Acido*pH*iles: a not- so-basic life

by

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ABSTRACT

There are conditions on the Earth that are completely inhospitable to humans. Macroscopic life forms in these conditions are extremely rare. Microscopic life forms, however, thrive. They are called extremophiles. One subset of extremophile called the acidophile live in acidic environments, at time even more corrosive than battery acid.

Acidophiles are microbes, and live together in hugely diverse ecosystems. Each species of acidophile fills a different niche. They survive in high acid environments using a number of methods, including a highly active proton pump, or five-ring structures called hopanoids that are imbedded in the cell membrane, among others. Acidophiles can be applied to many human questions. They are used in the process of bioremediation as applied to acid mine drainage sites. They are also useful in biomining. Because of their ability to flourish in near-otherworldly conditions, they have particular interest in the field of astrobiology, the search for extraterrestrial life.

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Red Eyes is a blemish on the Pennsylvania landscape. In summer, a bird's eye view shows two red-orange scars glaring up out of the lush green Gallitzin State Forest. But we approached the higher of the two streams, aptly named Upper Red Eyes, in the dead of winter, after ice skating in thick rubber boots down the frozen footpath.

The entire forest was dead, bare brown trees sticking up out of the hard ground like toothpicks. But there was something different about the trees looming out of the iced-over water flow before me. They were more brittle, like they hadn't felt green in a long time. Logs that used to be attached to trunks were strewn around, white like stone, and splintered. Try to grow a tree in acid and this is what happens.

Red Eyes, upper and lower, are streams of acid water flowing up from underground and over this formerly verdant landscape. The ground is rusty orange with iron, and the only sign of life is a bear print at the edge of the pool. All signs point to "barren wasteland."

But Dan Jones, a short, stocky Penn State graduate student, hunched down over a crack in the ice. With his bare, soon-to-be chapped hand, he scraped a pipette along the iron-coated bottom of the stream bed, sucking up some liquid and sediment as he went. The water here has about the same acidity as orange juice — not high enough to instantly melt skin off your bones, but pretty uncomfortable to take a long swim in. Definitely strong enough to destroy a few trees.

Jones squatted on the frozen flow, balancing on the balls of his feet, while he emptied the pipette into little sealed vials. These samples were going back to the Penn State lab. Jones was going to analyze and categorize their contents for inclusion in his thesis about communities of tiny organisms. Because thriving in just those few grams of soil and acid water are billions of microscopic life forms.

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Life as experienced by humans is fairly confined. We like our rooms at a nice 68 degrees Fahrenheit. If we hang out at freezing temperatures for too long, our limbs start to die. A prolonged heat wave of upwards of 95 degrees can be deadly. We can't drink too much salty water, we suffocate without oxygen, and an unfortunate many know the discomfort of excess stomach acid working its way back up the esophagus. The same is true for a lot of life on Earth. Plants and animals are generally comfortable in what we would consider normal environmental conditions. We like to avoid intensity.

But life is pervasive and tough, and where there is a niche to fill some life form will push its way in. There are some types of life on Earth, mostly the kind that can only be seen through a microscope, that embrace that stubborn persistence and not only survive but thrive in places we would fail — deep in the Dead Sea, nestled in the permafrost of the poles, bathing in the geysers of Yellowstone. And if they had a voice, they'd probably tell us that they're living normally, that in their view our lifestyles are abnormal.

These little guys are known as extremophiles — from phylos, meaning love. Thousands of extremes-loving species decorate the branches of the tree of life. Bacteria, algae, and fungi all have their representatives in the extreme classification pool. Halophiles stay hydrated encased in salt. Psychrophiles make their homes in the middle of glaciers. Thermophiles enjoy boiling baths. Xerophiles dwell in thirsty deserts. And then there are the acidophiles, microbes that have built a richly diverse ecosystem not just at Red Eyes, but at acidic environments all around the world. With amazing tolerance, they can flourish in acidic conditions even more toxic than battery acid.

Acidophiles have been found in some of the most corrosive places on Earth. And just as the ground we walk on is littered with microscopic cities of life, acidophiles form massive and diverse communities in their own soil and water. This wild diversity on Earth gives scientists hope that life can exist on the exotic landscapes of distant planets.

The acidophilic evolutionary storyline is similar to that of microbes we are familiar with, those things that make our wine or form slicks of algae at the bottom of our winter-sealed pools. But something happened in microbial history, some small tweak in the way certain microbes went about their lives that forced acidophiles to diverge from their neutral-condition-loving cousins. Acidophiles, Jones suggested, are microbial marsupials.

Millions of years ago, marsupial and placental mammals had a parting of the ways. Marsupials, like koalas and kangaroos, started giving birth to barely-formed babies, then tucking them away in pouches until they were grown enough to survive in the world. Placental mammals, us included, went on to have longer gestation periods. Both types of mammals continued on making their way in their separate parts of the world.

But the interesting thing is this: while placental mammals were evolving into cats and wolves and squirrels, the same types of animals were evolving as marsupials. They filled the same niches, they had the same habitats — they just gave birth in a different way. Similarly, the difference that sprang up between acidophiles and their more moderate relatives was their chosen neighborhood. From then on, they evolved to do the exact same types of things — just one of them was in acid.

Acidophiles exploded onto the research scene with the rest of their extreme posse in the mid 1960s, thanks to Thomas Brock, then a 40-year old professor of microbiology at Indiana University.

Brock was on his way to study marine habitats at Puget Sound near Seattle when he traveled through Yellowstone National Park. Something among the bubbling geysers and hot springs caught his eye — he saw life, and a lot of it. So he decided the boiling pools were worth deeper scrutiny.

In 1965, he gathered a team and some funding from the National Science Foundation and set up camp in West Yellowstone, Montana. For ten years, students and faculty came and went, collecting samples of microorganisms thriving in 122 degree Fahrenheit water, in liquid more acidic than the insides of a battery — the types of environments humans would find deadly.

Brock at first focused his studies on the microbes living in the super hot environments, but another location soon caught his attention. Some of the blistering geysers were coughing up some smelly acid steam, and he wanted to know, was life there, too?

"Acidic environments are really quite different from the big geysers and big hot springs," he says. "The acidic environments in Yellowstone are dominated by steam that rises to the surface." With the steam comes a good deal of hydrogen sulfide, leaking from the volcanic environment below. Some might associate it with the ever appetizing aroma of rotting eggs. The stench-causing gas drifts up from the bowels of the Earth to meet with oxygen, which then turns it into its straight elemental form of sulfur, carpeting the landscape in bright yellow crystals.

And here, Brock found more life. Life that ate the sulfur, no less. Bacteria living near the steam vents use the sulfur, add more oxygen to it, and turn it into sulfuric acid. "In nature, almost all the sulfuric acid that's made does come from bacterial action [...] so they have to deal with the acid or they kill themselves off," says Brock.

Brock's discovery prompted further exploration of the organisms, eventually dubbed extremophiles. Before his Yellowstone discoveries, a few species of microbes had been isolated in some severe conditions, like hot water or mild acidity. But the series of papers Brock published out of his years of research at Yellowstone blew the roof off the limits of how these microbes could survive. He even presented the idea that classifications could join forces, producing things like thermoacidophiles, acid-lovers in high heat.

The papers "just really opened peoples' eyes," says Michael Madigan, a former student of Brock's who studied with him in the 1970s. "Then people started saying, 'well, geez, the sky is the limit!'" Academics started probing every seemingly inhospitable corner of the Earth for thriving extremophiles. As Brock says, as long as there is water, there is life. This new branch of microbiology spawned conferences, journals, and today many hundreds of scientists around the world are investigating how the hardy microbes shape their landscapes and ecosystems. The rapid broadening of the field set the stage for the advancements of future generations of microbiologists, like Dan Jones.

The day after our Red Eyes outing, Jones stood behind a push cart surrounded by Dixie cups of lemon juice, crushed up bits of chalk, and five kids from a local fifth grade class. It was "Shake, Rattle and Rocks" day, an outreach program put on by the Penn State geology department and the kids were at Jones' station to learn about microbes.

"So what do you guys like to eat?" he asked.

A few of them shouted out their favorite eats: "Macaroni and cheese!"

"Right on. So, you guys eat macaroni and cheese and sugar and things like that, and you breathe in…" He paused.

"Oxygen!" They yelled.

"Right! So your body is taking that oxygen and reacting it with the food that you eat and then you're producing carbon dioxide that you breathe out as a waste product." The kids fingered the lemon juice cups as they studied Jones's faded black T-shirt advertising 2009's 15th International Congress of Speleology.

"So how many of you guys would like to eat…this rock?" He presented the kids with two chunks of butter yellow stone.

"Me!" One youngster raised his hand.

"Yeah. Well certain types of bacteria would like nothing more than to eat various types of rocks. Do you guys know what this kind of yellow-y rock is called?"

"Sulfur!"

"Exactly! Good job. How about this shiny golden one?"

"Fool's gold."

"Yeah, pyrite." He spun it in his hand so its shiny flecks, an iron-sulfur compound,twinkled in the light. "So these rocks, they have a lot of chemical energy in them. It's just that we can't use that energy, but different types of bacteria can."

He held up a beaker with a coating of crushed up sulfur rock and wiggled it, letting the pieces roll around the bottom. "So, you guys see this sort of yellow powder here? These guys can take this sulfur, react it with oxygen, and live off that, and then produce sulfuric acid as a by-product instead of carbon dioxide. So what do you think about that? Does that sound like an environment you'd want to live in? A bunch of sulfuric acid?"

"Noooooo," they chorused.

A steaming hot plate of freshly exposed brimstone or fool's gold is not appetizing to us, but as Jones explained to the kids, for some acidophiles it's a gourmet meal.

Rocks, like all matter, are made up of molecules bound tightly together. Molecules are made of atoms, and atoms consist of three smaller bits — protons, neutrons and electrons. And the electrons are what cells care about. Human cells break down the food we consume and use its electrons to produce the energy that keep us going. Some acidophiles also enjoy organics, like sugars or proteins found right in nature, using them to energize just like a human cell. Most acidophiles, though, prefer to extract their electrons from the sulfur sprinkling offered up by Jones.

Cells put these electrons to good use through an elaborate game of Hot Potato. Crammed inside a cell resides an army of molecules ready and waiting to grab electrons and pass them down the line, one after another. The last molecule-at-arms in line hoists the electron out to something known as a receptor. For humans and many acidophiles, the receptor is oxygen. Some acidophiles, however, toss the last electron out to be received by iron or sulfur. We breathe oxygen; some acidophiles, in a sense, breathe rocks.

This game of pass-the-electron generates a lot of energy, which gets released into the cell and triggers another cellular function — the movement of protons from outside of the cell to inside. Protons meander through the cell's interior, sucking up the extra energy and hoarding it until they are forced back out of the cell. When protons finally release their hold, they pass the energy to a protein in the cell that creates a molecule known as ATP, the ultimate rationer of energy. The rest of a cell's functions depends on energy doled out by this all-important molecule. This inevitably creates a by-product. Some microbes exhale carbon dioxide, much like us. Others produce different types of acid — usually sulfuric or ferric acid.

Most cells go through a version of this process. The differences between us and acidophiles arise in electron starting points and final resting places. If we tried to feast on stone, we'd be lacking all the nutrients that keep us alive.

Cruising down the winding highway from State College to Gallitzin State Forest, Bill Burgos, a civil and environmental engineering professor at Penn State, energetically opined on how exciting these microbes are.

"The bacteria are awesome because they can respire anything," he said to Jones, who was bundled up in the passenger's seat, ready to brave the bitter Pennsylvania January cold. They were explaining to me how electrons flow through the cells to create energy. They told me something that usually likes to eat iron and breathe oxygen can switch to an entirely different metabolic process halfway through its life, if for some reason its oxygen source disappeared. Microbes are metabolically diverse and flexible, Jones said.

"Maybe a little bit of a competitive edge," he continued.

Burgos agreed, with respect in his voice for the acidophiles: "Humans have big brains, but they have lousy gene expression." If you were locked in a room and all the oxygen you need to breathe was suddenly sucked out, "you'd die because you can't adapt," he said. But the acidophiles register the change and fix their metabolism, something humans could never do. One acidophile, named for the two-faced Roman god Janus, usually feeds on electrons pulled from sulfur. When it breathes, it transfers those electrons to oxygen. But when oxygen is scarce it will switch food sources entirely. Instead of sulfur, hydrogen becomes the new food source. And the electrons pulled from the hydrogen are handed over to the sulfur it used to snack on. Sulfur is now breathed and oxygen is no longer needed at all.

"Diversity of form is in all these higher organisms," Jones agreed, "But diversity of function..."

Harnessing that diversity of function may have played a large role in how these resilient little buggers came into existence. One of the best locations on Earth to study the question of acidophilic origins is a certain rich red Spanish river.

The Rio Tinto bleeds through 62 miles of southwestern Spain, both sickly scarlet and vibrantly orange. This powerful body of water has slowly worn away at rocks hundreds of millions of years old, naturally severing mineral bonds and producing acid. Fool's gold transforms into iron deposits the color of freshly spilled blood. Chalcocite morphs into blue copper streams. Many other heavy metal deposits similarly contribute to the watery mosaic. Here was haven for fledgling acidophiles who munched on the rocks and spit out what minerals they didn't want, hastening the creation of the acid and the deposition of those heavy metals.

For thousands of years, people have been mining along this massive river, exposing more rocks which in turn made the environment even more toxic, creating niches to be filled by more and more species of acidophile. Ancient Romans were the first to become unwitting beneficiaries of the microscopic Rio Tinto communities. Copper deposits glistening in the rushing river caught the Roman eye. They scooped up the bounty, not knowing life forms tinier than they could imagine were to thank. The river has supported this livelihood ever since and today, bordered by lush forests and tucked between terraces created by modern day miners, the water is a colorful patchwork of deep cranberry, pumpkin, and butter, full of all kinds of miniscule life. In this way, dissolved metals and intense acidity have created a phenomenon called acid mine drainage, seen at the Rio Tinto and other mining sites around the world.

Linda Amaral-Zettler has spent much of her career on the banks of the Rio Tinto, which is surrounded by rocks with a similar mineral makeup to those that have been found on Mars. She is studying the river's microbial diversity to get a feel for the range of life these kinds of minerals can support.

"The Rio Tinto is dominated by microbial life," Amaral-Zettler says. So far, fewer than nine thousand acidophilic species have been identified. But that's only counting species that have been formally described, those who have had their genomes sequenced, read out like an alphabet book. Many more are expected to be discovered. Sites like Rio Tinto are perfect for analyzing microbial diversity because of fluctuations in acidity and mineral concentrations along their lengths.

Different types of rocks become more abundant as the river flows along. And depending on the prevalence of certain rocks, acidophile species will vary. Iron-eaters will inevitably hang around a lot of iron, and the same is true of sulfur eaters. Moreover, it seems as though they all work together.

"We do tend to find some species co-occurring…[and there's] pretty strong evidence for symbiosis, [but I] don't think we've appreciated the extent to which it happens," says Amaral-Zettler.

She and her team studying the Rio Tinto have noted a certain interdependence among species, with each having its own special role to play. Some acidophiles eat the sulfur in pyrite, leaving behind the iron and breathing out acid. Others need a lot of acid to survive, so they live downstream from the sulfur-eaters. Still more eat that iron produced upstream to produce a different by-product, which another acidophile needs…and the sequence continues.

Some acidophiles provide protection instead of food. These guardian species defend themselves and those nearby from harmful dissolved metals. Some do this through the production of biofilms, a gooey shiny secretion, which act as a dam holding back the flow of metals. "They essentially make it easier for their neighbors to survive," explains Amaral-Zettler.

"I have a nice little microcosm of the Rio Tinto in my lab," she shares. A glass container sitting in a patch of light on her shelf holds rock, acidic water, and some of the microbial life that make the red river their home. "It's a self-contained system, and everyone is there and they're doing their thing," she says proudly. "It's a self regulating system…they produce the acid, they live in the acid, its just the way they've grown…the biomass is tremendous."

There's no question that diversity reigns supreme. How that diversity got there in the first place, however, is still up in the air. Amaral-Zettler, Jones, and their colleagues throughout the extremophile community are pondering the evolutionary story that might have led to acidophiles.

There's the "everything is everywhere hypothesis," says Amaral-Zettler. Maybe these microbes were dormant in the soil, or alive but barely scraping by, during times of more relatively moderate environments. When things started to get more acidic, when the Rio Tinto started pulsing through the exposed rock for example, they kicked into high gear. "When the environment changes, those organisms can out-compete," she explains.

Or maybe neutral-condition-loving microbes had secret genes locked away, only to be revealed under exposure to acidic conditions, like X-Men-like superpowers. These neutrophiles may have co-mingled with other nearby microbes, blending their traits to create acidophilic superbugs. Many species of acidophiles have similar DNA to neutrophiles, lending some weight to this idea.

The answer could be blowing in the wind. Jones suggests that the microbes hitch rides from place to place on strong gusts. Statistically speaking, he says, there are so many acidophiles and they are all so small that they are likely to get airborne at some point. "Millions of these things transported by dust," he says. "But that's only one thought," he qualifies.

This could explain why similar species of acidophiles are seen around the world. The rock star genus of acidophile is the *Acidithiobacillus*, including the popular species *ferrooxidans* which eats iron and sulfur, or *thiooxidans* which focuses solely on sulfur munching. They're in all the microbial tabloids – everyone wants to study them. Their popularity overshadows their many equally successful acidophilic cousins. Both types of *Acidithiobacillus* have been seen and studied in sites like Red Eyes or Rio Tinto so frequently that Jones refers to them as "classic acid mine drainage lab rats. [It's] to the point where it's such a well known lab bug that it's kind of the go-to bug for studying acid mine drainage."

But they're not only found swimming around acid mine drainage sites. Jones has seen *Acidithiobacillus* represented throughout the extremely acidic Grotte di Frasassi, a natural system of sulfur-rich caves yawning in the Italian countryside on the upper calf of the boot. He's been taking a close look at these things called snottites — and yes, they look like what they sound like. This cave in Italy is covered with a pink boogery biofilm, and it turns out that the snottites are there thanks mostly to good old *Acidithiobacillus*.

"It seems to be really the only bug that is consistently present in snottites," he affirms. "And you can see it just oozes biofilms."

But snottites don't reside solely in Italy; they dangle from cave ceilings worldwide. Jones has compared some of the life forms pulled from the Italian cave to microbes found in Mexican caves with equivalent conditions. He wanted to determine the intercontinental relationship between the different microbial species producing the snottites. He examined how closely the two species, separated by thousands of miles, were related. Were they siblings or distant cousins? "It's a controlled natural experiment," he says. The environments are similar but the species are different.

Jones dove into the bowels of these steaming acid caves, scraped up slime samples, and found that these species, on two separate continents, are functionally identical. They do similar jobs, fill the same niches. But it turns out they were somewhere around third cousins; analysis of their DNA showed that they came from the same stock. But their genetic code differed enough to signal an evolutionary split some time in the distant past, which is why they became two separate and distinct species. This split probably happened when one group of acidophiles changed their locale.

"Isolation is a key part of evolution," Jones says. Give a species enough time and space and it'll develop into two similar but distinct groups of animals. This evolution-by-isolation is responsible for war-like chimpanzees and peace-loving counterparts, bonobos. As for acidophiles, similar but not exactly identical species of *Acidithiobacillus* crop up in different places around the world doing all the same things. Soaring off from Italy, they dropped off some of their buddies as they passed through on their way to Mexico, or perhaps vice versa. Either way, each new population evolved separately to master the same positions in their own ways, similar to the differentiation of the Romance languages. There's French, Spanish, Italian — all stemming from Latin. They get the same job done, but in slightly different ways.

Jones is trying to pinpoint the moment of the genetic split to further understand the distribution and evolution of the population in general. So far, he has seen a correlation between distance and genetic relation — the further apart the caves, the more distant the acidophilic cousins.

Though one version or another of *Acidithiobacillus* seems to dominate all the snottite communities in caves worldwide, they are not working alone. Plenty of other species of acidophiles are active participants. Working together, they produce the slippery, pink biofilms drooping from the cave ceilings like stalactites. Like at the Rio Tinto, the community is expansive. Down one street beyond the sulfur snottite neighborhood reside groups of microbes that eat sulfur and produce acid. Across the way some other microbes feast on the waste product of their upstream associates. Lining one microscopic cul-de-sac are those usually inclined to indulge in iron. But these sulfur caves leave much to be desired in that regard, so they adjust their metabolisms to get their nutrients from all the organic stuff that's oozing from the snottite. Nutrients cycle through this sulfuric suburbia and no one goes hungry.

In other ecosystems, *Acidithiobacillus* isn't even the big man in the acid pond. "In reality, it's not necessarily the dominant organism," says Jones. In fact, there are some acidophilic algae, photosynthetic stuff, which tends to perform better under extremely high acid situations. Microbes are always in competition with each other for important resources. Higher temperatures might ensure the success of one species of acidophile. The temperature drops, and it's another acidophile's turf. Just like a jungle, there's a food chain, and many different kinds of acidophiles are needed to maintain order. The diversity in ability to survive is staggering; these communities are as huge and dynamic and beautiful as any macroscopic ecosystem.

As we sped down Pennsylvania's Interstate 99 on our way to the sites, I noticed a series of rock piles along the highway at regular intervals. While I was thinking they were a failed art exhibition, Bill Burgos started telling me a story about a road cut gone wrong.

In 2003, the state was in the middle of a huge project, extending the very highway we were traversing. But workers came across a pretty big road block. They unearthed a huge section of pyrite, the very fool's gold rock that acidophiles like to turn into sulfuric acid. Soon enough, water, oxygen, and acidophiles got to work, and the state had a problem on its hands.

It "created gnarly, gnarly acid mine drainage," Burgos enthused, which set them back ten years and billions of dollars, all because of some little bits of pyrite. The rock piles I had noticed on the side of the road were pebbles of limestone covering the pyrite. Limestone is a base, the opposite of an acid, and the road engineers laid it out in the hope that it would counteract some of the corrosive effects of the acidic water.

These acid mine drainage problems, the build-up of toxic metals and high acidity stemming from certain exposed rocks, are frequently seen around old, abandoned mines, particularly in places across the mid-western United States. Both Upper and Lower Red Eyes arose thanks to local strip mines. Pyrite is the usual culprit. Gashes in the earth expose the rock to the hungry microbes, which use the pyrite's sulfur for energy, leaving the leftover iron to coat the stream bed in thick, rusty layers.

But not all acid on the Earth is man-made. As with Yellowstone, sulfur caves, and the baby beginnings of the Rio Tinto, acidic environments can crop up naturally. Tom Brock noted the acid-caused destruction of the landscape near the sulfur-rich, acidic pools of Yellowstone in a book on his research experiences. "During the 10 years that I was at Yellowstone, I actually watched several large rocks disintegrate, and the boulder [...] about the size of an automobile, split in two and collapsed," he wrote.

Rocks are solid; centuries-old stone castles still stipple the hills of Europe. Monuments persist in the dry winds of Egypt. These things last, but a little exposure to acid can cause boulders to simply crumble away.

"What do you guys think about acid?" Jones posed another question to the 10-year olds crowded around him in rapt attention.

"I think of fruit, like lemon!"

"Yeah." He held up a steely gray hunk of rock. "This is a chunk of limestone, like most of the rocks around here. What happens when limestone comes in contact with acid?"

"It'll melt?"

"It'll melt, it'll dissolve, it'll break up. So everybody grab a cup of lemon juice. Lemon juice is highly acidic."

"Can I drink it?"

"No, don't drink it, it's really gross," he laughed. He gestured to pieces of blackboard chalk I had just finished smashing apart with a hammer. "Just everybody take some chalk here. Chalk is limestone. What do you think is going to happen when you put it in?"

An eager voice chimed in. "It's gonna dissolve!"

"Let's try it out." Jones watched, pleased, as the kids plunked their chalk chunks into the Dixie cups. Immediately, white bits started flying off the piece, carbon dioxide bubbling up and out like it was about to boil away. Listening closely, there was a faint fizz.

Giggles and gasps made their way around the circle — "It's like Alka-Seltzer!"

Jones hefted up the limestone again, this time with a little squeeze bottle in hand. "Here I've got some more concentrated acid. This is the type that's in your stomach; it's called hydrochloric acid."

"It's inside us right now too?"

"And it's pretty strong, not as strong as this," he indicated the bottle of hyper-concentrated hydrochloric acid, "but it's helping you digest your food. Now, ready?" He squiggled a line of acid across the surface of the limestone. There was an audible hiss as the acid snapped the bonds holding the surface molecules of the rock together and a white foam formed along the top.

"Oh my gosh!"

"Whoa!!!"

"It just goes away!"

"It's like that thing that spies do!"

"Oh yeah, spies and geologists are pretty similar," Jones quipped.

What is it about an acid that makes car-sized rocks crumble, that makes limestone fizz before our eyes?

It's all about the protons, the tiny bits of matter at the center of all atoms – specifically the proton at the center of a hydrogen atom. Hydrogen is a pretty simple element. At the center of its atom is one positive proton, which is orbited by only one electron — a tiny, negatively charged particle.

The atom of one element links up with another element by trading electrons. To secure the meet-up — seal the deal, so to speak — one element can lend electrons to the other, like a neighbor borrowing sugar. The element that donates the electron is left with a positive charge, and the borrower gets a negative charge. When a hydrogen atom donates, all that's left is one positively charged proton.

These protons are key; they're what make an acid acidic. When acids hang out in water, they release their hydrogen atoms – which, because the atoms already gave away their electrons, are essentially protons. The higher the number of protons packed in each cubic centimeter, the more acidic the water. Acidity is measured on the pH scale, a gauge that ranges from about 14 to less than 0. Clean, pure water comes in at a neutral 7. Bases, the opposites of acids, including ammonia and bleach, are on the alkaline side of things and register in the upper part of the scale. Acids are anything below a pH of 7. Acidity rises logarithmically as the numbers on the pH scale start to fall, so pH 6 (milk) is ten times more acidic than pH 7. The pH 5 of black coffee is ten times more acidic than that, and so on down the scale.

Floating in a fluid, an acid's excess protons work themselves between molecules of other matter, like limestone. The protons force the elements making up the rock to interact with them instead of the other elements they are supposed to be hooking up with. Rocks aren't alive, so when these extra protons come smashing through, all the rocks can do is break apart – there's not really much they can do.

So, how can microbes exist under such a horrifying onslaught? If their cytoplasm builds up too many protons, organelles will start to break down. But over the past couple billion years of existence, acidophiles have gotten pretty good at figuring out what to do with those extra protons. They've managed to come up with ways to keep their cytoplasm as neutral as water while they swim in acid, sometimes in a pH as destructive as 0, which has ten million times the proton concentration found inside their cytoplasm.

All cells have the capability to filter out protons through one way or another, but acidophiles are the masters of their craft. They've had time to hone their various skills to perfection. "From an evolutionary perspective, it's not that difficult," says Linda Amaral-Zettler, the Woods Hole researcher. It was just a matter of figuring out how to make what they already had a bit better.

The combinations of skills used by acidophiles are still not fully clear to researchers, but they have narrowed the methods down to several candidates — some exclusive to certain types of life, others common in all cells. "It might be that these organisms are using a variety of methods for doing it," says Amaral-Zettler. "There doesn't seem to be one overarching mechanism that's obvious."

All cells have the ability to shuttle protons across their membranes, usually to aid in the generation of energy. Because acid is an excess of protons, acidophiles have upgraded this mechanism to include a filter system that helps to clean out the cell's insides. Recall that certain proteins help to push protons out of the cell, creating energy in the meantime. In acidophiles, that mechanism is in overdrive, ejecting not just protons but dangerous acid-soluble metals that can also make their way into the cell's environment.

Acidophilic cells expel protons at a rapid rate. As a result, they are making a lot of energy. But they are also using a lot of energy, to keep up this high pace. "There's a tradeoff [for] being able to survive in an environment where others can't," says Amaral-Zettler. And that tradeoff is expending all that energy just to keep things acid-free. Amaral-Zettler and her colleagues believe acidophiles ease the tax on their energy supplies by combining multiple acid-clearing methods.

One such protective mechanism is found in many species of bacteria, from normal to extremophilic. Tucked inside the membrane of bacterial cells sits a series of five little rings bound together. They are hopanoids; plants and animals have similar structures called sterols. They act as a gatekeeper to the cell — they decide what can get in, and how much of it is allowed. Researchers are using what they know about hopanoids to understand how they manage to keep acidophiles alive. They have seen that, like bouncers at an exclusive nightclub, hopanoids in acidophiles have taken their job to a whole new level.

Paula Werlander, a post-doc at MIT, is paying close attention to these structures to figure out what makes them tougher in acid-lovers. "What environmental conditions induce them to be expressed?" she is asking. The bacteria making the hopanoids are very diverse. In acidophiles, it seems like they're primarily used to keep out an influx of unwanted protons. "They're maintaining membrane impermeability," she says.

The story of the creation of tougher hopanoids must have exactly paralleled the history of acidophiles themselves. Early in their evolutionary chain, some bacteria must have been exposed to acidic stresses. Some cells' membranes let the protons pour in, their cytoplasm turned acidic, and the cell became sickly. "You have an imbalance [of protons] ...and the cell can't respond that well," Werlander explains. A successful few (the eventual acidophiles) had stronger hopanoids imbedded in their membranes, arms linked in preparation for a miniscule game of Red Rover. Some protons rammed their way through, but just enough to power the cell's normal energy production. This mechanism improved over time, giving us the acidophiles that thrive in human flesh-eating conditions today.

In one experiment, Werlander removed hopanoids from an unsuspecting bacterium to see what difference it would make in its daily life. She saw that without its line of defense, the bacterium was more sensitive to everything that came its way. Now it's a matter of figuring out which types of hopanoids are weakened in which kinds of environments to better understand the evolutionary pathways of these barriers.

As another defense, some acidophiles will tuck protons away where they are useful, onto a buffer molecule like a bicarbonate molecule. Bicarbonate is a base, meaning it's ready and willing to accept the protons that are being tossed around by acids. Such molecular bases are used to soak up the excess protons everywhere from inside cells to inside the human stomach, which is itself home to its own brand of acidophile.

Some acidophiles have integrated these methods into their being so thoroughly that they now need the acid to survive. Because of their extensive adaptations, neutral environments will kill them. Generally speaking, acidophiles are most comfortable sitting in a pH of about 2.5, and they'll make do anywhere between pH 1 to pH 4. One acidophile, however, announced in 2000, broke records with its ability to survive at pH 0. *Ferroplasma acidarmanus* was found in an old California copper mine suffering from abandon and sulfuric acid. This dominating microbe at Iron Mountain near Redding, California, prefers baths at pH 1.2, but can still perform adequately at a greater acidity than the stuff of car batteries. Since then, an even more acidophilic organism was introduced — *Picrophilus*. It survives in a negative pH, but, more amazingly, if this microbe is exposed to anything higher than pH 4, its membrane will disintegrate and it will die.

"[People] think '...God, they must be barely hanging on, must be struggling, just barely making it,' and that's not what's going on at all," says Michael Madigan. These organisms were made for those conditions. They thrive in them, they love them.

This amazing ability to adapt to almost all (indeed, some think virtually all) cases of acid has led to a great diversity. Tom Brock notes that Yellowstone alone has a huge number of acid-loving microbial species. But the more the team focused on the microscopic, the more they realized that the little guys were not alone. In some cases, they seemed to be the base of a relatively complex ecosystem.

In his exploration, Brock found a type of purple algae layering some of the acid ponds. He called it *Zygogonium*. The *Zygos* clump together like algae. They're purple on top, to better deal with the high levels of ultraviolet radiation they're subjected to daily, sort of like a sun tan. Below the top layer, they are yellow-green, like many other photosynthetic life forms.

The microbes forming these mats are acidophiles, cleansing their insides by throwing out protons. But nestled in their mats are the eggs of a fly — *Ephydra thermophila*. The flies buzz around the acid algae mats for their entire life span, munching on the purple beds. Their closely related cousins, *Ephydra bruesii*, are strictly confined to neutral environments. Any exposure to acid is lethal. But *E. themo* persists. And on the shores of these acid lakes, feeding on the acid flies, are acid birds, acid spiders, acid beetles. These animals and insects aren't true acidophiles — they don't need the acid to live — but they are surviving in an ecosystem completely inhospitable to their relatives.

After a long day of getting 10-year olds excited about rocks, Jones was about to show me some acidophiles up close and personal. He moved quickly through the lab while I sat in a corner of a darkened room that housed two huge microscopes hooked up to computers. He was collecting all the odds and ends we'd need to zoom in on the microbes and see what was in the samples we collected during our field trip.

"No food or drink in the lab," he said, winking as he gulped his open cup of coffee. "Safety first." He smiled as he set the cup down and motored back into the other room to grab more equipment.

He gathered together the test tubes full of the samples we had scraped up the day before, some from the pyrite-rich Upper and Lower Red Eyes, and a few from a sulfur-heavy environment quite suitably named Sulfur Run. We hunched over a lab table and carefully squeezed our specimen into little trays — three rows of circles surrounded by yellow Teflon to prevent the samples from bleeding together. Each pocket was carefully labeled with date and location in Jones's large grid notebook. Then, we bathed each sample in special molecules that bound to DNA. When viewed under fluorescent lights, the molecules would glow in a unique color for each species of acidophile. Four probes — green, red, orange, and blue. Then, it was finally time to slip the slide under the lens to see what we were dealing with. The acidophiles glowed for the camera, and we took their picture.

It was a breathtaking firework of color. I immediately thought of the Hubble Deep Space field, the overwhelming mosaic of colorful galaxies extending beyond the horizon. As we viewed sample after sample, Jones kept murmuring under his breath. "There are all betas, man, just a handful of gammas," or "there are thin wiggly things, and fat helicoptery ones" and "that one appears to be dividing." There were biofilms, big green three-dimensional blobs in the lens. There were *Euglena*. There were *Ferrovum*, gammas, betas, *Acidithiobacillus*. Twenty percent of one slide was made up of "mystery green blobs that we don't know what they are," Jones laughed. The diversity in each two milliliter sample was staggeringly beautiful.

This diversity is important not just to the survival of acidophile communities, but to those hoping to harness the incredible power of the microbes for their own benefit. Acid mine drainage is more than just a convenient place to grow a lot of microbes. As strangely beautiful as the environments are, water from the sites is dangerous when it gets into local water supplies, as was the case with the last stop in our field trip — Sulfur Run.

Sulfur Run was born fifty years ago when an old, abandoned mine shaft began to flood. As the water pooled in the gaping hole, it came in contact with the pyrite and sulfur and got highly acidic. That acid started eroding out heavy metals detrimental to the health of most organisms, particularly humans. Eventually, the toxic waters began to trickle through underground pathways and into local water tanks, polluting the locals' drinking water.

The state of Pennsylvania recognized the problem and sent a crew of engineers to solve it. To remedy the situation, they punched a hole in the ground near the mine and siphoned the acidic water up and out, sparing the community water supply. Unfortunately, the resulting 1,000 gallon-per-minute flow out of the borehole flooded into a close-by creek. The near-pH 3, heavy-metal-saturated water has been coursing into the steam ever since. Where the two flows join is a definitive line — one side is blue green coming in at a respectable pH just below 5. The other is a rusty orange color typical of acid mine drainage, with reading of pH 3.6. From this point, the blended waters surge for eight miles as an unhealthy dusty desert color at a pH of 3.77.

Eight miles isn't close to the longest length affected by acid mine drainage. The Rio Tinto, for example, runs 62 miles. But no matter the length, the acid and metals destroy whatever environments they come in contact with, decimating everything from trees and plants to fish. Acid mine drainage is considered to be the worst environmental disaster in the eastern United States, and in many countries around the world. A number of clean-up methods are being bandied about, and right now the front-runner involves filtering affected sites with blocks of limestone. Engineers hope the base will soak up some of the extra protons that are making the water so acidic. The limestone works, to a point. The acidic water flow gathers oxygen as it cascades across a landscape. By the time the water hits the limestone barrier, it is so saturated with oxygen that it starts to push dissolved iron out of the water and on to the filter. Instead of the acidic stream flowing through the pit of stones, the limestone pores "get all clogged up and full of the orange precipitates," says Burgos. "The water just skims over the top and misses the treatment completely."

To solve this problem, environmental engineers, including Bill Burgos, are looking into an innovative and inexpensive solution that uses what is already at home — the acidophiles. Burgos is working mostly with acidophiles that live on iron. These microbes grab an electron from the dissolved iron and force it to shift into a solid, mimicking the precipitation process taken on by the oxygenated water flowing downstream. The iron comes crashing down to the ground far upstream from the limestone filters, allowing those filters to do their jobs uninhibited. But there's a catch; as the iron shifts from being dissolved in the water to a solid on the stream bed, it eats up any alkalinity in the water, meaning it drives the environment towards becoming more acidic. "So even though we've removed iron, we've lowered the pH of the water...which means this stuff absolutely needs to be run through limestone," says Burgos.

"We're trying to better understand the process to exploit it for treatment purposes," says Burgos. He is focusing on the Red Eyes for now, and the sites' two main iron eaters — those bacteria Jones was looking at under the microscope, *Ferrovum*, and the ever-present *Acidithiobacillus*. So far, he has seen that the more iron dissolved in the water, the greater the occurrence of *Ferrovum*. But at a certain point iron levels get too high for *Ferrovum*, and it starts to decline. That's when *Acidithiobacillus* takes over. Jones and Burgos are trying to figure out which species is better at forcing iron into a solid. Once they determine a winner, they will try to engineer a way to use that information to their advantage.

So far at Upper and Lower Red Eyes, Burgos has seen that by the time the water flow gets to the limestone treatment, the concentration of dissolved iron has been cut in half. "So that's a huge amount of treatment and a huge cost savings with respect to otherwise trying to deal with that clogged and armored limestone system," he says.

Next step is to see if this same model is true for other sites similar to Red Eyes, starting with other acid mine drainage systems in the Pennsylvania area. For this particular *Ferrovum* vs. *Acidithiobacillus* model to work, the sites need to have comparable distributions of microbes to those at Red Eyes. "We want to know to what extent geographically separated [sites] have different microbiota just because they're geographically separated," as opposed to completely chemically different, Jones says. Right now, they can't assume that all acid mine sites will have exactly the same microbial communities, and figuring this out definitively has important implications for future bioremediation strategies.

Thousands of miles away at the Rio Tinto, Amaral-Zettler's colleague, Angeles Aguilera, is working on a different bioremediation technique involving acidophilic biofilms. She is studying their capacity to act as a road block to heavy metals. Some of these gooey secretions hold back the harmful minerals, preventing them from flowing further downstream. Aguilera wants to develop a method of spreading this capability along the entire length of the river.

While acid mine drainage is a major environmental problem in many parts of the world, the group of people studying microbial clean-up is not large. "There ain't many [of us]!" Burgos says. He estimates that only a couple hundred people worldwide are looking at bioremediation. "But that's exciting because if we do good work, we'll get recognized for it," he says.

This community studying methods for cleaning up acid mine drainage doesn't represent the sole effort to manage microbes to suit the needs of humans. People have relied on acidophiles, knowingly or not, for thousands of years. Dating back before the ancient Romans, who harvested minerals from the banks of the Rio Tinto, societies have made use of the by-products of acidophiles' metabolisms. It was only a few decades ago, though, that people realized they could deliberately manipulate acidophiles' mealtimes and reap the benefits. In the mid-1980s the first mine that used acidophiles to yield minerals was put into operation. They called the process "biomining."

Biomining boils down to the basics of acidophile biology. Many acidophiles eat rocks and leave mineral by-products. Those by-products are what interest miners. If copper is melded together with a type of sulfur, acidophiles gorge on the sulfur and leave the copper. The same is true for blends of gold and sulfur, cobalt and sulfur, and so on. Miners realized that they could apply acidophiles' natural affinity for mineral separation to modern mining techniques.

Ripping minerals apart through traditional smelting requires large tanks filled with pressurized, heated air and chemicals. This intense atmosphere inside the tanks forces the electron transfer that acidophiles and other microbes do so well. When acidophiles are added to the mix, they organically initiate the mineral separation, using the same processes they employ in their own habitats, thus reducing the need for chemicals and high-energy.

Biomining comes in many forms. The methods and machineries can vary slightly, under the names of biooxidation and bioleaching; sometimes the process happens in large series of tanks, other times in mineral heaps. But the overall techniques are similar. Acidophiles are given a slurry mix of the ore, some liquid, and what nutrients they need to stay alive. They then go to work, eating what they like and leaving behind what the miners most covet.

Minerals separate from each other under varying conditions. Some fall apart at extremely high temperatures. Some need extremely low pH. BIOX, a reactor successfully used in South

Africa since 1986, operates best between pH 1.2 to 1.8. Acidophile diversity has allowed biominers to perfectly match microbes to minerals, optimizing their performances. Not surprisingly, various species of *Acidithiobacillus* have proven to be a strong force in many a biomining reactor.

Biomining can be used independently, but traditional and new methods are frequently paired. In that case, acidophiles act as a pre-treatment for the mineral ore, allowing the inorganic processes to work a little more efficiently. The usable metals left over in the reactors are then treated, purified, gathered up, and brought to market. Commercially, biomining is mainly used for yielding gold and copper, but it could theoretically be used to harvest any minerals locked together with an acidophile food source.

Mining plants and researchers around the world are refining the processes of biomining, from the ravaged Rio Tinto to the mines around Chile and South Africa, where the technique in its various forms has been implemented for about twenty-five years. "It offers a more environmentally friendly way of harvesting resources from the environment," says Amaral-Zettler, since using microbes significantly cuts down on the energy use and pollution of traditional mining and smelting.

The very existence and persistence of acidophiles around the Earth is helping researchers address questions on an even grander scale — how life started on our planet, or even what they mean for life other planets. "Life needing human conditions is completely false," Jones says. "Life didn't need to evolve under what we think of as normal conditions."

Early Earth was a tumultuous place. Volcanoes were spewing ash, chemicals, and various gases into the oxygenless atmosphere. Carbon dioxide ruled. It was a completely alien world. But as things calmed and cooled, one of those gases, water vapor, settled down and became liquid, contributing to the formation of the defining feature of our planet — our oceans.

And life arose. Probably deep underwater near pillars of hot chemical steam surging from far below the surface, without oxygen, without light — with nothing but the ability to eat chemicals to keep it going. These ecosystems still exist, and researchers have turned to these modern environments of extremophiles for answers to how ancient life could have survived. While these early systems may not have been completely acidic, understanding the niches within acidophile communities can provide valuable insight to early Earth researchers.

The realization that life is far more resilient than humans had previously imagined has led to the development of a field called astrobiology — the search for life off of our blue, mostly moderate planet. It's a fairly infant field (NASA's Astrobiology Institute was started in 1998), but it's growing fast.

"There are journals of astrobiology, there are people now getting PhDs in astrobiology," says Amaral-Zettler. "It's a pretty vibrant field, a pretty active field." Extremely diverse, as well. Amaral-Zettler thought she was immersed in the most interdisciplinary research possible as a graduate student studying marine microbiology. While she still researches ocean microbes, she was taken by the teamwork necessary within astrobiology. "It's sort of one level up," she says. "It's a vastly interdisciplinary field, from the origin of life to exoplanets. [There's] something for everyone if you're willing to work and think outside the box."

Studies of acidophiles and extremophiles in general are important to astrobiology. They show that the world is much bigger than humans are familiar with. "Conditions on the surface of the Earth are not the norm in the solar system," says Jones, who actually does his research in a lab funded in part by the NASA Astrobiology Institute. Fully understanding where life can not only manage, but dominate on Earth can help broaden the search for life, even microbial life, on other planets.

Lynn Rothschild is a very vocal extremophile researcher with NASA's astrobiology effort. She's written a number of reviews and articles published in a variety of venues on the terms and conditions life needs to form — and those terms are coming with fewer and fewer limitations.

"We're defining a minimum envelope for life," through extremophile studies, she says. "It may certainly be broader, but at least we know this is the minimum." Several decades ago, scientists thought Earth's acid sites were sterile voids. But now they know these pools not only sustain some life; they're the base of entire ecosystems. So now, researchers know not to count such places out. "If you find boiling battery acid on Venus or anywhere else in the solar system, it's worth checking," she says.

One of the places previously discounted for life, past or present, is our cosmic neighbor. Mars was assumed to be too cold and too dry. Its geology bears traditional markers of land thought to be too acidic to ever have supported life. But in recent years, opinions about the potential habitability of the planet have shifted.

“We have this remarkably new picture of Mars,” says Andy Knoll, a bespectacled Earth and Planetary Sciences professor at Harvard. He’s part of the joint MIT/Harvard Astrobiology group, and he is trying to figure out how the Rio Tinto formed and changed throughout the last couple billion years. He focuses on comparing how well the Earth site’s chemical composition matches Martian dirt. He says that over the last several years, a study of Martian geology has shown that though it wasn’t abundant, there was water on Mars.

“It’s been cold and dry and awful for most of its history,” he says. That part is true. Some of the minerals on the planet’s surface are 3.5 to 4 billion years old. They’ve been there since the planet was an infant. If those particular rocks were covered in vast oceans for a couple billion years, they would have weathered away fairly quickly. The fact that they did not means water on Mars was not long-lived. It was around for only a few brief, shining wet moments in its past. Mars “couldn’t have seen more than [a few million years] of water,” he says. But it was there, and that means life could have been there as well.

In the hopes that researchers will be well prepared for any future survey of Martian life, microbial studies are being conducted all around Earth in locations that encapsulate some of the most (previously assumed) restrictive characteristics of the red planet — in dehydrated deserts, at the subzero poles, and at the pH 2 Rio Tinto.

The acidophile community analysis being done at Rio Tinto by Amaral-Zettler and other associates of the Astrobiology Institute is bolstering hope that if life can survive, it will. Scientists are so hopeful, in fact, that NASA has planned a new Mars rover called the Mars Science Laboratory to be launched in the fall of 2011. The project’s main purpose is to get the lay of the land and figure out exactly what Martian soil is made of, what chemicals are there that could support life.

In anticipation of such Martian missions, Amaral-Zettler is compiling a library of biological signatures unique to microbial life at the Rio Tinto. Her records can be compared against any potential sign of life that might be found on Mars. Certain fats found in cells are so recognizable that if they were found in any capacity on the red planet, they could be distinguished using listings like Amaral-Zettler’s. “We want to produce a catalogue of things one might...see, [or] fossilized remains of what was once alive,” she says. “We’re not only interested in extant life, but extinct life or life that is no longer present… [the library could be] a mechanism for looking back in time.”

But there are some questions as to whether or not such a library will be applicable. Even if the chemical composition of Mars' dirt has all the ingredients for life, that doesn't necessarily mean it is there, or ever was there. As tenacious as acidophiles can be, there are some conditions even they can't survive. Regardless of the combinations of methods acidophiles apply to keep extra protons out of their system, "there's a cost," says Amaral-Zettler. "And the cost is to keep your internal pH [neutral]. You want to be able to survive and out-compete, but not to the point where [you need to use all this energy] to do so." So at some point, when the pH plummets too far below 0, there will be no life.

Understanding this limit will help to inform studies of life on Earth and beyond. "The more we can learn about life living in the extremes on Earth, or at least the extremes from our perspective, [the better]," Jones says. "It's more likely the extremes on Earth are going to be the norm on other planets."

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I smelled Sulfur Run before I saw it. As we approached the final destination of our freezing cold January field trip through Pennsylvania acid mine drainage sites, I got a nice nose-full of that distinct rotten egg smell. I was expecting another Red Eyes — rusty dirt under sheets of ice dotted with dead trees. We reached the borehole gushing out sixteen gallons every second and I saw green, everywhere a vibrant green rich enough to rival the best maintained summer lawn, swimming in water hovering around pH 3.5.

Long ago, sulfuric acid was known as vitriol and sulfur was brimstone. With all the associations of hell and hate cast upon it, sulfur and acid were written off as lifeless. But here, fertile algae-like life clung to rocks in tight clusters where the water was softly drifting through; it streamed in long banners where the hard flow pulsed down into the nearby stream. Surrounded by rich red iron coated rocks, it was an otherworldly, oil painting oasis surrounded by the barren, dead forests of winter.

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