A radon-resistant microbial community

Roberto P Anitori
Ruth M Henneberger

Australian Centre for Astrobiology
Department of Biological Sciences

Macquarie University Biotechnology
Research Institute
Macquarie University
North Ryde NSW 2109

Tel: (02) 9850 8221
Fax: (02) 9850 8245
E-mail: ranitori@rna.bio.mq.edu.au

Introduction

Over the past few decades, microbes have been discovered in a positively bewildering array of extreme environments. Bacterial and archaeal extremophiles exist, and often thrive, in conditions that would quickly kill eukaryotes, such as extremes of temperature, pH, pressure, salinity and radiation.

Deinococcus radiodurans is the champion with respect to the latter, being able to withstand doses of ionising radiation that would kill us feeble humans many times over; it can survive acute doses of gamma-radiation as high as 1,700,000 rads, whereas 500-1000 rad is lethal to humans [1, 2]. Other examples of radiation-resistant microbes are known (e.g. other *Deinococcus* sp., *Kineococcus radiotolerans,* some *Rubrobacter* sp.), but none, including *D. radiodurans,* has been associated with high levels of radioactive radon. Recently, we were the first research group to describe a diverse microbial community flourishing in a radon-rich, thermal environment, the Paralana hot spring [3,4].

Paralana and Astrobiology

Paralana is located near Arkaroola, in the Northern Flinders Ranges of South Australia. The hot spring system consists of two small, interconnected pools that contain obvious microbial mats and biofilms (Figure 1).

Water which issues from the sandy bottom of the smaller pool is at 60-63˚C (pH 7); this drops off to 48˚C in the larger pool. The water is believed to be heated by both geothermal processes and radioactive decay. Gas which bubbles into the pool contains radon (from the radioactive decay of radium), 88% nitrogen, 12% carbon dioxide, and trace helium and hydrogen. The radon levels are higher in the gas than in the spring water: 29000 Bq/L versus 2000-5800 Bq/L, respectively [5, 6]. The radon level in the gas is approximately two million times the average background reading in outdoor air [7]. The alpha radiation emitted by radon is known to cause DNA damage in both prokaryotic and human cells [8,9]. In humans, it is recommended that exposure be kept below approximately 0.15 Bq/L to avoid an increased risk of lung cancer [10].

Paralana is of particular interest for Astrobiology because the conditions – a hydrothermal system, ionising and

Figure 1. The Paralana hot spring. The source pool (upper photo), into which the water and radon gas (note bubbles) emerge through alluvial sand and gravel. The water then flows around and under a large boulder to form a larger pool (lower photo). Both pools contain macroscopic evidence of microbial communities, in the form of numerous floating and benthic mats and biofilms. The upper photo, which is a close-up from above the pool, covers an area of approximately 0.5x1.0m. *Photos ©Roberto Anitori, 03.*

ultraviolet *(uv)* radiation – are believed to be analogous to those present on the early Earth. It therefore represents an excellent laboratory for studying the origin and evolution of life. For example, studies conducted there may shed light on the currently popular theory that life evolved in a hydrothermal environment.

Where are the radiation-resistant microbes?

Molecular-based 16S rRNA studies using both bacterial and archaeal-specific primers indicated that Paralana supports a diverse microbial community. In general, the microorganisms identified (representing either previously cultured or uncultured microbes) are those expected in a hot spring. Examples include cyanobacteria (e.g. *Oscillatoriales, Stigonematales),* proteobacteria (e.g. *Hydrogenophilus thermoluteolus),* green sulphur and green non-sulphur bacteria [3], and thermophilic crenarchaeota [4]. Figure 2 shows a filamentous cyanobacterial sample from Paralana. There is currently no data on the resistance of these microbes to radon.

Whilst these results are not particularly remarkable for a thermal pool, the presence of radon means that Paralana is definitely not your standard, run-of-the-mill hot spring. We were therefore somewhat surprised that no previously described radiation-resistant microbes were identified (e.g. the thermophiles *Deinococcus geothermalis* and *D. murrayi* [11]). However, there were some tantalising hints – identification of a 16S rRNA sequence 93% homologous to a sequence originally identified in the anaerobic drain waters of uranium waste piles [12]; the presence of archaeal halophile sequences, interesting because it has been demonstrated that a halophile, *Halobacterium salinarum*, is resistant to ionising radiation [13]; and the identification of a gene sequence coding for a protein with homology to a hypothetical *D. radiodurans* protein [M. Gillings, personal communication].

Nonetheless, there was no conclusive evidence of the presence of radiation-resistant microbes in Paralana. To investigate further, we designed *Deinococcus* species-specific 16S rRNA primers with which to analyse the DNA extracted from the hot spring samples. No *Deinococcus* sequences were identified. Primers specific for other species resistant to ionising radiation will be tested in the future.

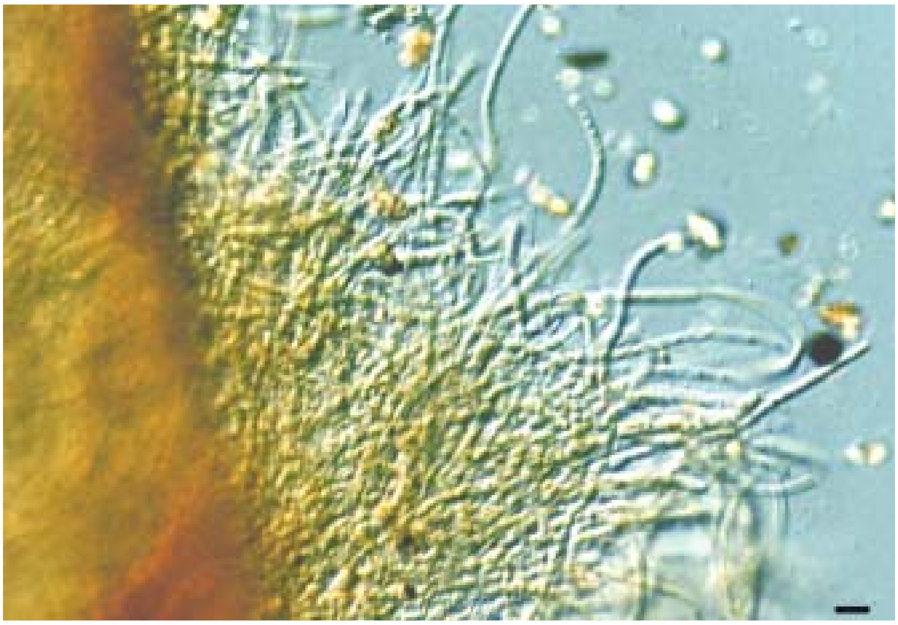


Figure 2. Differential interference contrast micrograph (wet mount) of a mat composed of filamentous cyanobacteria. This sample was collected from the source pool. Scale bar = 10 µm. Photo ©Roberto Anitori, 2003.

Of course, it is possible that no such 'classical' radiation-resistant microbes are present in Paralana. An intriguing possibility is that the spring represents an environment in which microbes not commonly associated with resistance to ionising radiation have evolved mechanisms that allow them to live in the presence of radon. For example, it will be interesting to determine whether the Paralana cyanobacteria are more radiation-resistant than their cousins in similar, but radiation-free, environments (e.g. Shark Bay in Western Australia, or New Zealand hot springs).

Fortunately, we will soon be able to test this hypothesis. The first author is a member of a NASA team, headed by Dr Lynn Rothschild, which plans to isolate highly radiation-resistant microbes from a number of different extreme environments, including Paralana. The aim of this NASA Astrobiology Institute funded 5 year study is to test the ability of microbes to survive the harsh space environment, which is extremely cold, and is subject to unfiltered solar *(uv)* radiation, solar wind and galactic cosmic radiation. The most radiation-resistant microbes will be exposed to this environment by flying them in experiments on-board satellites and the International Space Station. The results of this study will be used to test the theory that life can (and has) hitchhiked between solar system bodies, such as Earth and Mars, on objects such as meteorites.

Conclusion

The Paralana hot spring is an intriguing site with astrobiological implications as an analogue for conditions on the early Earth. The research conducted thus far has identified a diverse community of mostly thermophilic microbes. Whilst it is possible that the resident microbial community is unaffected by the radon, we consider this the least likely possibility. More probable is that the microbes have adapted to the environment, having evolved mechanisms that allow them to survive in the presence of ionising radiation. If this is the case, then the NASA collaborative study has a good chance of identifying a highly radiation-resistant microbe. So, remember to occasionally look up into the heavens over the next few years – that bright streak might just be carrying Australia's first official 'micronauts'.

Acknowledgements

For funding: the Macquarie University Research Development Grants Fund, the Australian Centre for Astrobiology, and the University of Auckland Research Grants Fund; Prof Peter Bergquist and Prof Malcolm Walter for professional guidance and (MW) collection of samples.

References

1. Daly MJ, Ouyang L, Fuchs P & Minton KW. In vivo damage and recA-dependent repair of plasmid and chromosomal DNA in the radiation-resistant bacterium *Deinococcus radiodurans*. *J Bacteriol.* 1994; 176:3508-17.
2. Travis J. Meet the superbug. *Science News* 1998; 154:376.
3. Anitori RP, Trott C, Saul DJ, Bergquist PL & Walter MR. A culture-independent survey of the bacterial community in a radon hot spring. *Astrobiology* 2002; 2:255-70.
4. Anitori RP, Trott C, Saul DJ, Bergquist PL & Walter MR. The microbial community of a radon hot spring. *Aust. Soc. Pac. IAU Symp. Series.* Vol. 213. 2004 (In press).
5. Grant K. The radio-activity and composition of the water and gases of the Paralana hot spring. *Trans. Roy. Soc. S.A.* 1938; 62:357-365.
6. Beverley EIS. *Beverley Uranium Mine Environmental Impact Statement. Main Report.* Heathgate Resources Pty. Ltd. Adelaide, 1998.
7. US Environmental Protection Agency. 2001; http://www.epa.gov/ttn/uatw/hlthef/radionuc.html and 2001; http://www.epa.gov/iaq/radon/radonqa1.html
8. Brena M, Tavera L & Balcazar M. SOS induction by low activity alpha sources:microdosimetric considerations. *Int J Radiat Biol.* 1996; 69:219-23.
9. Rydberg B, Heilbronn L, Holley WR, Lobrich M, Zeitlin C, Chatterjee A & Cooper PK. Spatial distribution and yield of DNA double-strand breaks induced by 3-7 MeV helium ions in human fibroblasts. *Radiat Res.* 2002; 158:32-42.
10. National Research Council report. Health effects of exposure to radon:BEIR VI. *Committee on Health Risks of Exposure to Radon (BEIRVI).* US National Academy of Sciences. 1999.
11. Ferreira AC, Nobre MF, Rainey FA *et al.* *Deinococcus geothermalis* sp. nov. and *Deinococcus murrayi* sp. nov., two extremely radiation-resistant and slightly thermophilic species from hot springs. *Int. J. Syst. Bacteriol* 1997; 47:939-47.
12. Radeva G & Selenska-Pobell S. Bacterial diversity in drain waters of several uranium waste piles. In: Bernhard G (Ed). *Annual Report Institute of Radiochemistry.* Dresden, Germany 1999, p.56.
13. Shahmohammadi HR, Asgarani E, Terato H, Ide H & Yamamoto O. Effects of 60Co gamma-rays, ultraviolet light, and mitomycin C on *Halobacterium salinarium* and *Thiobacillus intermedius*. *J. Radiat. Res.* (Tokyo) 1997; 38:37-43.

Lipids, biomarkers and the history of life

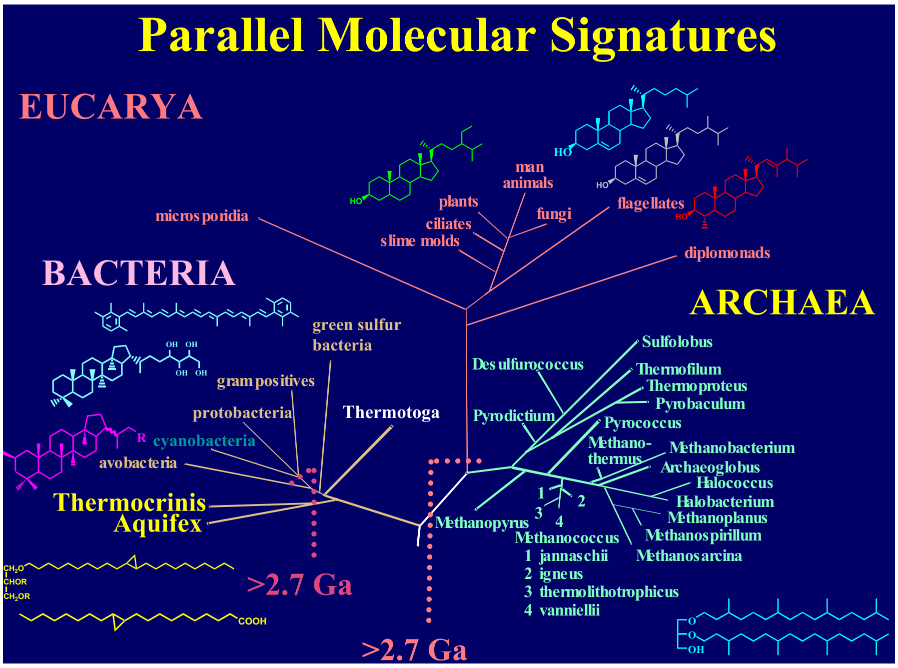
Roger E Summons

Earth Atmospheric and Planetary Sciences
Massachusetts Institute of Technology
77 Massachusetts Ave E34-246
Cambridge MA 02139 USA

Tel: (1) 617 452 2792
Fax: (1) 617 253 8630
E-mail: rsummons@mit.edu

Introduction

Organic matter is the stuff of life. Its qualities reflect the biochemical pathways used for its synthesis and, to a degree, the phylogeny and physiology of the organism. Organic matter is also readily fossilised and, under opportune circumstances, provides us with an accessible record of the history of life.



Biomarkers, isotopes and sedimentary rocks

Molecular methods are providing new windows through which we can observe biodiversity and study microbiologically-driven processes. DNA, cloned from living organisms, has given us the well known 'tree of life' and a depiction of the genetic distances between all living organisms [1]. This tree also encodes a sense of evolutionary relationships.

The inheritance and expression of lipid biosynthetic pathways follows a comparable pattern in which a range of specific and diagnostic chemical structures in membranes and other lipids are largely confined to particular taxa

Figure 1. Universal phylogenetic tree in rooted form [1] and annotated with the structures of a selection of lipids that are diagnostic for specific taxa within the tree. Oldest known occurrences of the hydrocarbon cores of the 2-methylbacteriohopanepolyols (2-methylhopanes) and the algal sterols (24-alkylsteranes), and the assumption that lipid biosynthesis is a conserved trait, suggests a minimum age for the appearance of cyanobacteria and Eukarya of 2700 Ma [7].