop 7.0.

Ionizing radiation exposure
Cultures (grown in the appropriate medium) of D. radiodurans (OD600 0.12–0.19), D. radiopugnans (OD600 1.5–1.8), rec30 (OD600 1.4) and E. coli (OD600 0.23) were irradiated to a dose of 5000 Grays using a Model 484R 60Co irradiator (J. L. Sheppard & Associates, San Fernando, CA). Controls were kept at room temperature during irradiation exposure of experimental samples. All samples were incubated at the appropriate conditions immediately after irradiation. All of the cells were harvested after 2 hours of incubation and prepared for microscopy as described above.

Survival curves
Cultures were irradiated using the irradiator described above, and survival established by serial dilution of irradiated cultures and plating on appropriate growth medium. Three independent trials were conducted for each species examined with three replicates per trial. Sigma Plot software was used to create the survival curve.

Authors’ contributions
JR B and JMZ conceived and designed the experiments. JMZ performed all of the experimental studies. JRB and JMZ wrote the paper.

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