Trapped in salt for 250 million years

This story may sound like a science fiction blockbuster, but is actually an authentic report from the field of geomicrobiology. Fifteen years ago, a team led by US researcher Russell Vreeland discovered the oldest reported living organisms, microbes that had been trapped in a salt crystal for 250 million years before they were extracted in the lab and successfully revived. We talked to *Russell Vreeland* about his extraordinary findings, their role in science and implications for understanding the evolution of life.

BY Juliana Troch

To kick off: were these findings just a lucky shot?

Contrary to popular belief, I don't think there's ever a «Eureka moment» in science. We sometimes act as if there is one, but in my opinion, that never happens. «Lucky shots"»almost always turn out to be proved wrong.

Is there any previous record of microbial life being found in salt crystals?

During a class on halophiles back in 1985, a student asked me how long a halophile could live in salt. I had no idea except for some anecdotes about 100 year-old salt in museums that had microbes inside. That student became my graduate student, and identified a site that could offer the possibility to study living halophiles. This was a salt mine that had been accidentally flooded by a drill rig. The water that entered the mine was fresh water, and five years later it had become saturated brine. We were able to get a water sample and found that it contained an extremely halophilic Archaeon – a single-celled microorganism. That microbe could not have been in the water entering during the flooding. So that gave us a hypothesis: the microbe had been in the salt that dissolved to make the brine. We did not realize at the time that the salt was 125 million years old.

How can we imagine the state of the bacteria before they were reactivated in the lab? How long can bacteria stay in this state?

We did some experiments with this particular group of microbes and found that when the salt concentration increased, they quickly formed spores. As spores, these organisms became «cryptobiotic», so they had no need to metabolize or produce wastes. They can exist in this state – well, I guess, indefinitely. Of far greater interest are the microbes that don't form spores. We started experiments with some extreme halophiles and were amazed to see them swim into the inclusions as the crystals formed. They allowed themselves to become trapped! We realized it was a survival mechanism that would evolve.

Anyway, without spores it becomes really hard to envision, but there is a thing in bacteria called «starvation survival», wherein they slowly shut down metabolism and reproduction. As to how long something can stay like this, I have no answer. We did a hypothetical analysis using ten times the amount of damaging conditions that could be expected and came to something like 1.5 billion years, if you started with a million microbes. Unfortunately, this experiment can't be done in the lab because there is no way to speed up time, which is the one true constant.

If microbes can survive for 250 million years in salt, could there be even older microbes surviving out there?

Without a doubt. We humans make the mistake of thinking we are special, and we talk about «life on Earth». That is totally wrong: we should rather consider that «the Earth itself is alive». Microbes have populated it for all but about the first 600 million years of its existence. As the Earth goes through its geological cycle, live organisms are carried underground and later brought back up to the surface. So I do think there are older microbes out there and I am pretty certain we run into them all of the time.

What properties does salt have that make it such a great container for these traces of former life?

The first thing we recognized was the simple axiom that «without liquid water there can be no life». In order for an organism to survive long-term, it must have liquid water. Salt crystals meet that criterion because as they form they

trap some of the surrounding fluid in inclusions and they preserve it in the liquid state. Think of it as water dissolved in salt, but now the freezing and boiling points are so altered that the water stays as a liquid. NASA has even found that water in a crystal will remain liquid on Mars!

Second, if an organism is trapped and unable to metabolize, there are many nasty things that will kill it. One is oxygen, which damages our cells. But a salt crystal lattice is so tight that oxygen cannot penetrate. Another thing is radiation, both from sunlight and radioactive materials. Once the crystals are underground and covered, sunlight doesn't reach them strongly enough to do any damage. As the salt content of the brine increases, heavy metals like uranium become insoluble and precipitate before the salt crystals form, so those are not present either. The only radioactive material that is present are 40K-isotopes, but in a single 0.1 mm inclusion, there would only be about 12 such molecules and only one of these might emit radiation in our time frame.

Is the survival of such old organisms a common event throughout the Earth's history?

Truthfully I don't know the answer to that. People are still looking, and that is good. I do think this wonderful Earth preserves more than we give it credit for. Look at the recent literature: we have now found DNA and proteins in dinosaur bones, Neanderthal DNA and DNA frozen in ice. So I do think there is more out there – or maybe I should say «Life will find a way to survive».

What does that mean for the evolution of life?

I would say that it means that the evolutionary goal of life is survival. I also think that as living things adapt, they have the record of everything that has gone before. That doesn't mean that every gene ever needed has already been invented; in the case of microbes, they are experts at picking up DNA, using it if it is good, modifying it as needed and developing new capacities as those become available. You need only look at antibiotics, polychlorinated biphenyls and DDT: all of these were new to the biosphere 100 years ago and all can now be degraded by naturally evolved microbial pathways.

How did the general public react to your findings?

They were really excited, probably because the story sounded a bit like the ‹Jurassic Park› movies. The fact that we were drilling into crystals and finding life had everyone talking. Also the other authors and I took the position that, since the funds came from public taxes, we would agree to speak in any public venue available, and we did.

...and the scientific community?

The scientific reaction varied literally by discipline and in a surprising way. Geologists initially tended to be doubtful, but when they examined the data, they accepted the age of the formation. They did question the crystals a bit, but once those answers were provided, they accepted the results and considered it rather a break-through. Microbiologists and biologists looked closely at the isolation procedures and wanted answers about the geology but, again, once they got answers, they said yes, this is the best evidence yet for long-term survival.

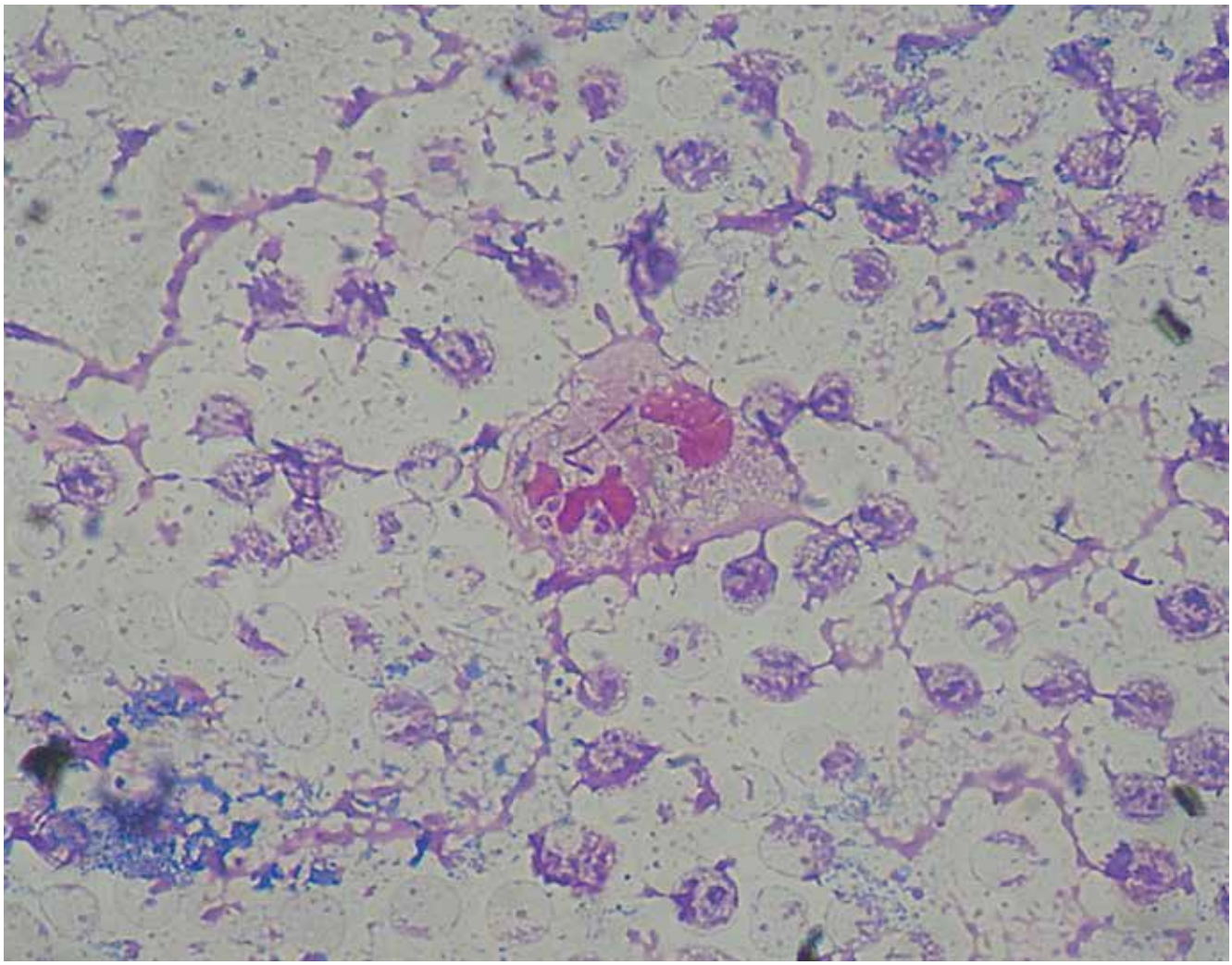
Molecular biologists responded in a completely different way. They basically ignored all of the data and argued that since these data did not agree with their computer simulations, we had to be wrong. They actually wrote papers along these lines. Essentially what happened was that, based on their theories, the organism's 16S rRNA sequence was «too similar» to that of modern organisms. We continued to point out that we hadn't used the sequences to justify the organism's age, and that the objection was therefore irrelevant. I now simply ignore them – I have always felt their reaction was because our findings challenged their own preconceived hypotheses.

What is the status of the research field now?

Well, three years ago I attended a halophile meeting in Connecticut and they had a session where five papers in a row – even a molecular one – demonstrated that we were correct. At the end my friends were high-fiving me and saying how good it must feel – and they were right, it did. There are now several labs pursuing what we started, examining the survival mechanisms and even sampling younger material moving toward our old salts. I am hoping that this work will continue and I hope that young people like you will take it up, do it better and move forward.

Cryptobiosis

is an ametabolic state of life entered by an organism in response to adverse environmental conditions such as desiccation, freezing, and oxygen deficiency. In the cryptobiotic state, all metabolic processes stop, preventing reproduction, development, and repair.



Phagocytosis of P. aeruginosa by neutrophil in patient with bloodstream infection (Gram stain); «Pseudomonas aeruginosa smear Gram 2010-02-10» by Paulo Henrique Orlandi Mourao – Own work.