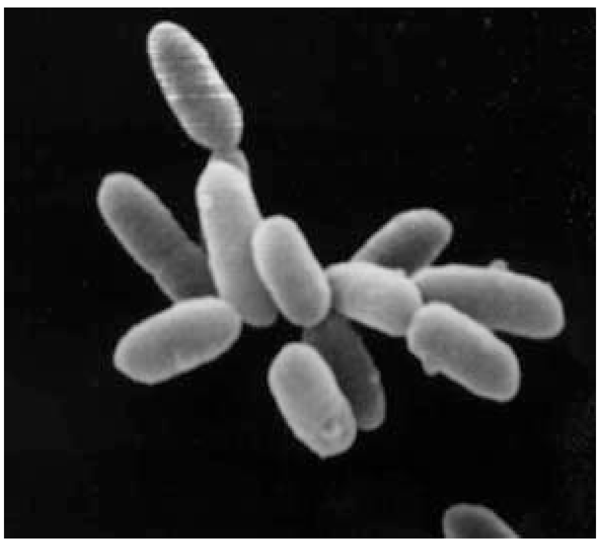
Physiological responses of the halophilic archaeon *Halobacterium* sp. strain NRC-1 to desiccation and gamma irradiation.

Kottemann M, Kish A, Iloanusi C, Bjork S, and DiRuggiero J.

Extremophiles. 2005 Jun;9(3):219-27.

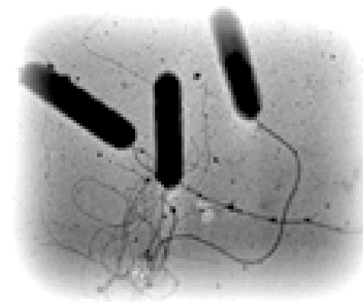


Halobacterium

Dead Sea,

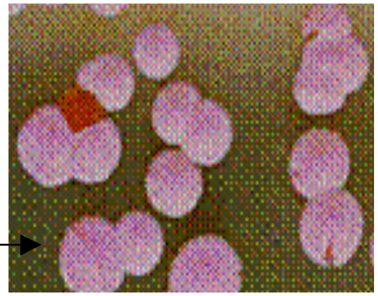
Salt Ponds CA, USA

Halobacterium sp. strain NRC-1



- Halophilic archaea
  - extremophiles that grow optimally under conditions of extremely high salinity (10x of sea water)

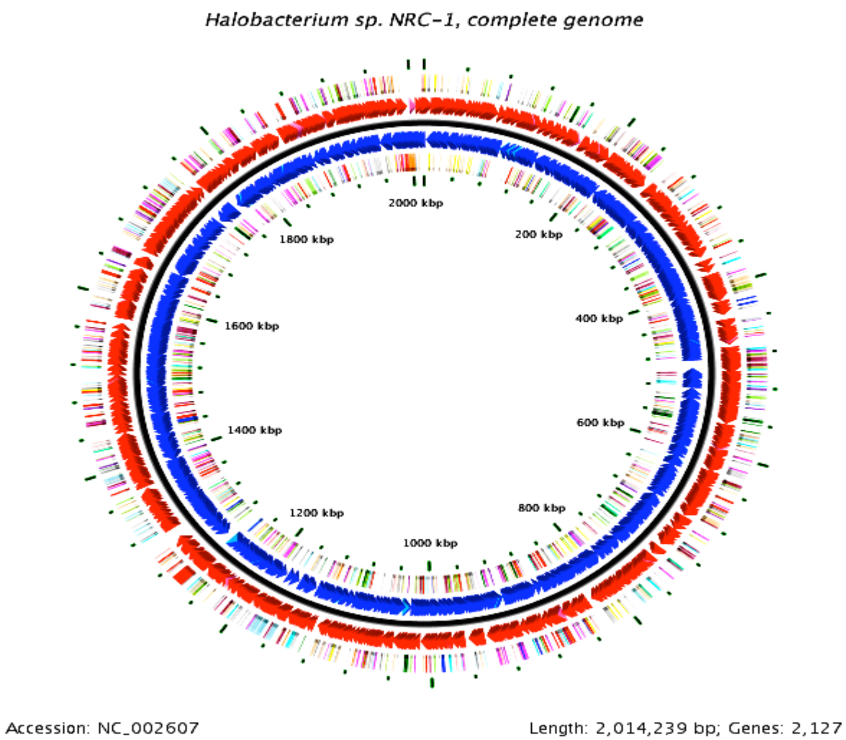
- Metabolically versatile
  - Aerobic metabolic capacity
  - Facultative growth via anaerobic respiration utilizing dimethyl sulfoxide (DMSO) and trimethylamine N-oxide (TMAO), and via arginine fermentation
  - Have phototrophic capability via the proton pumping activity of bacteriorhodopsin
  - Highly motile
  - Synthesizes gas vesicles for buoyancy and flotation, and sensory rhodopsins for phototaxis.

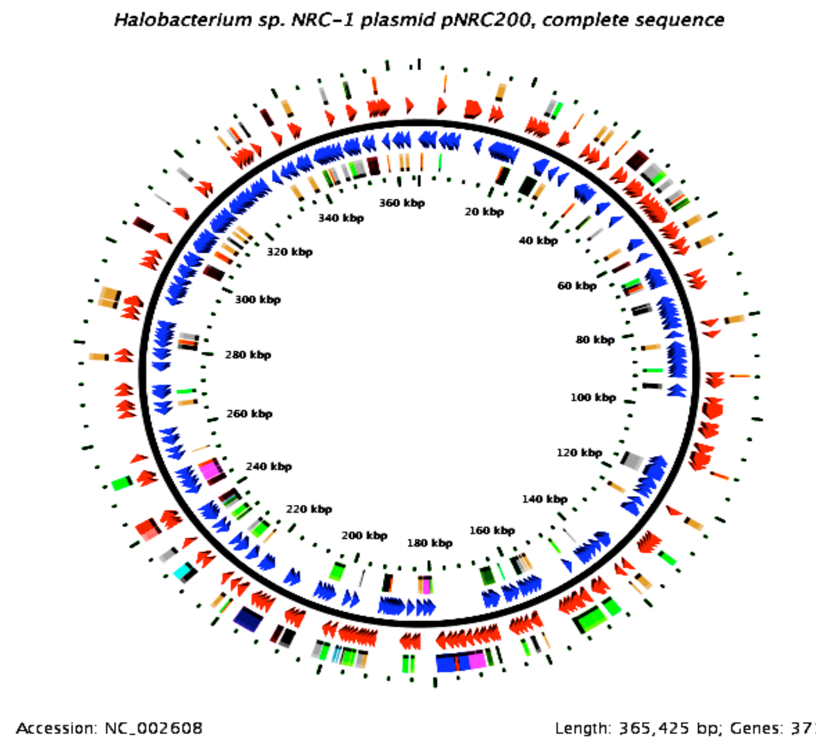


Pigmented colonies of *Halobacterium* sp. NRC-1 on salt agar plates

Halobacterium sp. strain NRC-1

- Sequenced genome, 2000 (Ng et al.) - 2.571 Mbp's, composed of 3 circular replicons
  - 2.0 Mbp large chromosome and plasmids, pNRC200 (366 Kbp) and pNRC100 (192 Kbp)





Halobacterium as an experimental organism

- Sequenced genome - 2000 (Ng et al.)
- Culturing is simple, with a 6 hour generation time at 42°C (aerobic and mesophilic)
- Genetically tractable
  - being transformable at high-efficiency
  - good selection of cloning and expression vectors available (mevinolin resistance),
  - Gene replacement and knockout strategies : selectable and counterselectable *ura3* gene
- Whole-genome DNA microarrays - to interrogate patterns of gene expression
- Biochemical analyses
  - *Halobacterium* sp. NRC-1 cells are easily lysed in hypotonic medium, releasing both soluble and membrane proteins

Hypersaline Environments and challenges in growing in them

- Hypersaline conditions
  - High ionic strength (10x that of sea water)
  - Elevated temperatures
  - High levels of UV radiation
- Periodic evaporation of water
- Concentration of salts
  - Cells exposed to desiccation
  - DNA damage

Physiological responses of the halophilic archaeon *Halobacterium* sp. strain NRC-1 to desiccation and gamma irradiation.

Kottemann M, Kish A, Iloanusi C, Bjork S, and DiRuggiero J.

Extremophiles. 2005 Jun;9(3):219-27.

Challenges : desiccation & high vacuum

- Evaluated survival due to desiccation at ambient pressure & high vacuum (10^{-6} Pa)
  - Medium formed large salt crystals in which cells were encased (Figure 1a, b)

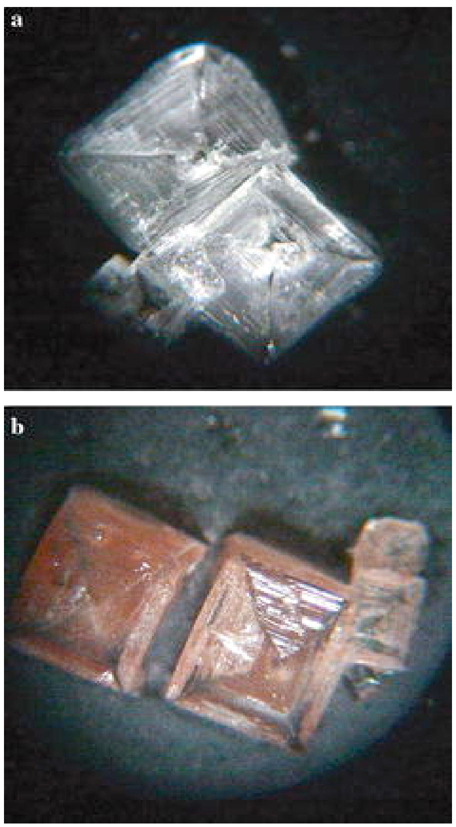


Fig. 1 Salt crystals from GN101 culture medium after 2 days of desiccation (a) un-inoculated and (b) inoculated with *Halobacterium* sp.

(a) un-inoculated

(b) inoculated

Survival due to Desiccation and High Vacuum

- Both challenges - slow decrease in survival, 25% cells viable after 20days
- No significant difference in survival to either desiccation or high vacuum
  - Salt crystals offer some level of of cellular protection

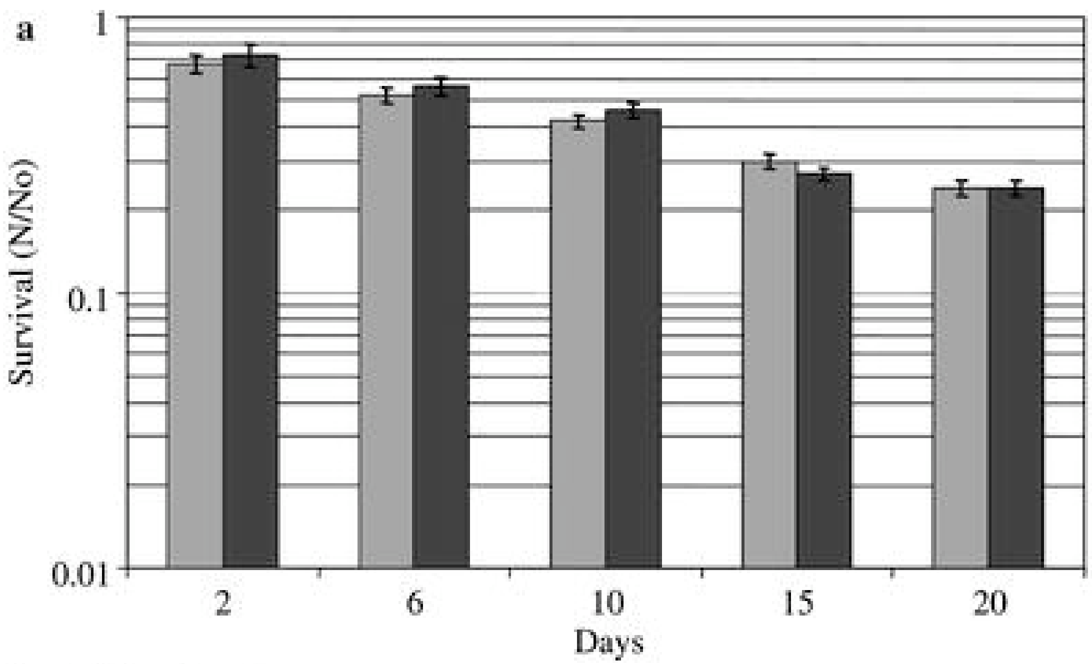


Fig. 2a: *Halobacterium* sp. survival following desiccation **(dark bars)** and high vacuum (light bars)

Survival due to ^{60}Co Gamma irradiation

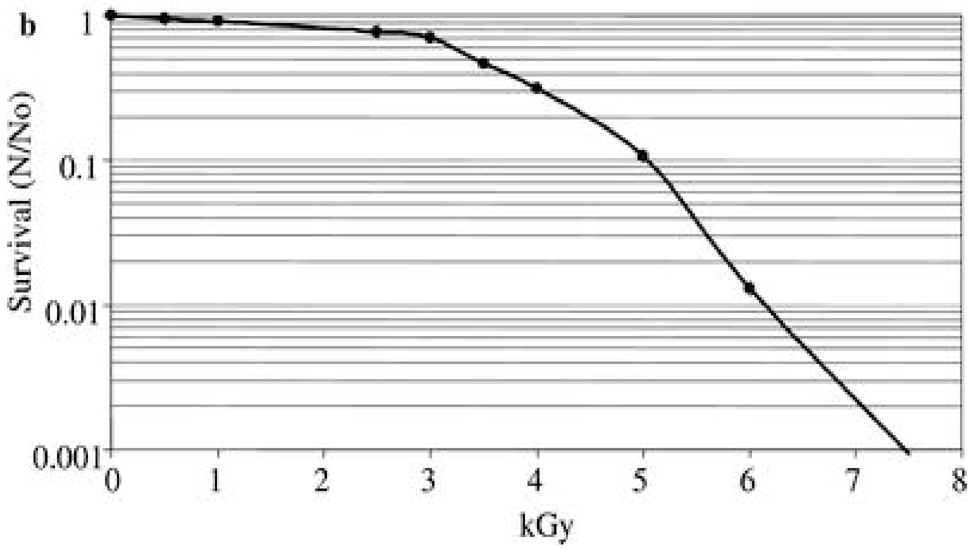


Fig. 2b: *Halobacterium* sp. survival following ^{60}Co gamma irradiation

- No loss of viability uo to 2.5 kGy, D_{10} value of 5kGy
  - D_{10} value - dose necessary to effect a 90% reduction in CFU's

- D_{10} value for *E.coli* is 0.25 kGy (gamma sensitivity), D_{10} value for *D. radiodurans* is 10 kGy (gamma resistance)

Survival to gamma irradiation at different growth stages

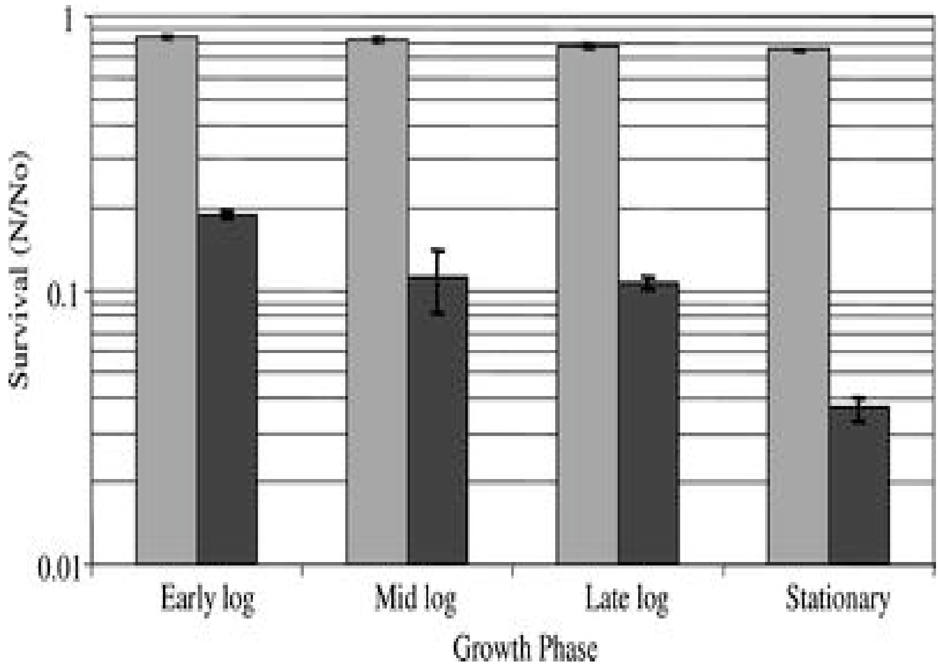


Fig. 3 Survival to 2.5 kGy (light bars) and 5 kGy **(dark bars)** of irradiation at different growth stages.

OD600 nm values 0.2, 0.4, 0.8, and 1.0 represent early log, mid log, late log, and stationary phase, respectively

- See increased sensitivity to irradiation at stationary phase
- Removal of ROS - might be more efficient in actively growing cells - increase in survival during exponential phase

Repairment of DNA DSB's - Desiccation

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

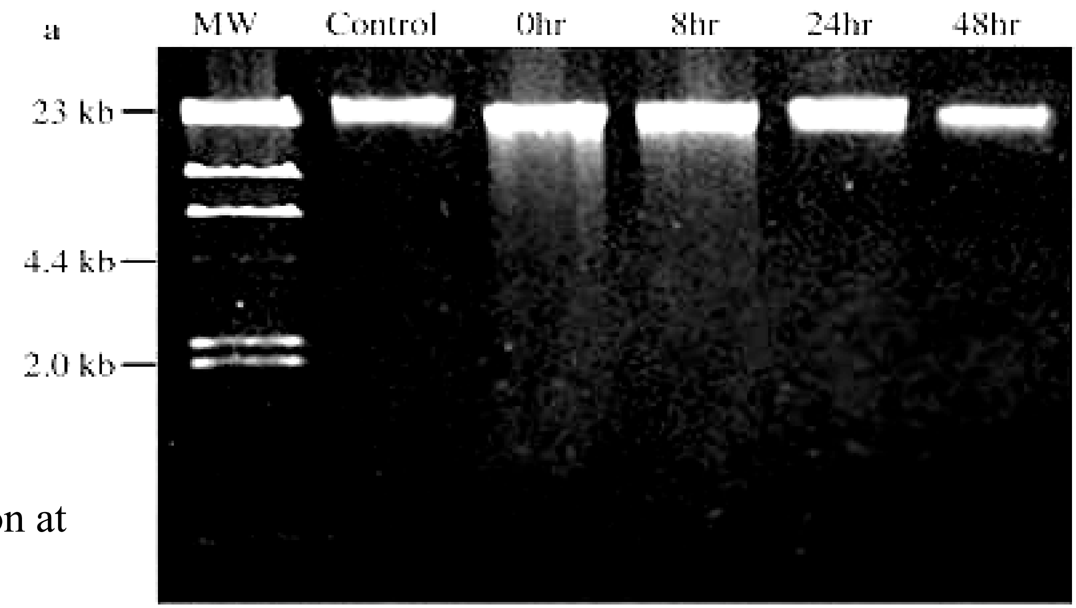
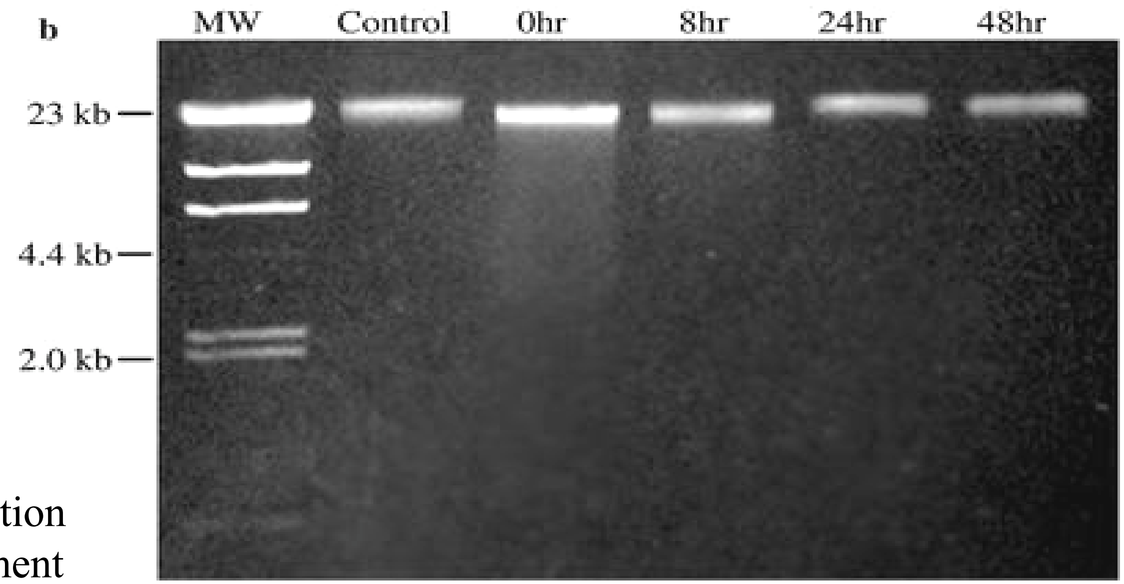


Fig. 4a : Agarose gel electrophoresis of *Halobacterium* sp. DNA following a 144 h desiccation

- DNA DSB's (shown as smear under the genomic DNA band ~23kb)

Repairment of DNA DSB's - Irradiation



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

Fig. 4b : Agarose gel electrophoresis of Halobacterium sp. following a ^{60}Co gamma irradiation at 7.5 kGy.

- Both desiccation/irradiation causes DNA DSB's

Conclusion on DNA DSB Repairs

- Direct DNA damage - Gamma ray photons
  - Indirect damage: 80% radiolysis of water and production of ROS
- Damages by ROS are limited
  - Catalase, peroxidase, superoxide dismutase
  - Thioredoxin oxidases, NADH oxidase & DNA repair proteins
    (all encoded for in the *Halobacterium* genome)
- Damage end results - DSB's
  - Typically repaired by RecA mediated homologous recombination/HR
  - *Halobacterium* sp. has homologs to HR
  - Suggests, HR might be the major pathway for DSB's repair

DNA repair inducible pathways?

- Tested potential for acquired radiation & desiccation tolerance after low-level gamma irradiation
- No increased survival - due to desiccation & irradiation after pre-exposure

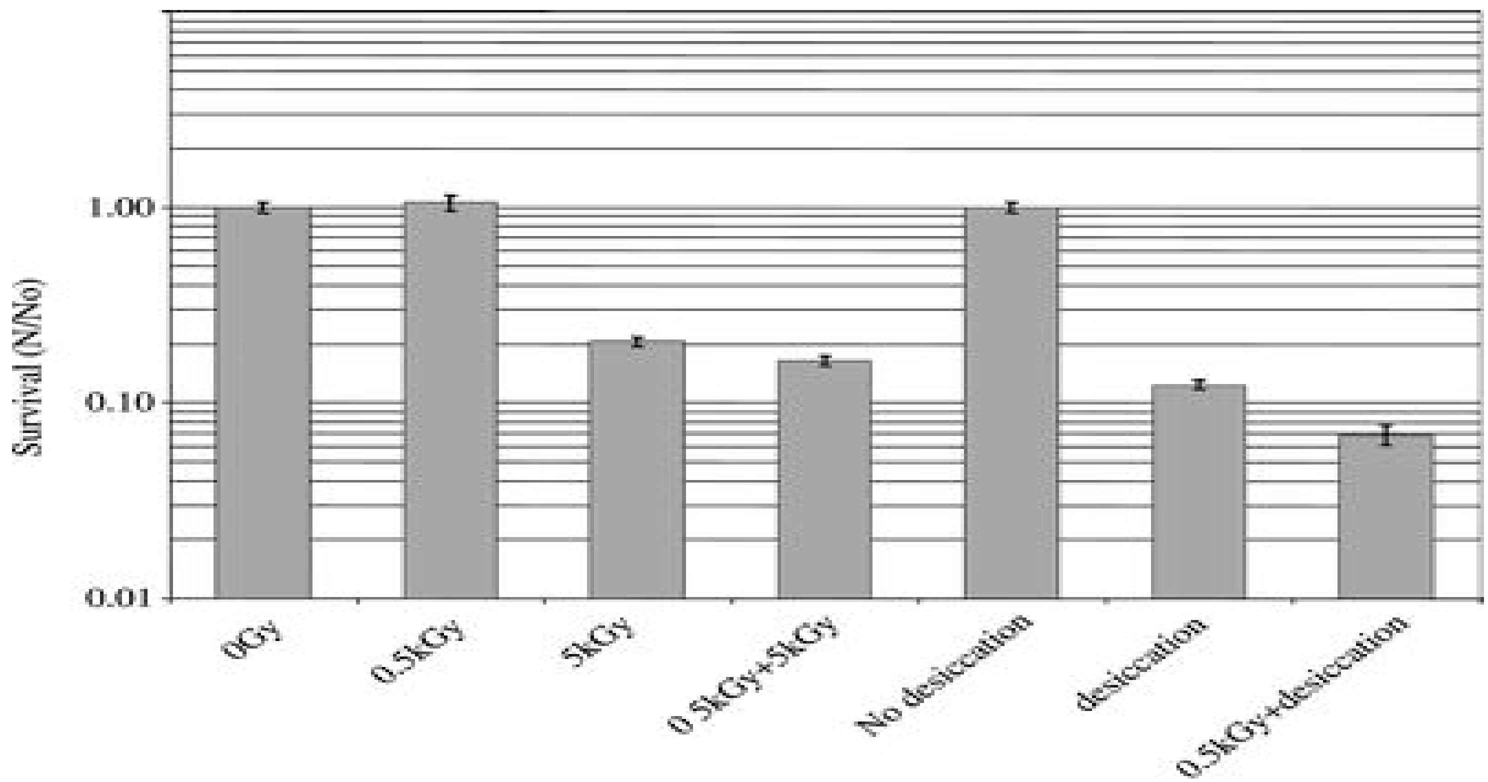
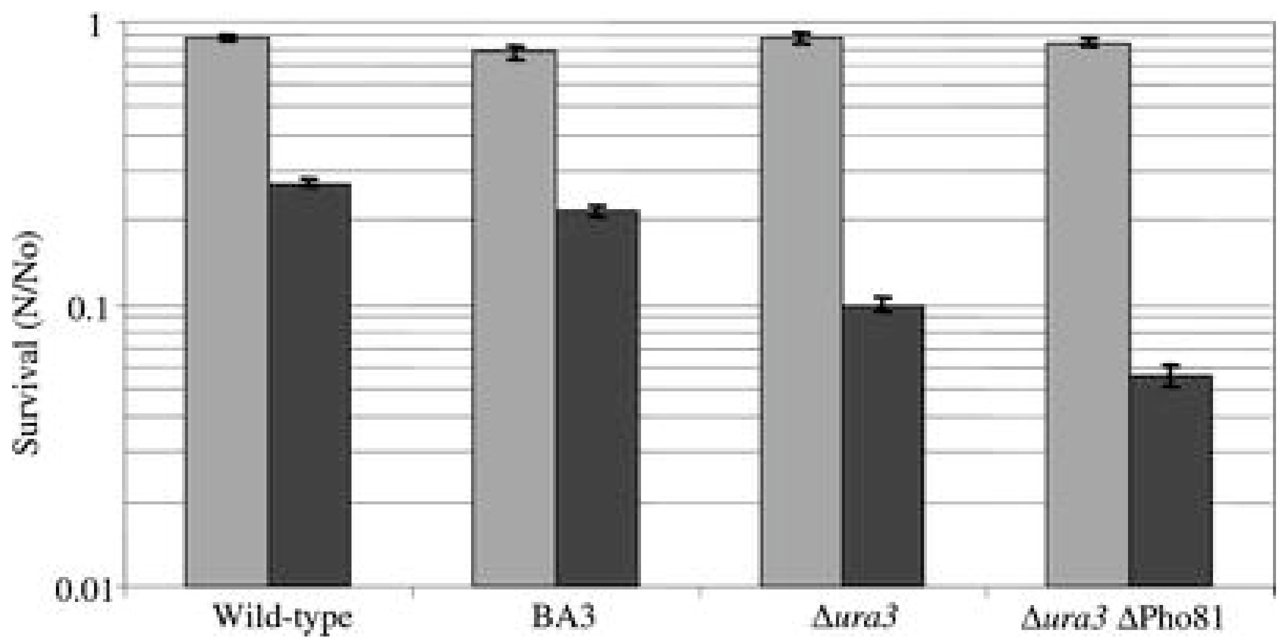


Fig. 5 *Halobacterium* sp. survival after irradiation at a non-lethal dose (0.5 kGy) prior to either irradiation at 5 kGy or desiccation for 12 days.

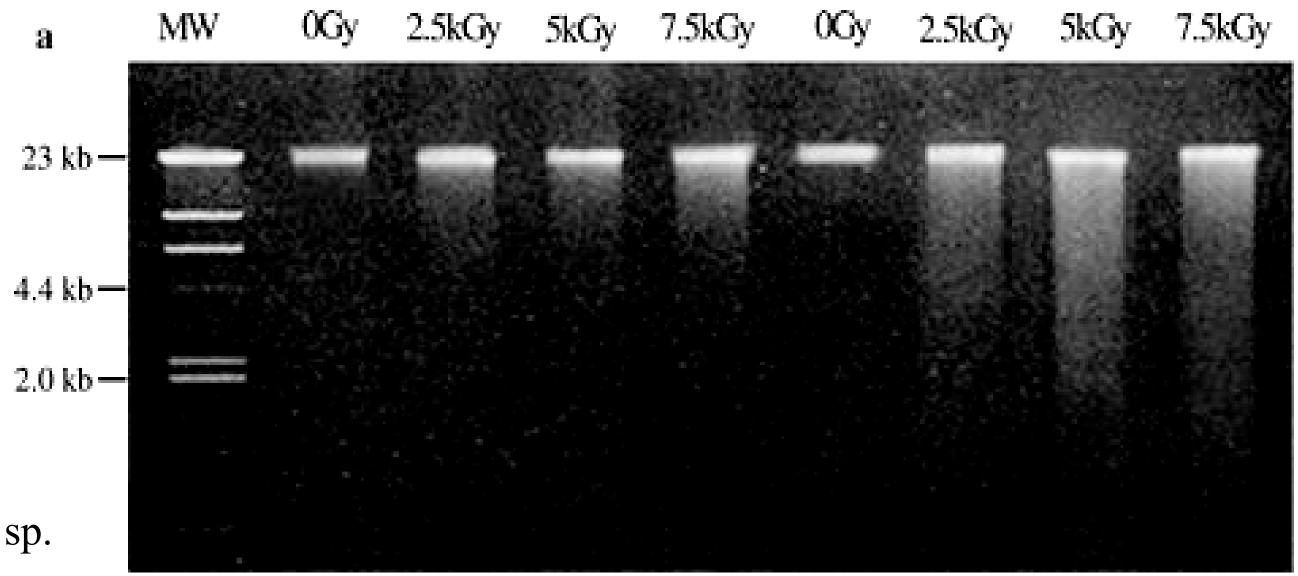
Fig. 6 Survival of Δ Pho81 (deletion of four rhodopsin pigments), BA3 (EMS-generated colorless mutant), Δ *ura*3 deletion background strain and *Halobacterium* sp. wild type strain to 2.5 (light bars) and 5 kGy (dark bars) of ^{60}Co gamma irradiation.



- Pigments help protection against ionizing radiation
- Protection of red membrane pigments have been shown earlier to lessen damaging effects of gamma irradiation

Protective Effects of Salts

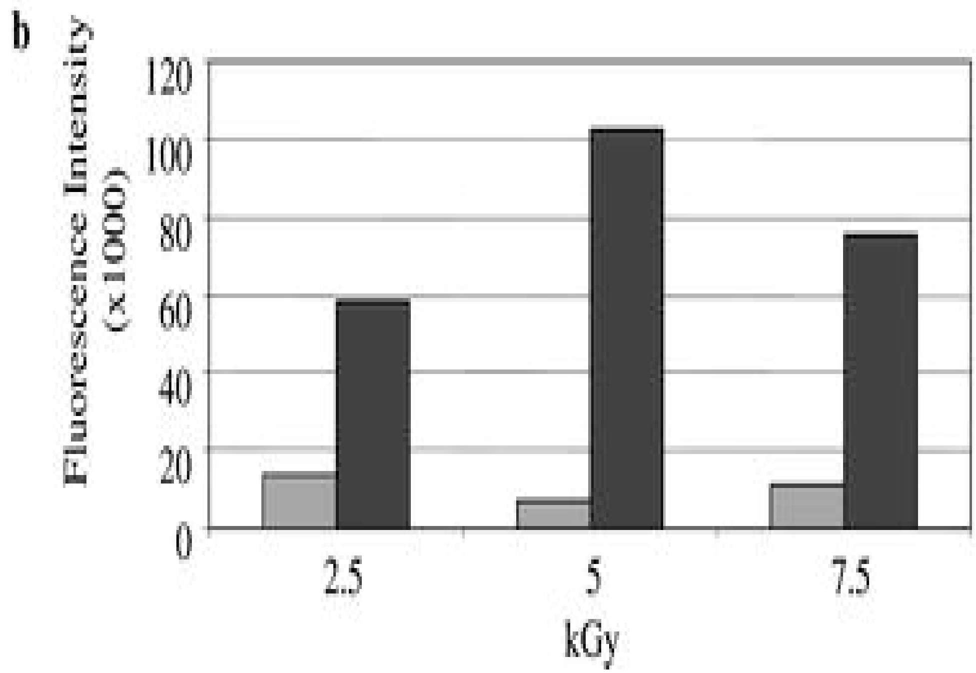
- Gamma resistance of *Halobacterium* sp. grown in 4.3 M NaCl were compared to *E. coli* grown in 0.1 M NaCl
- Chromosomal fragmentation of *Halobacterium* sp. was less severe than *E. coli*



Lane 1 - 50 kb ladder;
lanes 2–5 - *Halobacterium* sp.
lanes 6–9 - *E. coli*.

Fig. 7a: 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.

Fig. 7 b: Fluorescence intensity of *Halobacterium* sp. (light bars) and *E. coli* (dark bars) DNA gel electrophoresis



- Increase in fluorescent act. as gamma irradiation increases in *E.coli*, indicating DNA fragmentation while that of *Halobacterium* sp. remained low

Conclusions

- *Halobacterium* sp. is resistant to desiccation, high vacuum & gamma irradiation
  - Consequence to it adaptation to desiccating conditions
- Chromosomal fragmentation was repaired in hours
- No acquired tolerance to desiccation or gamma irradiation
- Pigments and high salts offered protection
- Loopholes
  - Salt crystals
  - Densitometry analysis