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ORIGINAL PAPER

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Physiological responses of the halophilic archaeon *Halobacterium* sp. strain NRC1 to desiccation and gamma irradiation

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Abstract We report that the halophilic archaeon *Halobacterium* sp. strain NRC-1 is highly resistant to desiccation, high vacuum and ^{60}Co gamma irradiation. *Halobacterium* sp. was able to repair extensive double strand DNA breaks (DSBs) in its genomic DNA, produced both by desiccation and by gamma irradiation, within hours of damage induction. We propose that resistance to high vacuum and ^{60}Co gamma irradiation is a consequence of its adaptation to desiccating conditions. Gamma resistance in *Halobacterium* sp. was dependent on growth stage with cultures in earlier stages exhibiting higher resistance. Membrane pigments, specifically bacterioruberin, offered protection against cellular damages induced by high doses (5 kGy) of gamma irradiation. High-salt conditions were found to create a protective environment against gamma irradiation in vivo by comparing the amount of DSBs induced by ionizing radiation in the chromosomal DNA of *Halobacterium* sp. to that of the more radiation-sensitive *Escherichia coli* that grows in lower-salt conditions. No inducible response was observed after exposing *Halobacterium* sp. to a nonlethal dose (0.5 kGy) of gamma ray and subsequently exposing the cells to either a high dose (5 kGy) of gamma ray or desiccating conditions. We find that the hypersaline environment in which *Halobacterium* sp. flourishes is a fundamental factor for its resistance to desiccation, damaging radiation and high vacuum.

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Keywords Halophiles · Ionizing radiation · Desiccation · Double-strand breaks · DNA repair

Abbreviations DSBs: Double-strand breaks · ROS: Reactive oxygen species · EMS: Ethyl methanesulfonate · Gy: Gray

Introduction

Hypersaline environments are characterized by extremely high ionic strength, up to ten times the salinity of seawater, by elevated temperatures and by high levels of UV radiation. These conditions lead to periodic evaporation of water resulting in concentration of salts, exposure of cells to desiccation and DNA damage (Potts 1994; Martin et al. 2000). UV radiation promotes the formation of two types of photoproducts; (6–4) photoproducts and cyclobutane pyrimidine dimers. In the halophilic archaeon *Halobacterium* sp. strain NRC-1, the majority of lesions are cyclobutane pyrimidine dimers: these lesions are removed via photoreactivation using a photolyase encoded by the *phr2* gene (McCready 1996; Baliga et al. 2004). Stress response to intense UV exposure in *Halobacterium* sp. include a down-regulation of all genes involved in the production of gas vesicles, causing the cells to sink in the water column therefore avoiding the deleterious effects of UV irradiation (Baliga et al. 2004).

Induction of DNA double-stranded breaks (DSBs) in microorganisms has been observed under both desiccating conditions and gamma irradiation (DiRuggiero et al. 1999; Mattimore and Battista 1996). The mechanism for DSB formation after exposure to desiccation is poorly understood. In contrast, ionizing radiation is a well-characterized exogenous source of free radicals produced via the radiolysis of water, which account for more than 80% of the DNA damage, whereas less than 20% is the result of direct effects of γ-photons (Riley 1994). Reactive oxygen species (ROS) are able to modify

DNA bases into such forms as the well-characterized 8-hydroxy-2′-deoxyguanosine resulting in mismatches, apurinic/abasic sites and strand breaks (Riley 1994). These damages often occur in clusters within two helical turns of the DNA on opposite strands resulting in the formation of DSBs during attempted base excision repair (Blaisdell and Wallace 2001; Dianov et al. 2001). Furthermore, hydrogen peroxide (H_2O_2) can react with Fe^{2+}, by the Fenton reaction, to produce the highly reactive hydroxyl radical that is able to attack and damage proteins, lipids and nucleic acids. Scavenging of ROS is the function of superoxide dismutase, catalase and peroxidase that aid in preventing cellular damages by ROS (Salin and Brown-Peterson 1993). Low intracellular concentrations of Fe^{2+} combine with high concentrations of Mn^{2+} have been identified as factors that limit the formation of hydroxyl radicals in *Deinococcus radiodurans* from H_2O_2 via the Fenton reaction (Daly et al. 2004).

Mechanisms for the maintenance of genome fidelity under extremely DNA damaging conditions are especially vital for the survival of life in hypersaline environments. Adaptations, for survival of desiccation, of many prokaryotes include sporulation (Nicholson et al. 2000) and the production of extracellular polysaccharides (Potts 1994), neither of which is utilized by *Halobacterium* sp. Instead, genome analysis showed that *Halobacterium* sp. contains putative genes for all major DNA repair pathways (nucleotide excision repair, base excision repair, photoreactivation, homologous recombination, translesion synthesis, and mismatch repair) found in both Bacteria and Eukarya (Ng et al. 1997), suggesting a major role for DNA repair in *Halobacterium* sp. resistances to extreme conditions such as desiccation. Efficient DNA repair upon rehydration is essential for maintaining DNA fidelity in other desiccation-resistant species such as *Chrococcidiopsis* sp. and *D. radiodurans* (Billi et al. 2000; Battista et al. 1999). Adaptations to desiccation, and the resulting DSBs, have been hypothesized as the source of gamma radiation resistance in *D. radiodurans* (Mattimore and Battista 1996) as cells on the earth's surface are not naturally exposed to ionizing radiation. A similar mechanism likely results in the ability of *Halobacterium* sp. to withstand high levels of gamma irradiation. Additionally, protective mechanisms such as membrane pigments, including C_{50} carotenoid, bacterioruberin and intracellular KCl were found to be important in UV and gamma-ray protection in *Halobacterium salinarium* through the scavenging of hydroxyl radicals (Shahmohammadi et al. 1998).

In this work, we describe the effects of desiccation, high vacuum and gamma irradiation on the survival and recovery of *Halobacterium sp*. strain NRC-1. *Halobacterium sp*. is an excellent model organism for examining the cellular mechanisms involved in survival under damaging conditions and simulated space conditions (Mancinelli et al. 1998). It is an extremely halophilic archaeon that grows optimally under aerobic conditions in 4 M NaCl and maintains an internal salt concentration of K^+ and Na^+ to balance the extracellular salt concentration (Lanyi 1974; DasSarma 1995). Ease of culturing, a completed genome sequence (Ng et al. 1997) and efficient genetic and biochemical systems have enabled detailed investigations of the molecular response of this halophile model to a range of environmental conditions (Baliga et al. 2004; Mancinelli et al. 1998). This could also be extended to Martian conditions where high concentrations of sodium chloride and bromine compounds were found by the Mars Exploration Rovers in the soils of Meridiani Planum resulting from the evaporation over time of water from a brine solution (Rieder et al. 2004). *Halobacterium* sp. inhabits a challenging environment for the maintenance of genomic stability. Adaptive mechanisms for stress induction were investigated along with the protective effects of pigments and salt in vivo.

Materials and methods

Microbial strains and growth conditions

Halobacterium sp. strain NRC-1 (ATCC number: 700922), *Halobacterium* sp. mutants BA3 [colorless random ethyl methanesulfonate (EMS) mutant] and ΔPho81 [deletion mutation lacking *bop* (bacteriorhodopsin), *sop-1* and *sop-2* (sensory rhodopsins 1 and 2), and *hop* (halorhodopsin) prepared using the protocol by Peck et al. (2000); a gift from Nitin Baliga] and *Escherichia coli* K12 (ATCC number: 10798) were used in this study. *Halobacterium* sp. was grown in GN101 medium [250 g/L NaCl, 20 g/L $MgSO_4$, 2 g/L KCl, 3 g/L sodium citrate, 10 g/L bacteriological peptone (Oxoid brand)] with the addition of 1 mL/L trace elements solution (31.5 mg/L $FeSO_4.7H_2O$, 4.4 mg/L $ZnSO_4.7H_2O$, 3.3 mg/L $MnSO_4.H_2O$, 0.1 mg/L $CuSO_4.5H_2O$), at 42°C with shaking at 220 rpm (Innova 4080, New Brunswick Scientific). The GN101 medium was supplemented with 50 μg/L uracil for strains derived from a Δ*ura3* background (ΔPho81), and with 20 g/L Agar for solid medium. Basal salt solution (BSS) was used as a nonnutrient buffer during irradiation, and was composed of GN101 medium without peptone and trace elements. *E. coli* were cultured in Luria Broth (LB) and low salt medium [basal salt solution diluted 1/50 with the addition of 10 g/L oxoid brand peptone and trace elements solution (1 mL/L)].

EMS mutant construction

Halobacterium sp. cells were incubated with 0.076 M ethyl methanesulfonate (EMS) in basal salt solution at 37°C with shaking for 1 h (0.017% survival) after which an equal volume of 10% (w/v) sodium thiosulfate was added to the cell suspension. Cells were washed twice in BSS, recovered by centrifugation and

resuspended in the same buffer. Aliquots were plated onto solid GN101 medium and incubated at 42°C for 7 days. Colonies were transferred onto solid GN101medium grid-marked plates and further incubated at 42°C. 1,415 surviving colonies with varied colony size and appearance were selected for mutant screening including one colony exhibiting white pigmentation that was used in this study (BA3 mutant).

Exposure to desiccation and high vacuum

Halobacterium sp. cultures grown to an $OD_{600\ nm}$ of 0.8 (late log phase) were concentrated 20-fold in BSS, placed onto glass microscope cover slips (2×50 µl per cover slip as 4–5 dots) and allowed to dry completely. Cover slips were placed in six-well plates with perforated lids and exposed to high vacuum (10^{-6} Pa) using a high vacuum chamber at the NASA Goddard Space Flight Center (Greenbelt, MD, USA) for 2, 6, 10, 15, and 20 days (Saffary et al. 2002). For desiccation at ambient pressure, cover slips were placed in open glass Petri dishes with 36 g of Drierite desiccant (97% $CaSO_4$ and 3% $CoCl_2$) each, covered in tin foil and placed in a NAPCO model 5,831 vacuum oven at a pressure of 5 mm Hg at RT for 12 days. Cells were rehydrated by placing cover slips in 5 mL of BSS with moderate agitation for 30 min at room temperature (RT). The resulting cell suspensions were concentrated by centrifugation at 5,000×*g*, RT for 10 min, resuspended in BSS and plated onto solid GN101 medium to quantify cell survival by colony counts, after 4–7 days of incubation at 42°C. Control samples were processed in the same manner immediately after preparation and were not permitted to dry. For recovery experiments, cells were resuspended in GN101 medium following rehydration and incubated at 42°C with shaking. At time points 0, 8, 24, and 28 h of incubation postprocessing, the cultures were centrifuged at 5,000×*g*, 4°C, for 5 min, the supernatant was decanted, and the cell pellets stored at −20°C for subsequent DNA extraction as described below.

Gamma irradiation

All gamma-ray exposure experiments, unless otherwise stated, were conducted as follows: cultures of *Halobacterium* sp. grown to an $OD_{600\ nm}$ of 0.8 at 42°C were centrifuged at 5,000×*g*, at RT for 5 min. Cells were resuspended in one volume of BSS and exposed to 2.5–7.5 kGy of gamma-ray at 22°C (measured with an Omega Engineering Model HH 611PL4F Type K logging thermometer during irradiation) using a 26,000-curie (9.6E14 Bq) ^{60}Co source located at University of Maryland College Park Gamma Test Facility at a dose rate of 7.3 kGy/min. Controls were kept at RT. Irradiated samples and controls were diluted in BSS and plated on GN101 solid medium. Survival was quantified as described above. For analyses of chromosomal fragmentation repair, *Halobacterium* sp. were exposed to gamma-ray as described above, followed by incubation in GN101 at 42°C with shaking. Samples were removed for processing at 0, 8, 24, and 48 h. The control and 0 h samples were processed immediately after resuspension in GN101. Samples were centrifuged at 5,000×*g*, 4°C, for 5 min and the pellets stored at −20°C for subsequent DNA extraction as described below. *E. coli* cultures were processed in the same manner but using low salt medium for culture growth, irradiation and postirradiation incubation at 37°C.

Acquired resistance in *Halobacterium* sp. was determined by first exposing the cells to 0.5 kGy of gamma-ray in BSS, followed by 1 h incubation in GN101 at 42°C with shaking, and then either exposure to 5 kGy or 12 days of desiccation as described above. Controls with and without preirradiation were processed in the same way. Survival was quantified by colony count as described above.

DNA preparation

DNA was prepared according to Ng et al. 1995, with the following modifications: frozen cell pellets were thawed on ice and resuspended in 1/20 volume chilled BSS. Sterile water was added to 1/3 volume to lyse cells and a crude extraction of DNA was performed using 25:24:1 phenol:chloroform:isoamyl alcohol, subsequent precipitation in 95% ethanol and resuspension into Tris-Low EDTA (TLE pH 8; 10 mM Tris, 0.1 mM EDTA). RNAse ONE (Promega) was added to a final concentration of 0.3 units/µl, and the mixture was incubated for 75 min at 37°C followed by phenol–chloroform extractions and ethanol precipitation as described above. DNA was visualized by 0.8% (w/v) TAE agarose gel electrophoresis and ethidium bromide staining. Densitometry analysis was conducted using the ImageQuaNT5.0 software (Molecular Dynamics). Fluorescence intensity of 0 kGy was used as background and subtracted from the fluorescence intensity for 2.5, 5 and 7.5 kGy samples.

Results and discussion

Halobacterium sp. is highly resistant to desiccation, high vacuum, and ^{60}Co gamma-ray irradiation

The survival of *Halobacterium* sp. to desiccation at ambient pressure and high vacuum (10^{-6} Pa) was evaluated for up to 20 days of exposure. Under these conditions, the medium formed large salt crystals in which the cells were encased (Fig. 1a, b). For both challenges, a slow decrease in survival was observed over time, with 25% of cells viable after 20 days (Fig. 2a). There was no significant difference in survival

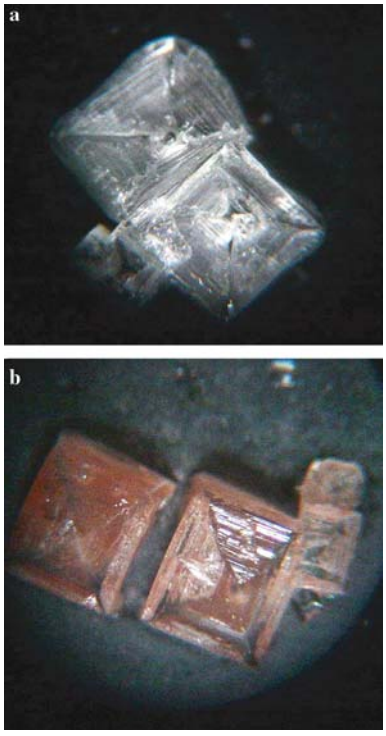

Fig. 1 Salt crystals from GN101 culture medium after 2 days of desiccation **a** un-inoculated and **b** inoculated with *Halobacterium* sp.

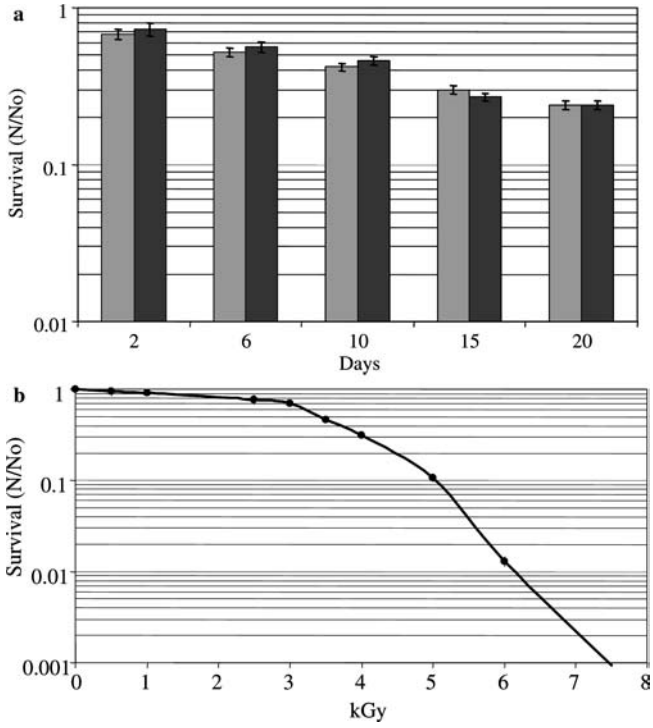

Fig. 2 *Halobacterium* sp. survival following **a** desiccation (*dark bars*) and high vacuum (*light bars*); **b** ^{60}Co gamma irradiation. *N* number of viable cells in challenged sample; *No* number of viable cells in control; *Error bars* represent standard error for at least three independent experiments

after exposure to either desiccation or high vacuum, suggesting that salt crystals themselves offer a level of cellular protection from environmental stresses. Indeed, viable halobacteria have been isolated from Triassic and Permian salt deposits (McGenity et al. 2000; Vreeland and Rosenzweig 2002), and more recently haloarchaeal 16S rDNA was detected in single-crystal and brine-inclusion-rich microsamples of ancient halite (Fish et al. 2002). The resistance of vegetative *Halobacterium sp*. cells is still less than that of *Bacillus subtilis* spores that were able to withstand exposure to 10^{-6} Pa at 77 K for 24 h with 75% survival, while *Halobacterium halobium* cells were completely killed (Koike et al. 1992; Nicholson et al. 2000).

Halobacterium sp. cells showed high resistance to ^{60}Co gamma-ray without distinct loss of viability up to 2.5 kGy (Fig. 2b) and with a D_{10} of 5 kGy. This is higher than the D_{10} reported for *Pyrococcus furiosus* (DiRuggiero et al. 1997) of 3 kGy and within the range reported for *Thermococcus gammatolerans* isolates with a D_{10} of 4–6 kGy (Jolivet et al. 2003, 2004). At the extremes of gamma sensitivity/resistance, *E. coli* has a D_{10}value of 0.25 kGy (Clavero et al. 1994) whereas *D. radiodurans* D_{10} is 10 kGy (van Gerwen et al. 1999). *Halobacterium salinarium* was reported to have a ten-fold lower tolerance for gamma irradiation than our strain of *Halobacterium* (Shahmohammadi et al. 1998), which is in contrast with the reported rates of UV survival that were identical between the two strains (Baliga et al. 2004; Shahmohammadi et al. 1998). This was surprising since Gruber et al. (2004) recently proposed that *Halobacterium* sp. strain NRC-1 be classified as a strain of *H. salinarium*. The survival curve for *Halobacterium* sp. is identical to that reported for the desiccation-resistant *Chroococcidiopsis* species of cyanobacteria (Billi et al. 2000), indicating a similarity in adaptation among organisms inhabiting desiccating environments, whether it is desert or hypersaline.

Halobacterium sp. resistance to gamma ray was growth phase-dependent with increased sensitivity to irradiation in stationary phase ($OD_{600\ nm} > 1.0$) when compared to exponential growing cultures (early, mid, and late log phase) (Fig. 3). This is in contrast to the growth-phase dependence observed for most *D. radiodurans* isolates that are more resistant to gamma irradiation during stationary phase than exponential phase with the exception of isolate four that was more sensitive to both gamma and UV irradiation in stationary phase (Keller and Maxcy 1984; Makarova 2001). Increased resistance of *D. radiodurans* to gamma irradiation in stationary phase suggested that the DNA was more tightly packed in nongrowing cells and therefore less accessible to damage (Bala and Jain 1996). However, active removal of ROS, which are the major cause for DNA damage by gamma irradiation, might be more efficient in actively growing cells thereby explaining the increase in the survival of *Halobacterium* cells during exponential growth phase.

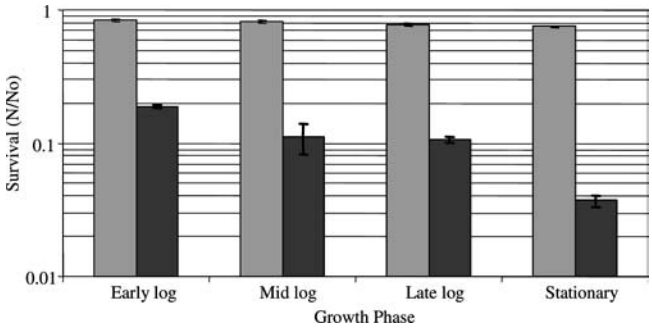

Fig. 3 *Halobacterium* sp. survival to 2.5 kGy (*light bars*) and 5 kGy (*dark bars*) of gamma irradiation at different growth stages. $OD_{600\ nm}$ values of 0.2, 0.4, 0.8, and 1.0 were used to represent early log, mid log, late log, and stationary phase, respectively. *N* number of viable cells in challenged sample; *No* number of viable cells in control; Error bars represent standard error for three independent experiments

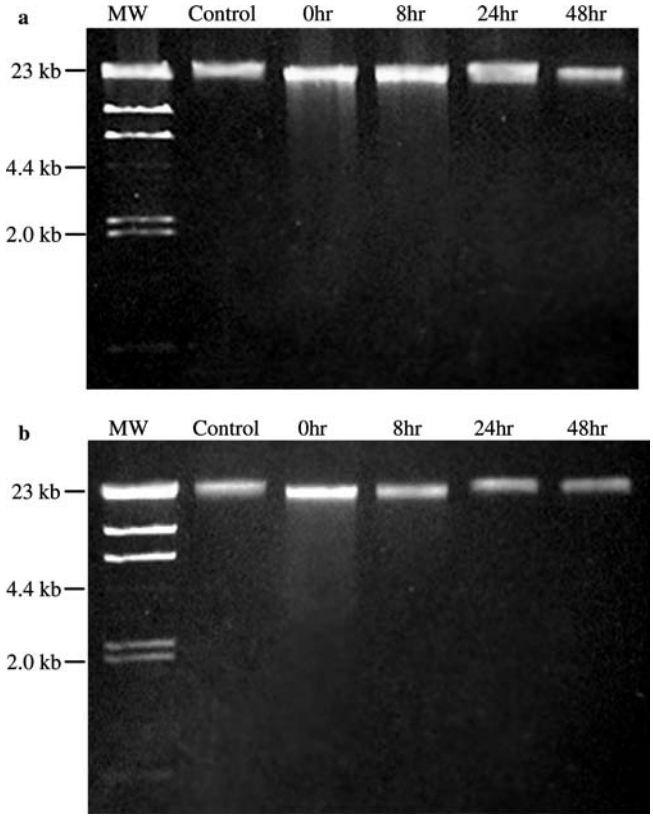

Fig. 4 Agarose gel electrophoresis of *Halobacterium* sp. DNA following **a** 144 h desiccation, and **b** ^{60}Co gamma irradiation at 7.5 kGy. Each lane contains DNA from approximately 2.2×10^8 cells. *Lane 1*, 50 kb ladder; *lane 2*, control non-treated; *lane 3–6*, 0, 8, 24 and 48 h of incubation at 42°C, respectively, following treatment

Halobacterium sp. repairs DNA DSBs produced by desiccation and gamma irradiation

It has previously been shown that *D. radiodurans* and the hyperthermophilic archaeon *P. furiosus* can efficiently repair DNA DSBs resulting from exposure to ionizing radiation (Moseley 1983; DiRuggiero et al. 1997). Here, we showed that both gamma irradiation and desiccation resulted in DNA DSBs in *Halobacterium sp.* and that those DNA breaks were repaired within several hours of incubation under optimal conditions (Fig. 4). In those experiments, DNA was liquid-extracted and the fluorescent smear under the genomic DNA band (at ~23 Kb) indicated additional DNA strand breaks due to exposure of the cells to ionizing radiation. Gamma-ray photons can cause direct damage to DNA, but the vast majority of the damage (over 80%) is the indirect result of the radiolysis of water and the subsequent production of ROS including the biologically destructive hydroxyl radical (Riley 1994). Damages by ROS are limited by free radical scavengers such as catalase, peroxidase, and superoxide dismutase (Riley 1994) that are found encoded in the genome of *Halobacterium* sp. along with thioredoxin reductase, NADH oxidase, and DNA repair proteins encoding genes such as specific glycosylases and endonucleases (Ng et al. 1997). The end result of damage by ionizing radiation are DNA DSBs that are typically repaired by RecA-mediated homologous recombination (HR) in *D. radiodurans* and in many Bacteria (Gutman et al. 1994; Daly et al. 1994; Minton and Daly 1995), although Ku-mediated nonhomologous end-joining has also been reported for several prokaryotes (Della et al. 2004). The genome of *Halobacterium* sp. does not encode a Ku-homolog but contain genes for several proteins involved in HR including RadA, the RecA/Rad51 archaeal homolog (Komori et al. 2000a), an archaeal-type Holliday junction resolvase (Hjr), previously characterized in *P. furiosus*, (Komori et al. 200b), homologs of the eukaryotic MRX complex, Rad51/Mre11, also biochemically characterized in *P. furiosus* (Hopfner et al. 2000), and a putative helicase, HerA (Constantinesco et al. 2004), suggesting that HR might be the major pathway for DSBs repair in *Halobacterium sp.*

We further investigated the molecular mechanisms for DSB repair in *Halobacterium* sp. by testing the potential for acquired radiation and desiccation tolerance following low-level gamma irradiation. Inducible stress systems are present in Bacteria and Eukarya (Crawford and Davies 1994, Ulsh et al. 2004) and acquired thermoresistance was reported for thermophilic archaea following exposure to nonlethal heat shock (Holden and Baross 1993; Trent et al. 1994). We did not find increased survival of *Halobacterium* sp. to desiccation exposure or ionizing irradiation when the cells were first exposed to a nonlethal dose of gamma irradiation (Fig. 5). In the case of desiccation, preexposure to 0.5 kGy of gamma ray resulted in a slight decrease in survival after desiccation, when compared to desiccation without preexposure (Fig. 5). The absence of an inducible system for the repair of gamma-induced DNA damage is consistent with the lack of DNA repair inducible pathways in *Halobacterium* sp. cells after UV irradiation, with the exception of a novel mismatch repair pathway (Baliga et al. 2004). No coordinated

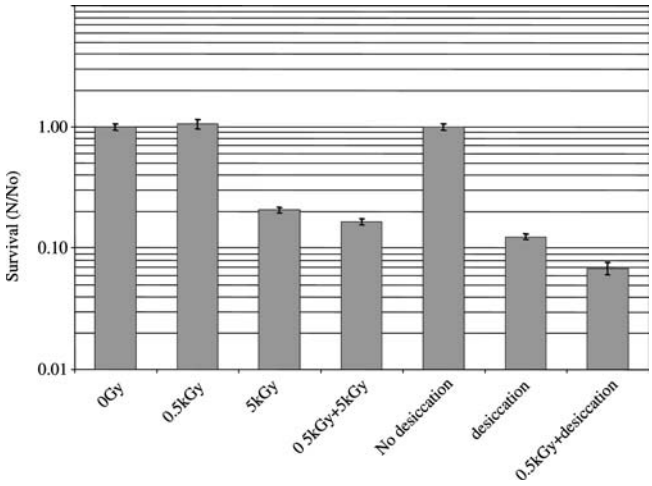

Fig. 5 *Halobacterium* sp. survival after irradiation at a non-lethal dose (0.5 kGy) prior to either irradiation at 5 kGy or desiccation for 12 days. *N* number of viable cells in challenged sample; *No* number of viable cells in control; *Error bars* represent standard deviation for six replicates

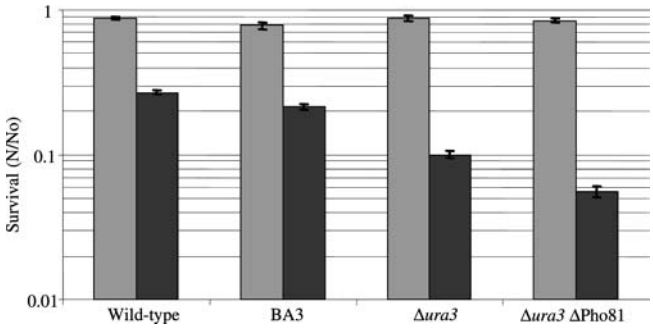

Fig. 6 Survival of ΔPho81 (deletion of four rhodopsin pigments), BA3 (EMS-generated colorless mutant), Δ*ura3* deletion background strain and *Halobacterium* sp. wild type strain to 2.5 (*light bars*) and 5 kGy (*dark bars*) of ^{60}Co gamma irradiation. *N* number of viable cells in challenged sample; *No* number of viable cells in control; *Error bars* represent standard error for three independent experiments

DNA repair response, similar to the bacterial SOS response to DNA damage was found in *Halobacterium* sp. following UV irradiation (Baliga et al. 2004), or in both *Halobacterium* sp. and *P. furiosus* following ionizing radiation (DiRuggiero, unpublished). Furthermore, *Pyrococcus abyssi* was also found to lack an inducible response for DNA repair following gamma irradiation (Gerard et al. 2001).

Resistance to high levels of gamma irradiation is unexpected in terrestrial organisms since there is no large natural source of gamma-ray on Earth. It has been proposed that *D. radiodurans* adaptation to desiccation accounts for its resistance to ionizing radiation due to the fact that the same types of DNA lesions, DSBs, are incurred by exposure to either condition (Mattimore and Battista 1996). *Chroococcidiopsis* strains from both desert and hypersaline environments were also found to be resistant to ionizing radiation (Billi et al. 2000), suggesting in turn that *Halobacterium's* adaptation to low water pressure resulting from water evaporation and high salt concentration in hypersaline pools, also accounts for its survival to high levels of gamma irradiation.

Halobacterium sp. DNA is protected against DNA double-stranded breaks

A mutant strain of *Halobacterium* sp. constructed by gene deletion from a Δ*ura3* background strain and lacking all four rhodopsin pigments (ΔPho81), and a colorless random EMS mutant (BA3) were examined for survival after gamma irradiation. Neither mutant exhibited a loss of survival at lower doses of gamma irradiation (2.5 kGy), whereas a modest decrease in survival was observed in both mutants at the higher dose of 5 kGy, when compared to their respective control strains (Fig. 6). Protection by red membrane protein pigments has previously been demonstrated to mitigate the damaging effects of UV and gamma irradiation in *H. salinarium* (Shahmohammadi et al. 1998) and *D. radiodurans* (Carbonneau et al. 1989). In previous studies, exposing *Halobacterium* sp. mutant strain BA3 to 200 J/m^2 of UV-C (254 nm) irradiation resulted in a two-fold decrease in survival when cells were allowed to recover in the dark, compared to wild-type cells, also suggesting pigment protection against photooxidative damage (DiRuggiero and Kottemann, unpublished data). In both *H. salinarium* and *D. radiodurans* a C_{50}-carotenoid, bacteriorubrin, was identified as being particularly effective in scavenging hydroxyl radicals thereby reducing the damage to nucleic acids. It must be noted that since the BA3 mutant was derived from EMS mutagenesis, and was not subjected to any further genetic characterization, the decreases in survival after both UV (dark recovery) and gamma irradiation could be the result of another non-observable mutation. Overall, at low doses of ^{60}Co gamma irradiation DNA repair mechanisms alone are adequate, but at higher doses repair mechanisms may get overwhelmed and the free radical scavenging activities of C_{50}-carotenoid pigments reducing the DNA damage caused by ionizing radiation becomes apparent.

The protective effects of salts were investigated by comparing *Halobacterium* sp. resistance to gamma to that of *E. coli* grown in 0.1 M NaCl, compared to 4.3 M NaCl for *Halobacterium* sp. Chromosomal fragmentation in *Halobacterium* sp. after gamma irradiation at 2.5, 5, and 7.5 kGy was far less extensive at the same dosage than that observed for *E. coli* (Fig. 7). *E. coli* survival after gamma irradiation was roughly 1% at 2.5 kGy, 0.1% at 5 kGy, and 0.001% at 7.5 kGy while *Halobacterium* sp. cultures proved considerably more viable with 85% survival at 2.5 kGy, 28% at 5 kGy, and 0.4% at 7.5 kGy (data not shown). Densitometry analysis of the agarose gel showed a large increase in fluorescence intensity corresponding to further DNA fragmentation

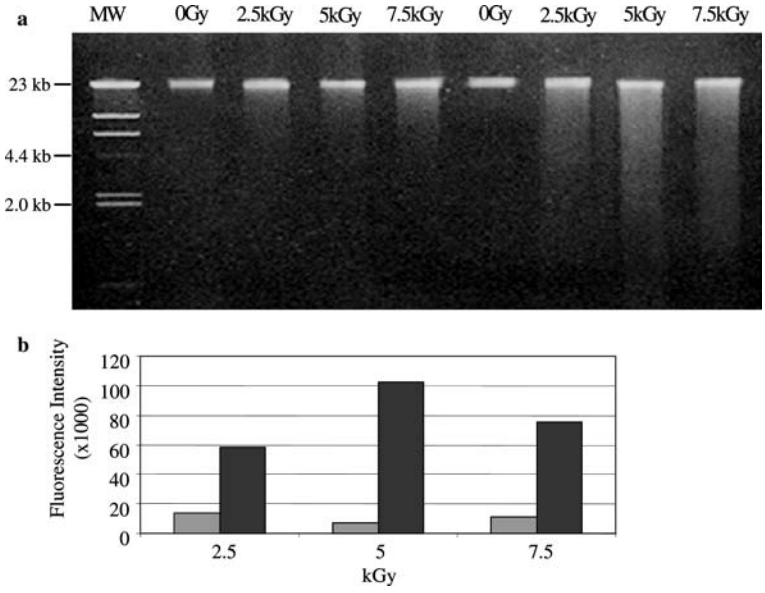

Fig. 7 **a** Agarose gel electrophoresis of *Halobacterium* sp. and *E. coli* DNA following exposure to 0, 2.5, 5, and 7.5 kGy of ^{60}Co gamma-ray. Each lane contains DNA from approximately 2.2×10^8 cells. Lane 1, 50 kb ladder; lanes 2–5, *Halobacterium* sp.; *lanes 6–9*, *E. coli*. **b** Fluorescence intensity of *Halobacterium* sp. (*light bars*) and *E. coli* (*dark bars*) DNA gel electrophoresis from **a** using ImageQuaNT5.0. The 0 kGy samples (*Halobacterium* sp. and *E. coli*) were used as background and subtracted from the respective samples

in *E. coli* as the dose of gamma irradiation increased while in *Halobacterium* sp. DNA fragmentation remained low (Fig. 7).

Intracellular salts such as KCl have previously been found to mitigate the effects of oxidative free radicals produced by the radiolysis of water by ionizing radiation in an in vitro study using plasmid DNA (Shahmohammadi et al. 1998). Chloride anions react with hydroxyl radicals and then with other chloride anions to produce chloride radicals, which are far less reactive than hydroxyl radicals. In hyperthermophilic species, high intracellular KCl was shown to reduce DNA cleavage at apurinic sites and to inhibit depurination (Marguet and Forterre 1998). A different protection strategy has been proposed for *D. radiodurans*. The accumulation of Mn (II) resulting in high intracellular Mn:Fe ratios were found to be important in radioresistance not only for *D. radiodurans*, but also for several other extremely radiation resistant bacterial species (Daly et al. 2004). Mn is a cofactor for the free radical scavenging Mn-dependant superoxide dismutase, whereas Fe participates in the Fenton reaction resulting in the formation of hydroxyl radicals from H_2O_2. Like other extremely radioresistant prokaryotes, *Halobacterium* sp. does not sporulate but, unlike *D. radiodurans*, Fe is a requirement for cell growth (Hubmacher et al. 2002). Thus the presence of salts rather than Mn accumulation is a vital factor in gamma radiation protection in *Halobacterium* sp., although like *D. radiodurans* the exact mechanism of protection is unknown.

Conclusions

We have shown here the extreme resistance of *Halobacterium* sp. to up to 20 days of desiccation, high vacuum and ionizing radiation ($D_{10} = 5$ kGy). No acquired tolerance to desiccation or gamma irradiation was observed. Chromosomal fragmentation induced by 144 h of desiccation or 7.5 kGy of gamma irradiation was repaired within hours. Similarly to *D. radiodurans*, *Halobacterium* sp. adaptation to desiccation secondarily allows for resistance to gamma irradiation as both conditions result in the formation of DSBs. Our data suggest that, in *Halobacterium* sp., bacteriorubrin and intracellular salts such as KCl aid in restricting DNA DSB formation resulting from exposure to desiccation and high doses of gamma irradiation. Survival within brine inclusions provides an extra layer of protection by which *Halobacterium* sp. cells can withstand both desiccation and high vacuum. Efficient DNA repair systems (likely HR) increase cell survival after gamma-ray exposure or desiccation. The mechanisms of radiotolerance with emphasis on DNA repair systems in Archaea require further study, for which *Halobacterium* sp. strain NRC-1 provides an excellent model system.

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