MICROBIOLOGY OF EXTREME ENVIRONMENTS

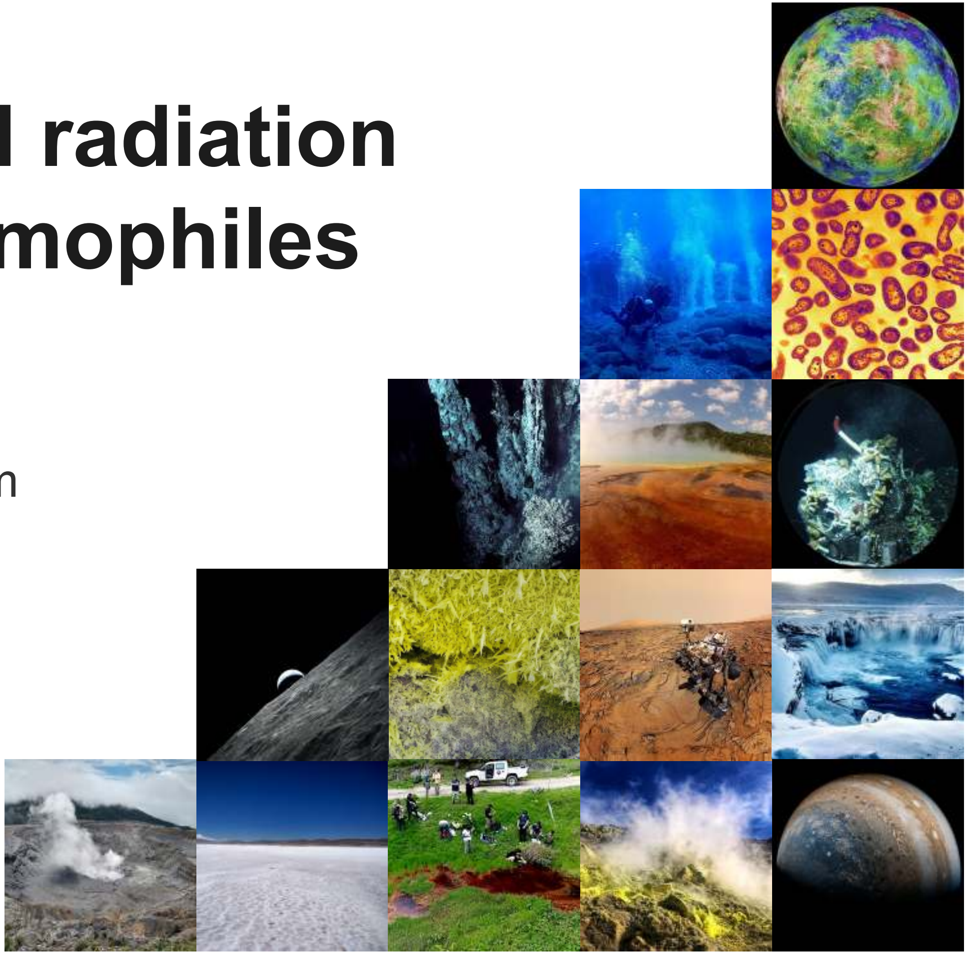
Xerophiles and radiation resistant extremophiles

Deborah Bastoni

deborah.bastoni@gmail.com

www.donatogiovannelli.com

#giovannellilab



Xerophiles

Microorganisms that have adapted to survive in environments in which water is scarce like hyper-arid deserts and desiccated foods.

Xerophiles

Microorganisms that have adapted to survive in environments in which water is scarce like hyper-arid deserts and desiccated foods.

- Archaea

Xerophiles

Microorganisms that have adapted to survive in environments in which water is scarce like hyper-arid deserts and desiccated foods.

- Archaea
- Bacteria

Xerophiles

Microorganisms that have adapted to survive in environments in which water is scarce like hyper-arid deserts and desiccated foods.

- Archaea
- Bacteria
- Fungi

Xerophiles

Microorganisms that have adapted to survive in environments in which water is scarce like hyper-arid deserts and desiccated foods.

Xerophiles

Xerophiles

Microorganisms that have adapted to survive in environments in which water is scarce like hyper-arid deserts and desiccated foods.

~~Xerophiles~~

Xerotolerant

Xeric stress limits

A xeric stress can be caused either by:

Xeric stress limits

A xeric stress can be caused either by:

- **Desiccation**, lack of water

Xeric stress limits

A xeric stress can be caused either by:

- **Desiccation**, lack of water
- **Hypertonicity**, excessive solute concentrations in the surrounding environment that removes water from cells

Xeric stress limits

A xeric stress can be caused either by:

- **Desiccation**, lack of water
- **Hypertonicity**, excessive solute concentrations in the surrounding environment that removes water from cells

How do we measure the amount of water available in an environment?

Xeric stress limits

Water activity

Xeric stress limits

Water activity

The vapor pressure of water in a system divided by that of pure water at the same temperature

Xeric stress limits

Water activity

The vapor pressure of water in a system divided by that of pure water at the same temperature

It's a measure of how much of that water is free and unbound, and thus available for microorganisms to use for growth

Xeric stress limits

Water activity

The theoretical water activity lower limits for life are:

Xeric stress limits

Water activity

The theoretical water activity lower limits for life are:

- 0.611 aw for archaea and bacteria

Xeric stress limits

Water activity

The theoretical water activity lower limits for life are:

- 0.611 aw for archaea and bacteria
- 0.632 aw for fungi

Xeric stress limits

Species	Lowest water activity (a_w) for cell division	Environmental source	Refs
Bacteria and archaea			
Haloarchaea GN-2	0.635	Solar salterns, Mexico	3
Haloarchaea GN-5	0.635	Solar salterns, Mexico	3
Halorhabdus utahensis DSM 12940	0.647	Salt Lake, USA	3
Halobacterium strain 004.1	0.658	Brine pool, UK	3
Halorhodospira halophila DSM 244	0.66	Salt lake, USA	3
Salinibacter ruber DSM13855	0.725	Solar salterns, Spain	3
Salisaeta longa DSM 21114	0.747	Dead Sea, Israel	3
Fungi			
Aspergillus penicillioides	0.585	Raisins, Australia	7,106
Xeromyces bisporus	0.637	Antique wood, Thailand	3,6,7,107
Aspergillus amstelodami FRR2792	0.656	Dates, Australia	7
Xerochrysium xerophilum FRR 0530 (formerly known as *Chrysosporium xerophilum*)	0.686	High-moisture prunes, Australia	7
Aspergillus chevalieri PIL 119	0.71	Soiled prunes, Australia	7

Lebre, P. H., De Maayer, P., & Cowan, D. A. (2017). *Nature Reviews Microbiology*, *15*(5), 285-296.

Where?

Where?


A
1
2


B
Hyper-arid deserts


Hyper-arid deserts


hypersaline aquatic environments

Where?


Salt-cured food

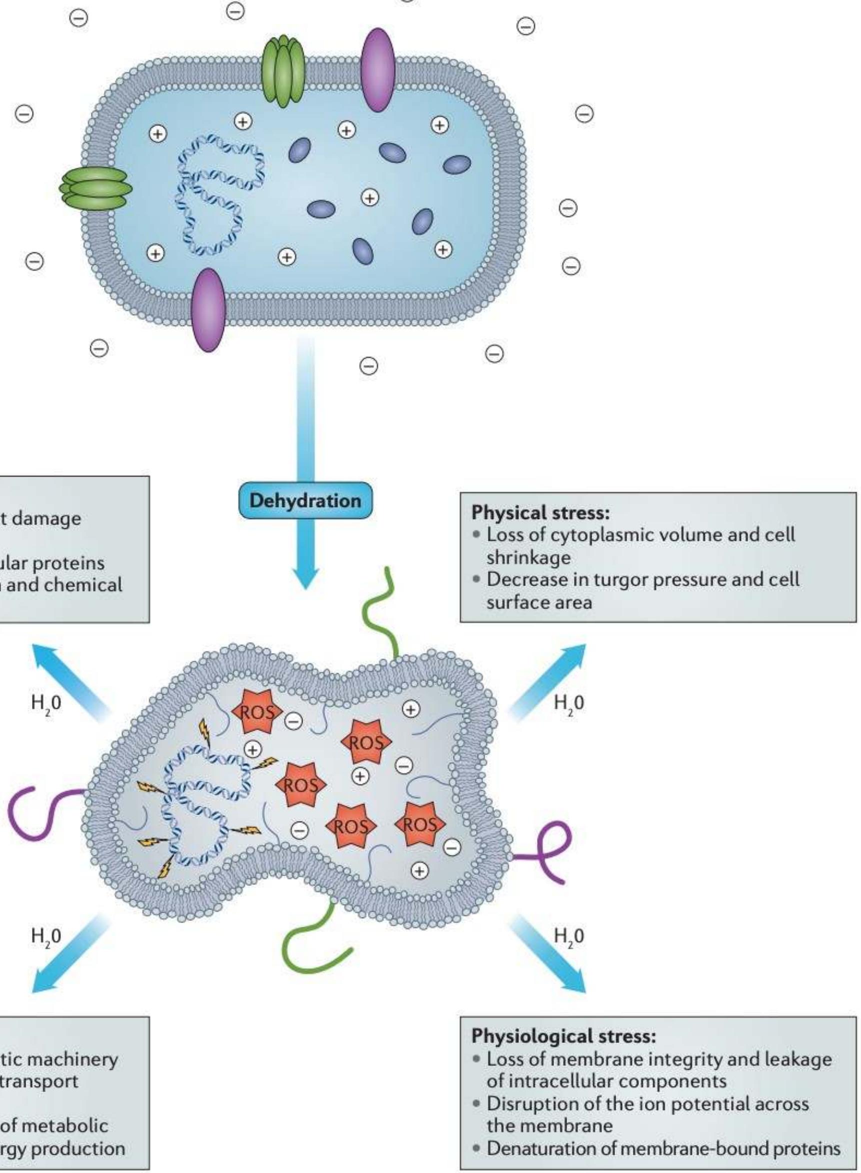

A
1
2


B
Hyper-arid deserts


Hyper-arid deserts


hypersaline aquatic environments

What happens when the water activity is too low?



Lebre, P. H., De Maayer, P., & Cowan, D. A. (2017). *Nature Reviews Microbiology*, *15*(5), 285-296.

How do they survive?

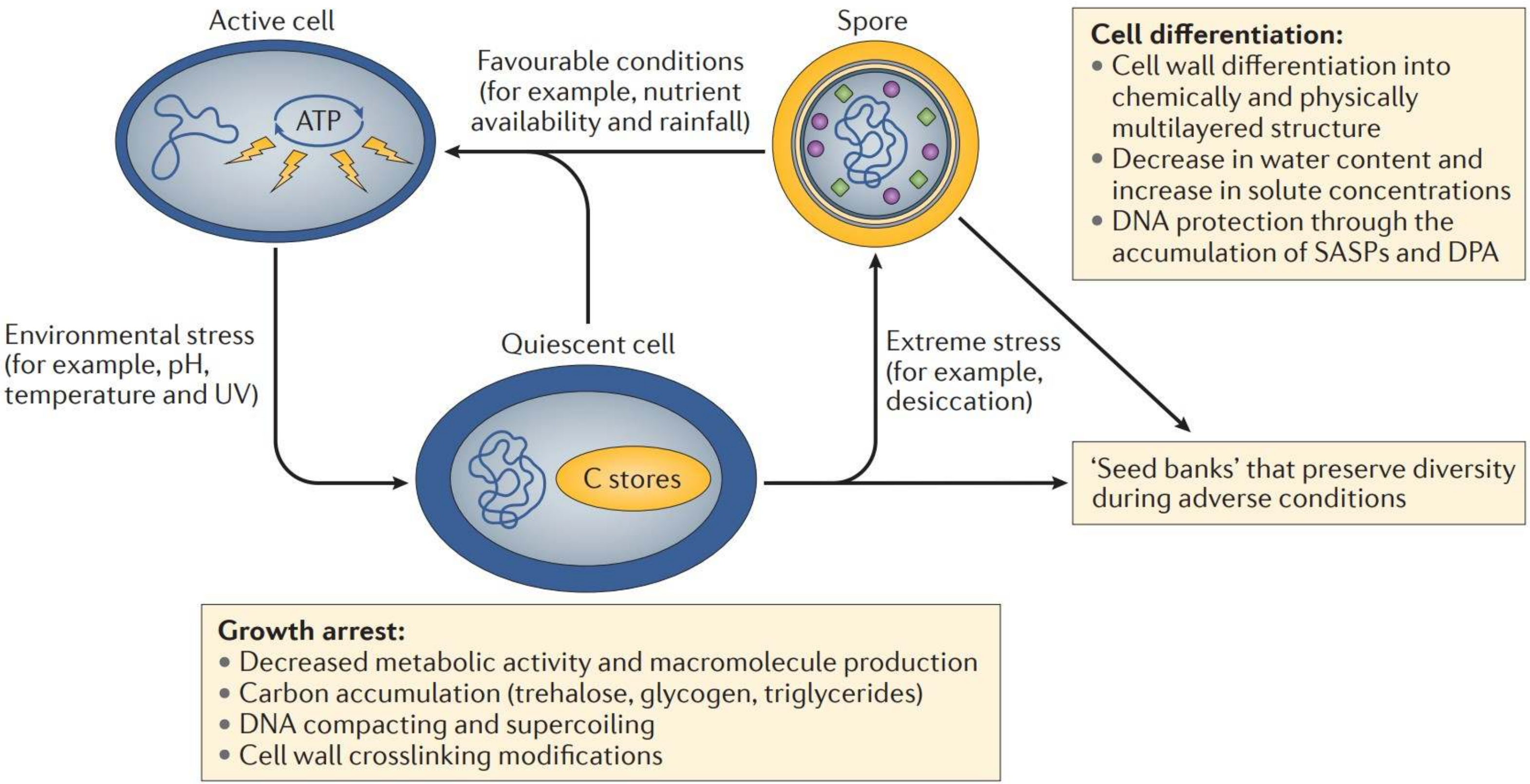
To counteract the negative morphological, physiological and biochemical consequences of desiccation, xerotolerant microorganisms have developed a broad range of adaptive strategies.

How do they survive?

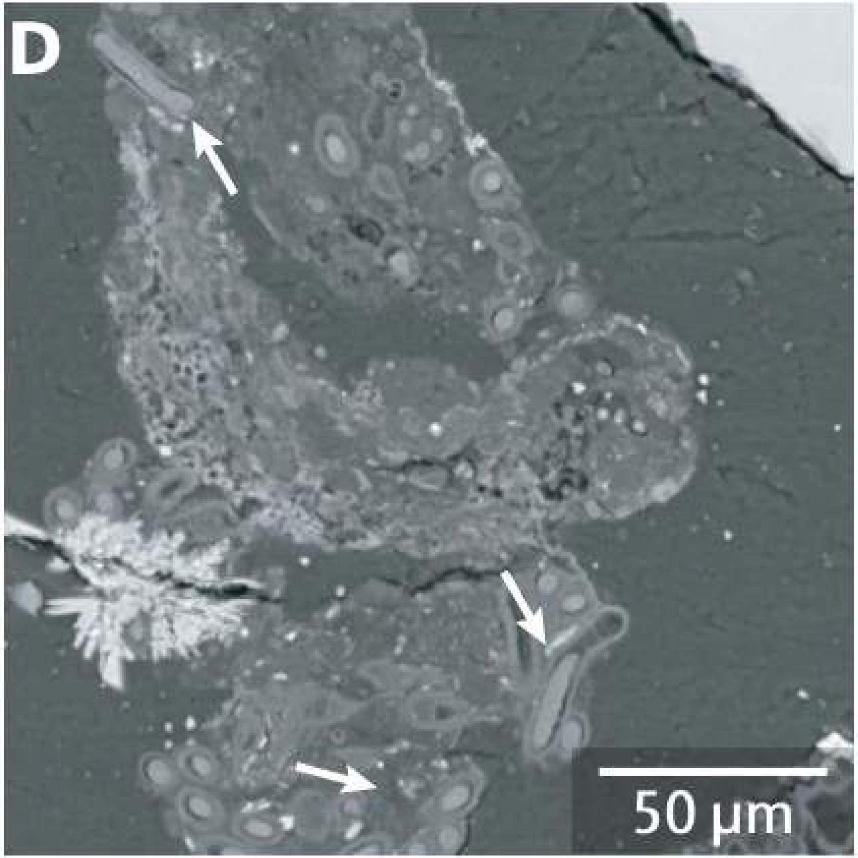
To counteract the negative morphological, physiological and biochemical consequences of desiccation, xerotolerant microorganisms have developed a broad range of adaptive strategies.

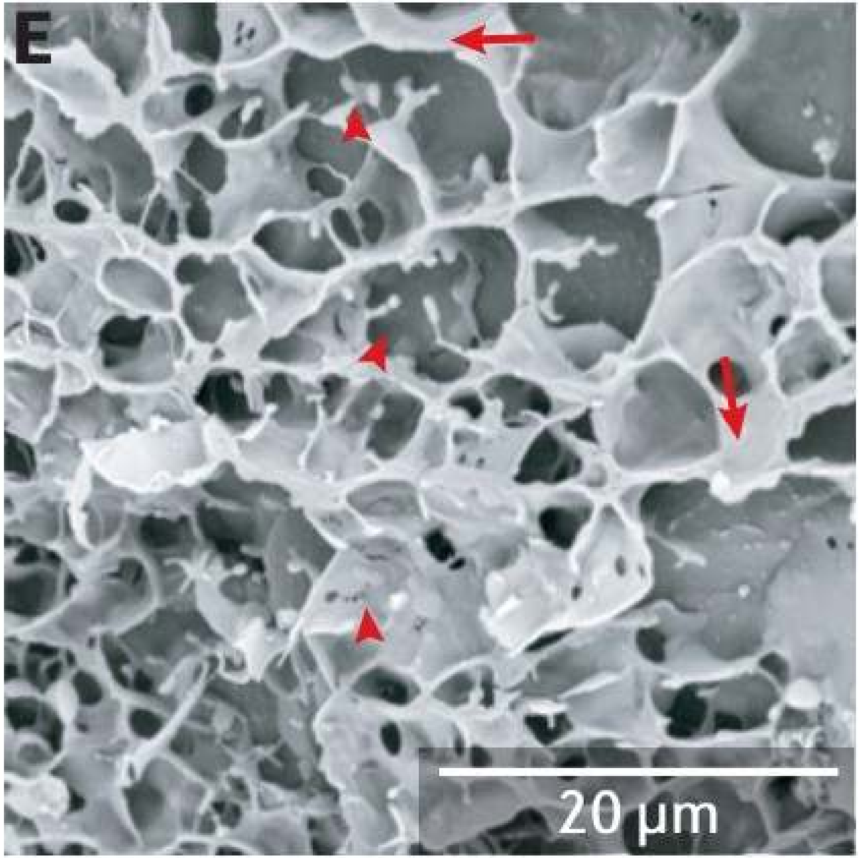
They have evolved a series of **behavioural, physiological and molecular** mechanisms

Behavioural adaptations: Dormancy and sporulation

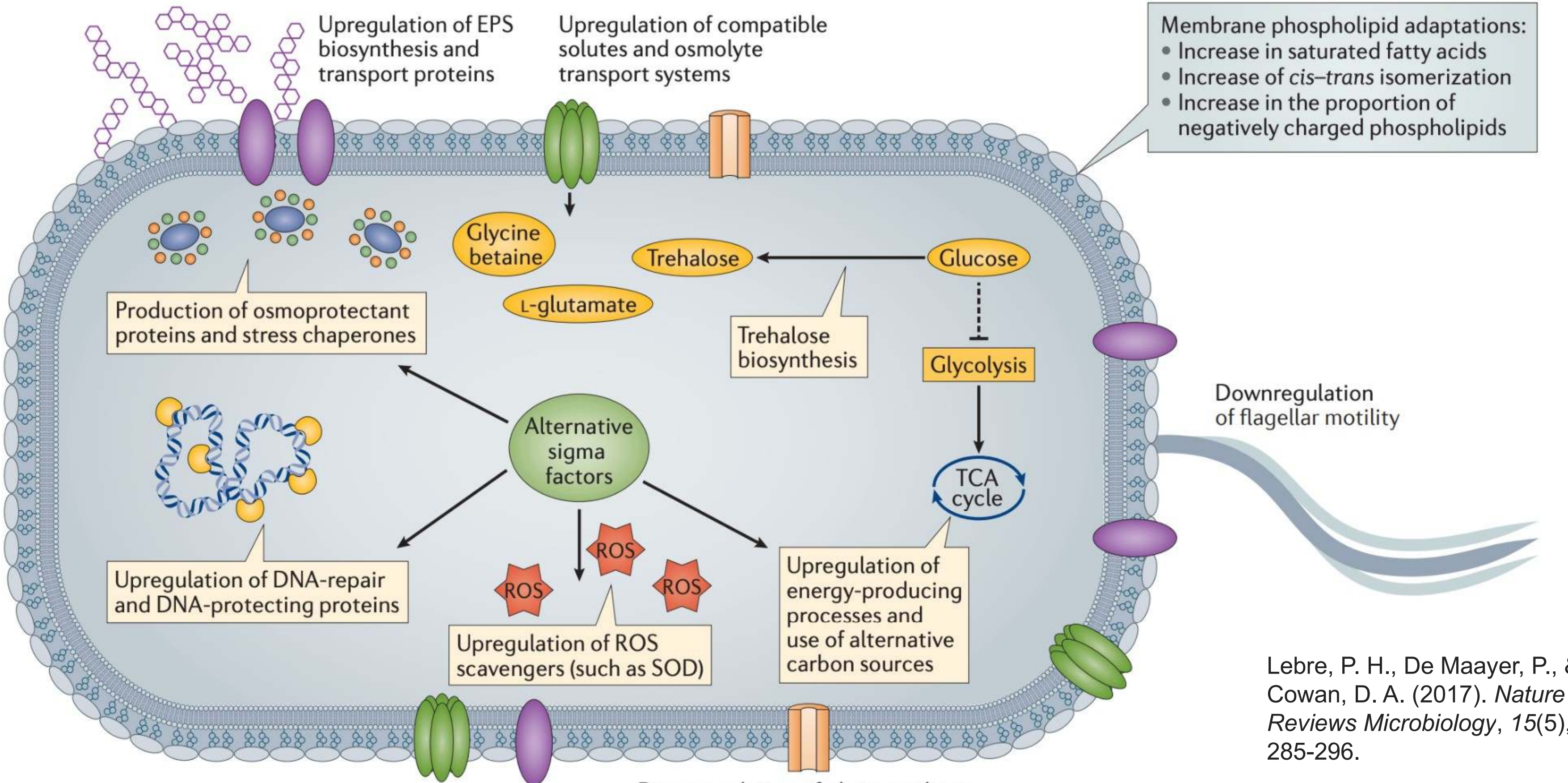


Behavioural adaptations: EPS and biofilm formation





Physiological adaptations: Cell membrane adaptations, accumulation and biosynthesis of compatible solutes



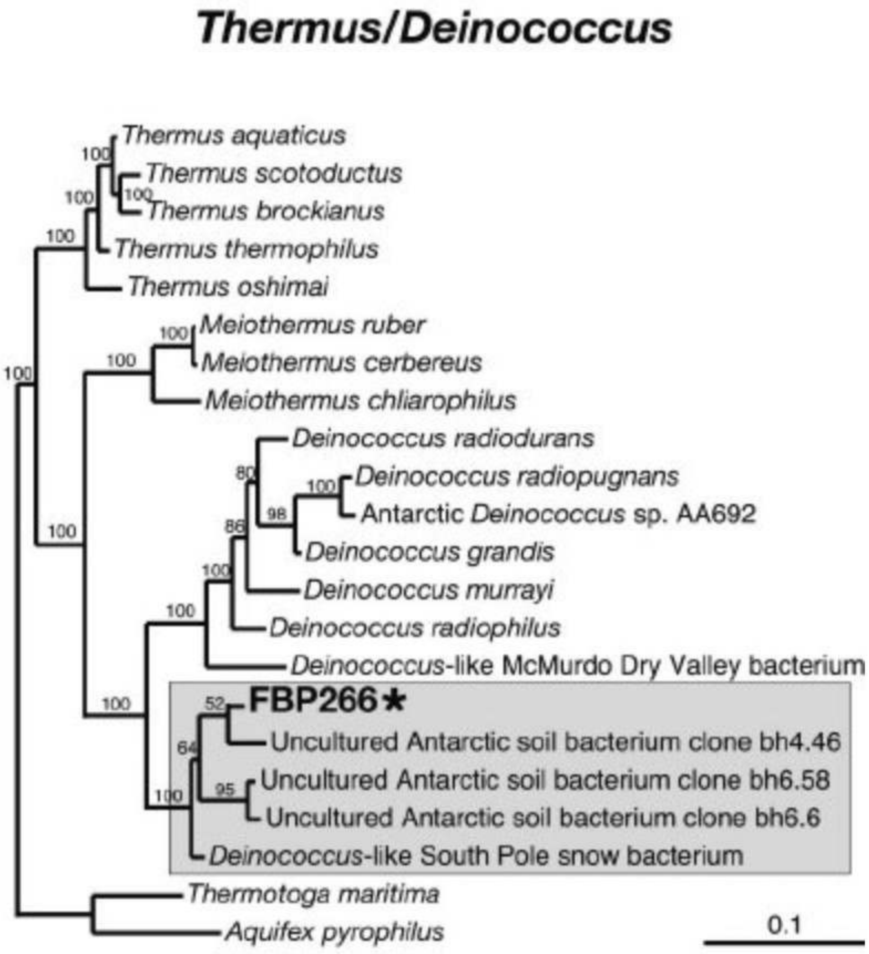
Lebre, P. H., De Maayer, P., & Cowan, D. A. (2017). *Nature Reviews Microbiology*, *15*(5), 285-296.

Metabolic and molecular adaptations

- They can differentially upregulate and downregulate various metabolic pathways, which can lead to a shift from anabolic to catabolic metabolism

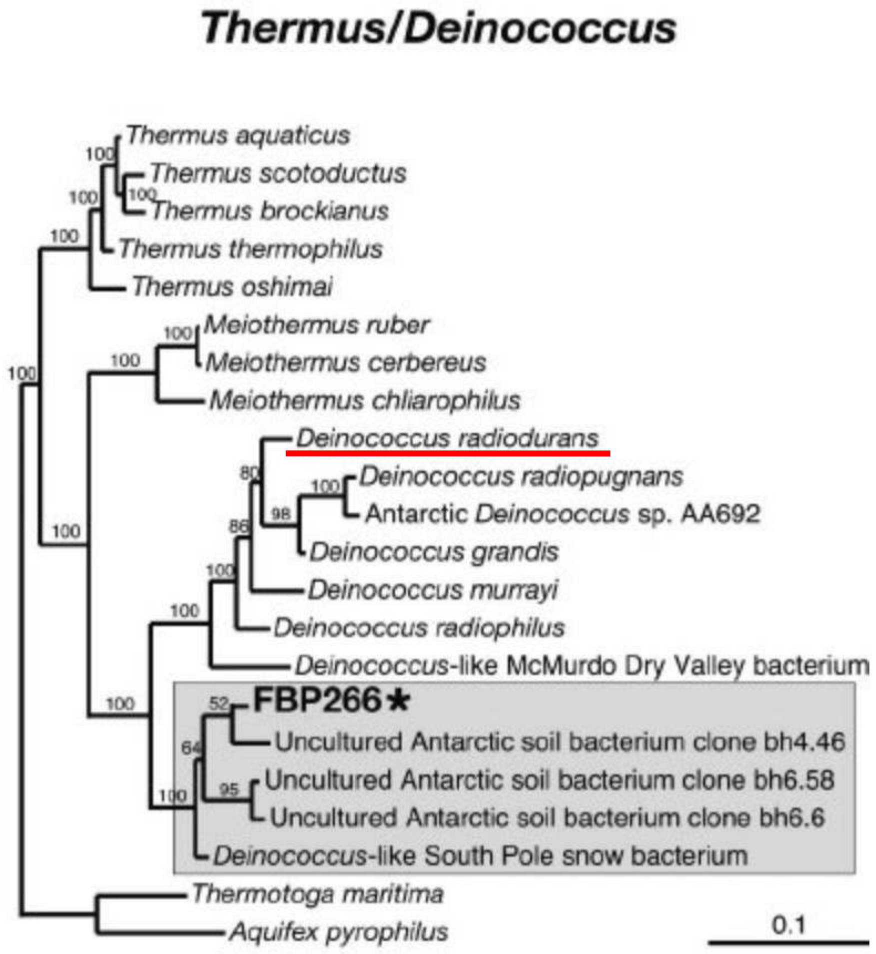
- They can express a range of proteins to counteract the effects of low water activity es. Late embryogenesis abundant (LEA)

Thermus/Deinococcus phylum



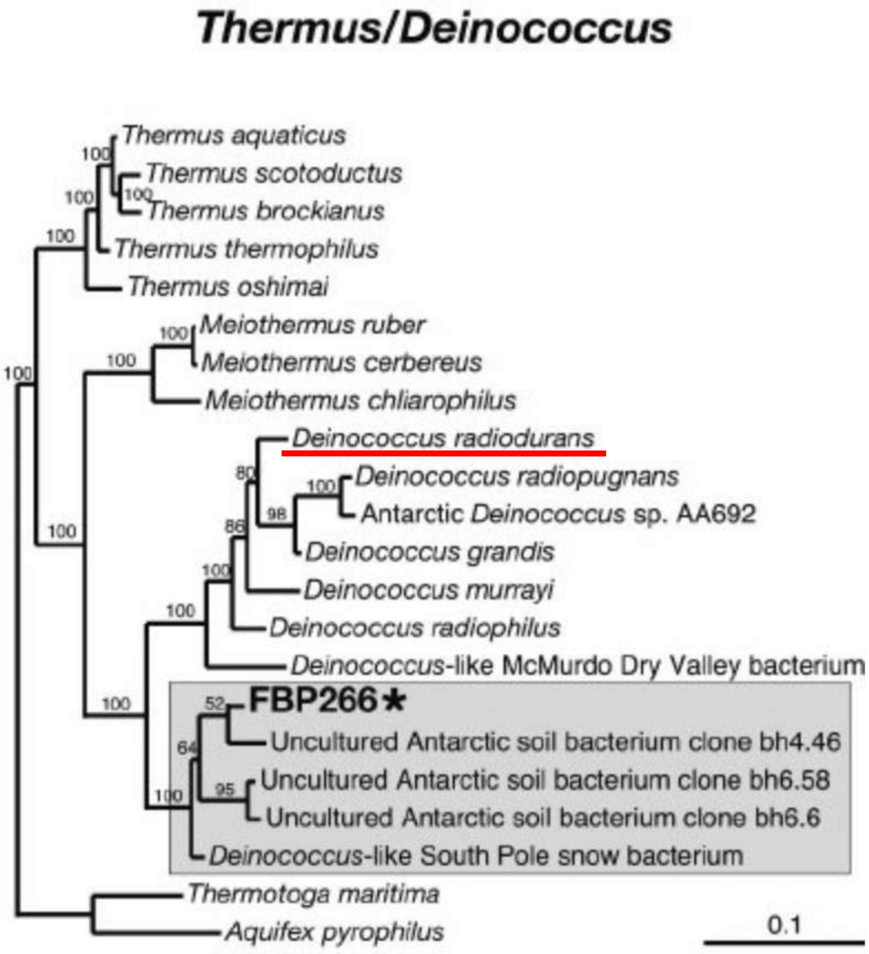
de la Torre, J. R., Goebel, B. M., Friedmann, E. I., & Pace, N. R. (2003). *Applied and environmental microbiology*, *69*(7), 3858-3867.

Thermus/Deinococcus phylum

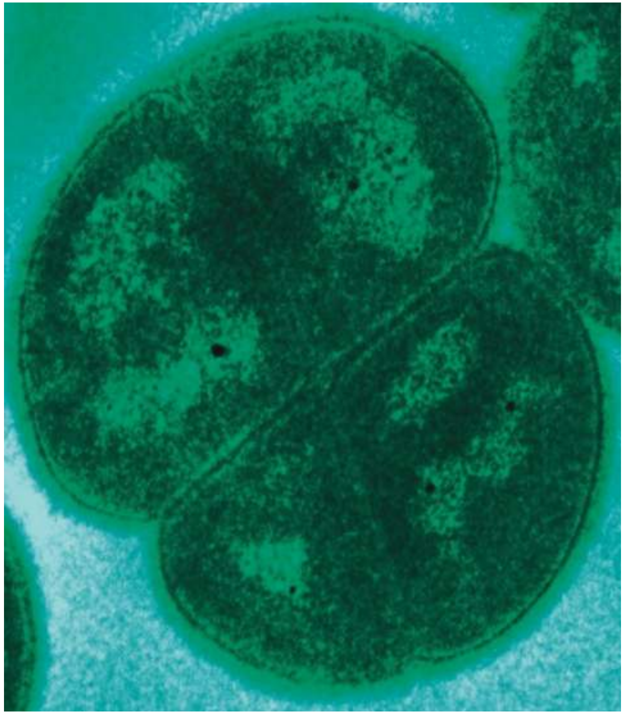


de la Torre, J. R., Goebel, B. M., Friedmann, E. I., & Pace, N. R. (2003). *Applied and environmental microbiology*, *69*(7), 3858-3867.

Thermus/Deinococcus phylum

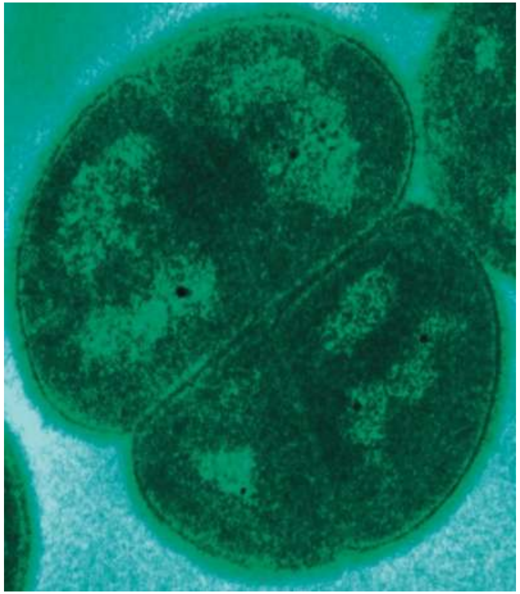


Deinococcus radiodurans



de la Torre, J. R., Goebel, B. M., Friedmann, E. I., & Pace, N. R. (2003). *Applied and environmental microbiology*, *69*(7), 3858-3867.

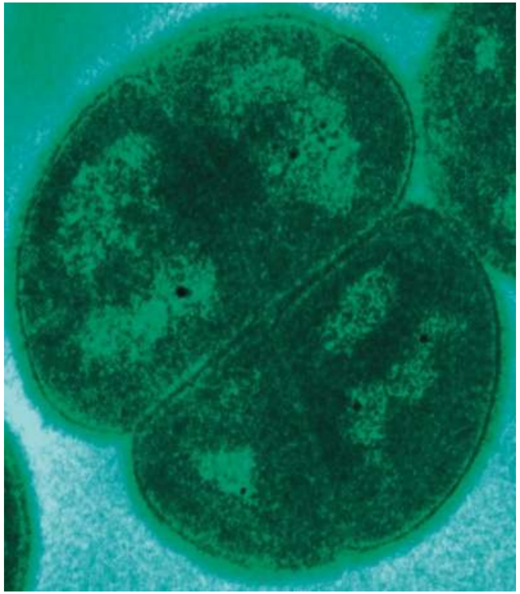
Deinococcus radiodurans



It is a **polyextremophile**:

- one of the most **radiation-resistant** organisms known
- It can survive cold, dehydration and vacuum

Deinococcus radiodurans



It is a **polyextremophile**:

- one of the most **radiation-resistant** organisms studied
- It can survive cold, dehydration and vacuum

- Humans cannot survive a whole-body exposure of 10 Gy
- 200 Gy is lethal for most bacteria
- Deinococcus radiodurans can survive acute exposures of 15,000 Gy and chronic exposures of 60 Gy/h

Radiation-resistance

UV radiation, X-rays and gamma rays are different types of ionizing radiation that can impact microbial cells.

Radiation-resistance

UV radiation, X-rays and gamma rays are different types of ionizing radiation that can impact microbial cells.

Radiation-resistance limits

- *Thermococcus gammatolerans* EJ3 and *Deinococcus hohokamensis* have been shown to resist up to 30 kGy of γ-radiation

Radiation-resistance

UV radiation, X-rays and gamma rays are different types of ionizing radiation that can impact microbial cells.

Radiation-resistance limits

- *Thermococcus gammatolerans* EJ3 and *Deinococcus hohokamensis* have been shown to resist up to 30 kGy of γ-radiation
- *Psychrobacter pacificensis* L0S3S-03b can resist up to 100–1000 J/m2 of UV254

Radiation-resistance evolution

There are no naturally occurring environments known that result in exposures exceeding 400 mGy per years

How it has evolved?

Radiations

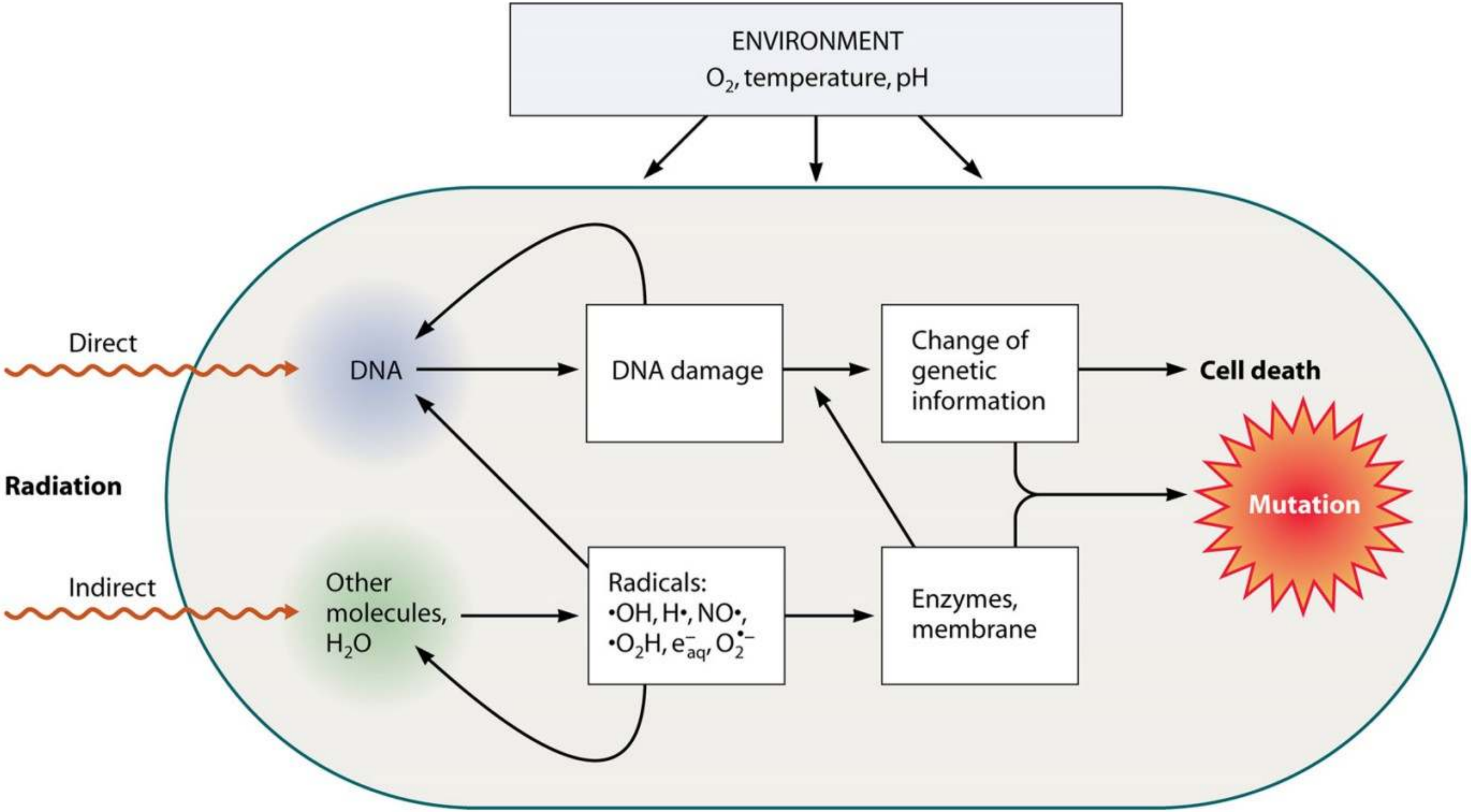
Other stresses like desiccation

They cause similar damages

The effects of ionizing radiations

The effects of ionizing radiation on living cells include:
the formation of large numbers of **ROS, DNA SSBs, DNA DSBs** and extensive **base modifications**; combinations of all of these induce the so-called **complex (clustered) DNA damage**

A dose of 6 kGy is expected to induce approximately **200 DSBs, over 3000 SSBs** and many more sites of DNA base lesions per D. radiodurans' genome. In addition, no significant differences have been found in genomic damage accumulation between radioresistant and radiosensitive bacteria.



Horneck, G., Klaus, D. M., & Mancinelli, R. L. (2010). *Microbiology and Molecular Biology Reviews*, *74*(1), 121-156.

Survival strategies

They need two main response systems:

Survival strategies

They need two main response systems:

- DNA and protein protection mechanisms

Survival strategies

They need two main response systems:

- DNA and protein protection mechanisms
- Efficient DNA repair mechanisms to restore genome functionality

Survival strategies

- **Small non-coding RNAs** (sRNAs) regulate DNA repair gene expression
- Genome redundancy
- Novel two-steps DNA repair processes

Published: 27 January 2009

A new perspective on radiation resistance based on *Deinococcus radiodurans*

Michael J. Daly

Nature Reviews Microbiology **7**, 237–245 (2009) | Cite this article

Abstract

In classical models of radiation toxicity, DNA is the molecule that is most affected by ionizing radiation (IR). However, recent data show that the amount of protein damage caused during irradiation of bacteria is better related to survival than to DNA damage. In this Opinion article, a new model is presented in which proteins are the most important target in the hierarchy of macromolecules affected by IR. A first line of defence against IR in extremely radiation-resistant bacteria might be the accumulation of manganese complexes, which can prevent the production of iron-dependent reactive oxygen species. This would allow an irradiated cell to protect sufficient enzymatic activity needed to repair DNA and survive.

Published: 27 January 2009

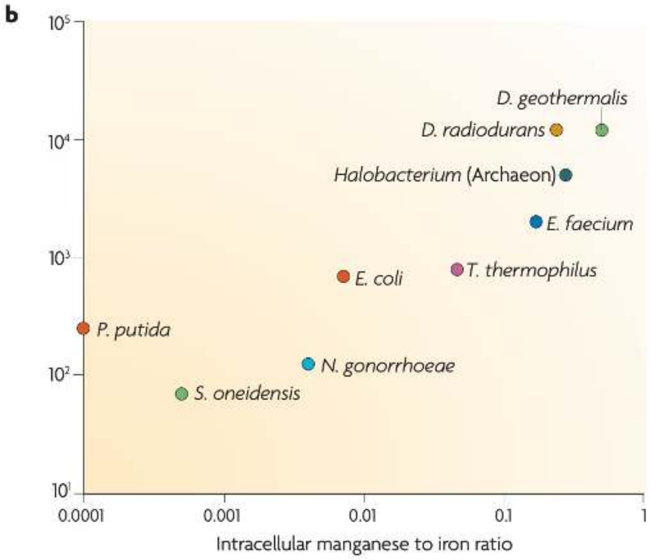
A new perspective on radiation resistance based on *Deinococcus radiodurans*

Michael J. Daly

Nature Reviews Microbiology **7**, 237–245 (2009) | Cite this article

Abstract

In classical models of radiation toxicity, DNA is the molecule that is most affected by ionizing radiation (IR). However, recent data show that the amount of protein damage caused during irradiation of bacteria is better related to survival than to DNA damage. In this Opinion article, a new model is presented in which proteins are the most important target in the hierarchy of macromolecules affected by IR. A first line of defence against IR in extremely radiation-resistant bacteria might be the accumulation of manganese complexes, which can prevent the production of iron-dependent reactive oxygen species. This would allow an irradiated cell to protect sufficient enzymatic activity needed to repair DNA and survive.



Published: 27 January 2009

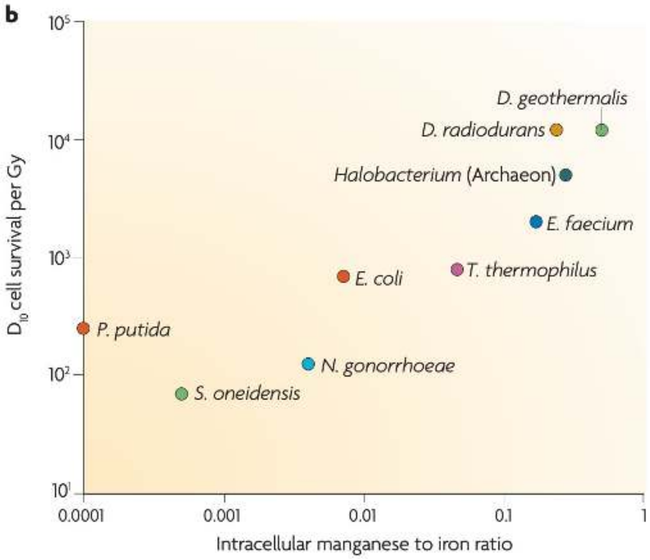
A new perspective on radiation resistance based on *Deinococcus radiodurans*

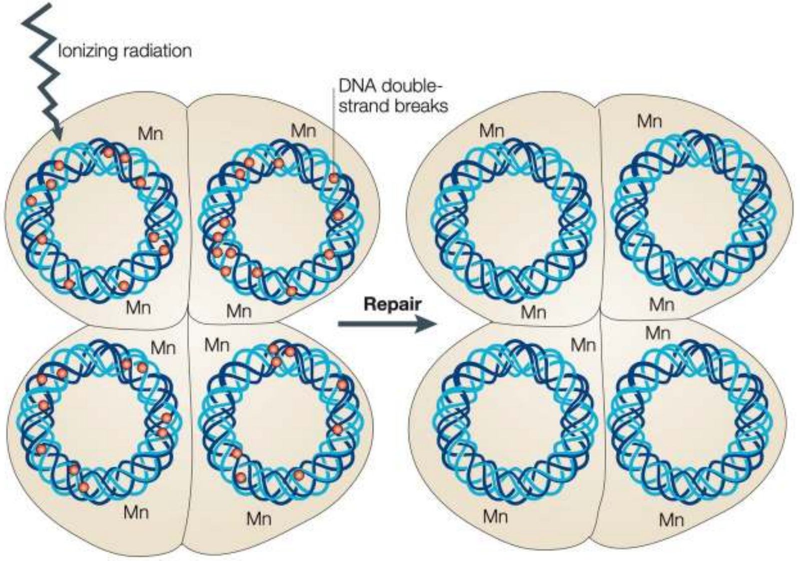
Michael J. Daly

Nature Reviews Microbiology **7**, 237–245 (2009) | Cite this article

Abstract

In classical models of radiation toxicity, DNA is the molecule that is most affected by ionizing radiation (IR). However, recent data show that the amount of protein damage caused during irradiation of bacteria is better related to survival than to DNA damage this Opinion article, a new model is presented in which proteins are the most importa target in the hierarchy of macromolecules affected by IR. A first line of defence agains extremely radiation-resistant bacteria might be the accumulation of manganese com which can prevent the production of iron-dependent reactive oxygen species. This wo allow an irradiated cell to protect sufficient enzymatic activity needed to repair DNA a survive.





Where?

Table 1 Radiation-resistant extremophiles isolated from different types of environments

Organism	Environment	Radiation	Reference
Cellulosimicrobium cellulans UVP1; *Bacillus pumilus* UVP4	Elevated land	UVR-type C	Gabani et al. (2012a)
B. pumilus SAFR-032	International Space Station	UVR	Vaishampayan et al. (2012)
Bacillus subtilis	Earth's megnetosphere	UVR	Nicholson et al. (2011)
B. subtilis HA101	Simulated Martian environment	UVR	Kerney and Schuerger (2011)
Hymenobacter xinjiangensis	Desert	UVR and gamma	Zhang et al. (2007a)
Rubrobacter radiotolerance	Unknown	Gamma	Terato et al. (2011)
Sphingomonas sp. RB2256	Unknown	UVR-type B	Joux et al. (1999)
Chroococcidiopsis sp.	Desert and hypersaline	X-ray	Billi et al. (2000)
Halobacterium salinarum	Unknown	X-ray	Robinson et al. (2011)
*Deinococcus radiodurans*R1	Canned meat	X-ray, UVR, and gamma	Shukla et al. (2007)
Bacillus megaterium	Lake	UVR	Zenoff et al. (2006)
Staphylococcus saprophyticus	Lake	UVR	Zenoff et al. (2006)
Acinetobacter sp. Ver3, Ver5, and Ver7	Lake	UVR-type B	Di Capua et al. (2011)
Streptomyces radiopugnans	Radiation–pollution	Gamma	Mao et al. (2007)

Gabani, P., & Singh, O. V. (2013).. *Applied microbiology and biotechnology*, *97*(3), 993-1004.

Table 2 | **Species of ionizing-radiation-resistant bacteria**

Species	**Representative D_{10} value***	**Phylum**	**Refs**
Methylobacterium radiotolerans	1,000 Gray	α-Proteobacteria	80,81
Kocuria rosea	2,000 Gray	Actinobacteria	4,8
Acinetobacter radioresistens	2,000 Gray	γ-Proteobacteria	82
Kineococcus radiotolerans	2,000 Gray	Actinobacteria	83
Hymenobacter actinosclerus	3,500 Gray	Flexibacter–Cytophaga–Bacteroides	84
Chroococcidiopsis spp.	4,000 Gray	Cyanobacteria	11
Rubrobacter xylanophilus	5,500 Gray	Actinobacteria	85
Deinococcus radiodurans R1	10,000 Gray	Deinococcus–Thermus	86
Rubrobacter radiotolerans	11,000 Gray	Actinobacteria	85

Cox, M. M., & Battista, J. R. (2005). Deinococcus radiodurans—the consummate survivor. *Nature Reviews Microbiology*, *3*(11), 882-892.

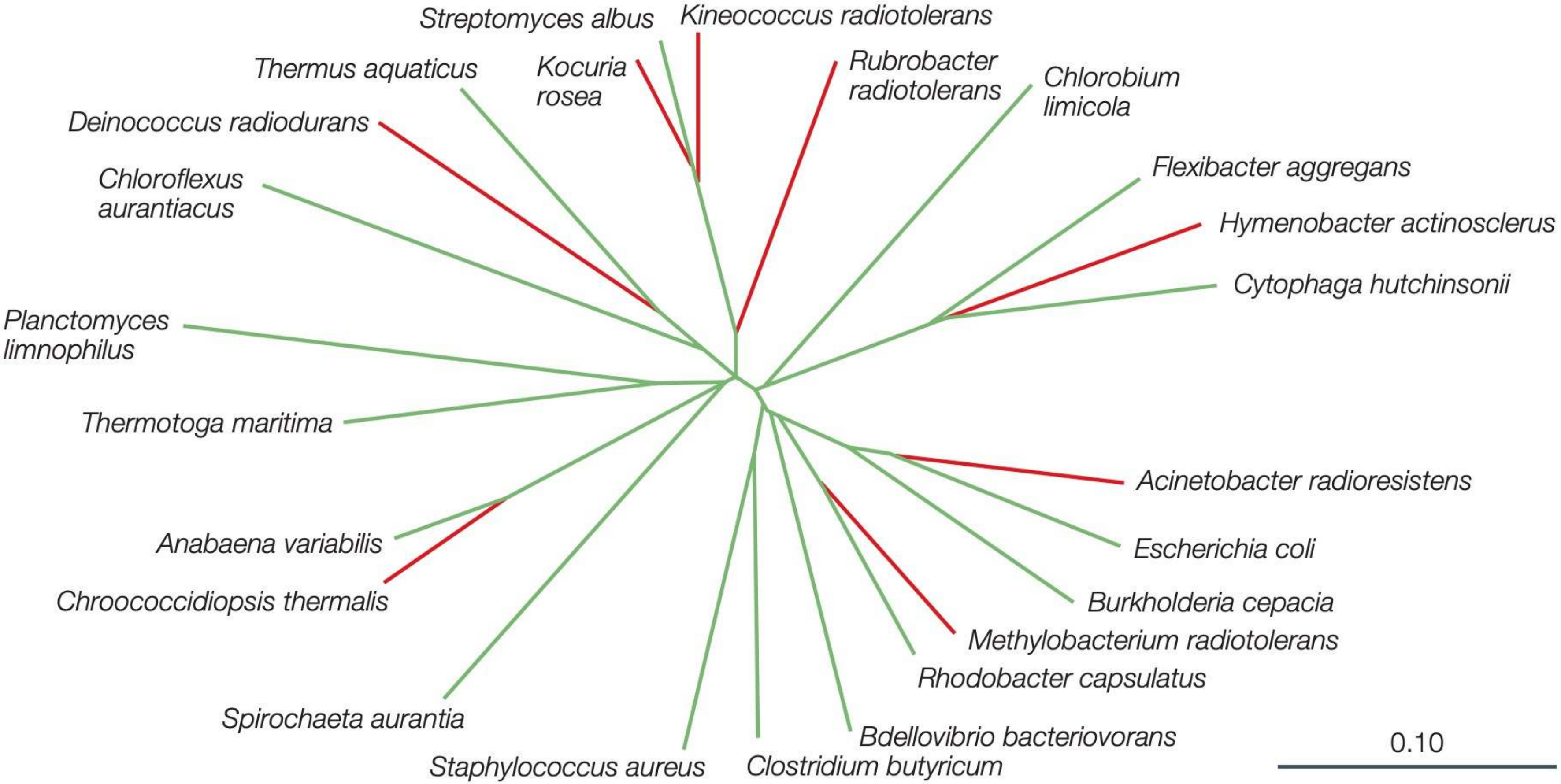


Figure 2 | **A 16S-rRNA-gene-sequence-based phylogeny of the main lineages of the domain Bacteria.** The branches in red are those in which ionizing-radiation-resistant taxa have been described. The scale bar represents 10 inferred nucleotide substitutions per 100 nucleotides.

Two hypotheses

Radioresistance is a vestige of DNA-repair mechanisms that were present in ancestral species retained in those organisms that continue to require this phenotype

This phenotype has arisen in unrelated species through horizontal gene transfer, or possibly convergent evolution

Applications

- Bioremediation of nuclear waste
- D. radiodurans has been proven effective in bioremediation of heavy metals from acidic and neutral water
- Several UVR-protective compounds have been isolated from UVR-resistant extremophiles

Reads

- Lebre, P. H., De Maayer, P., & Cowan, D. A. (2017). Xerotolerant bacteria: surviving through a dry spell. *Nature Reviews Microbiology*, *15*(5), 285-296.
- Daly, M. J. (2009). A new perspective on radiation resistance based on Deinococcus radiodurans. *Nature Reviews Microbiology*, *7*(3), 237-245.
- Gabani, P., & Singh, O. V. (2013). Radiation-resistant extremophiles and their potential in biotechnology and therapeutics. *Applied microbiology and biotechnology*, *97*(3), 993-1004.
- Cox, M. M., & Battista, J. R. (2005). Deinococcus radiodurans—the consummate survivor. *Nature Reviews Microbiology*, *3*(11), 882-892.
- Stevenson, A., Cray, J. A., Williams, J. P., Santos, R., Sahay, R., Neuenkirchen, N., ... & Hallsworth, J. E. (2015). Is there a common water-activity limit for the three domains of life?. *The ISME journal*, *9*(6), 1333-1351. (About water activity limits)