Live wires: The electric superorganism under your feet

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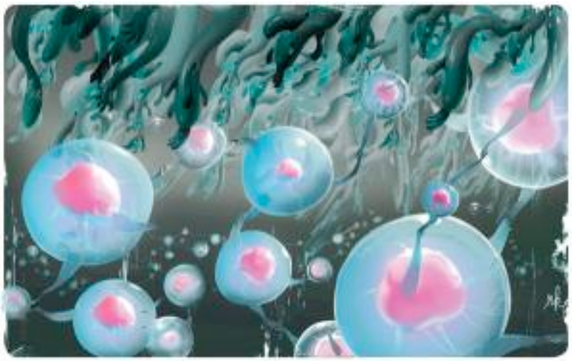
Life would be tough for lonely soil bacteria. That's why they wire themselves into their very own electricity grids



Living the life electric (Image: Ronald Kurniawan/Debut)

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READ this sentence, then pause awhile. Take a moment to listen to the soft rhythm of your breathing and the regular beat of your heart, the outward signs of the evolutionary wonder that is respiration. Lungs, diaphragm, heart, veins, arteries and the intricate molecular apparatus of our cells: all that kit working in concert to deliver one molecule - oxygen - to where we need it.

Keep that in mind as we now travel down into murky worlds beneath our feet. Here, in rust-red soils, sands and underwater sediments, oxygen is as scarce as daylight. And yet life, in the form of bacteria, respires and survives here too. How? As we slowly unravel the principles and apparatus of this other respiratory wonder, we are catching glimpses of a whole new model of life - a world where electricity is the base currency, where success means being plugged into the network, and where our cosy and convenient divisions of life into autonomous species rapidly become meaningless.

For most denizens of Earth's surface, oxygen is life. That is all down to a bald chemical fact: the element is a powerful "electron acceptor", readily sucking up electrons set free when enzymes within living cells break down organic foodstuffs. This oxidation process releases energy that fuels the organism.

Oxygen itself is chemically "reduced" in the process, taking up the liberated electrons and using them to form new bonds and molecules. For animals like us, the abundance of this chemical chameleon in Earth's atmosphere gives us the autonomy to move, forage and hunt without worrying where our next breath is coming from. Plants, too, rely on oxygen's omnipresent muscle to liberate the energy stored in their cells and power their growth.

Although aerobic respiration, using oxygen, is the most obvious choice for life on Earth, it is not the only option. Other chemical species, including sulphates, nitrates and some forms of iron and other heavy metals, have a similar hunger for electrons. Indeed, within the past century it has become clear that oxygen is toxic to hundreds of species of bacteria, which spurn it in favour of other respiratory tools such as dissolved sulphates and nitrates within their cell bodies. And in the late 1980s, researchers dredging up oxygen-poor lake and river sediments in the US found bacteria that respired entirely without the aid of oxygen or other internalised molecules. Instead, they dumped the waste electrons of respiration onto iron flakes in the surrounding sand.

Out-of-body experience

This was a challenge to established beliefs, says Kenneth Nealson at the University of Southern California in Los Angeles, who found one such iron-reducer, *Shewanella oneidensis*, in the sediments of Lake Oneida in upstate New York. "No one wanted to accept that bacteria could hand over electrons to a rock." Until then, respiration had been something that happened inside bodies, organs and cells. Now it seemed bacteria could engage in out-of-body respiratory experiences. One touch of an iron flake outside their cells and they had all the power they needed.

Shewanella turned out to be a particularly intriguing case. Reducing oxygen is its true métier, but deprive it of oxygen and it will reduce iron instead. In 2003, Yuri Gorby, then at the Pacific Northwest National Laboratory in Richland, Washington state, put *Shewanella* in a closed environment with scant iron and oxygen. By rights, the bacteria should have been slowly asphyxiated. They weren't. Instead, they started growing hairs.

It is not uncommon for bacteria to grow hairs. But why should *Shewanella* grow them at this point? Gorby had a hunch, and so he handed over a sample of the hairy bacteria to his colleague Svetlana Yanina. She is a dab hand at scanning tunnelling microscopy (STM), a powerful technique that uses electrons to probe surfaces at the atomic scale. In less than 2 hours she had the answer Gorby was hoping to hear. *Shewanella*'s hairs had an unusual feature: they could conduct electricity.

On the other side of the US, Derek Lovley's team at the University of Massachusetts in Amherst was seeing something similar. They were investigating iron-reducing *Geobacter* bacteria, scions of a genus that Lovley had discovered some 15 years before when dredging the Potomac River near Washington DC. *Geobacter* also grew hairs when placed in an iron-poor environment, and they too were conductive. What's more, when the team created a mutant strain that couldn't produce the hairs, it stopped reducing iron (*Nature*, vol 435, p 1098).

> When the bacteria were deprived of oxygen and iron, they should have died. Instead, they grew hairs

For Gorby, the implication was clear. As the bacteria broke down foodstuffs within their cells, they needed somewhere to dump the electrons or their respiratory cycle would grind to a halt and they would suffocate. Sensing the lack of any appropriate electron acceptor in their immediate environment, they grew the hairs as the electronic equivalent of a snorkel - a breathing tube down which they channel electrons to dump them on an electron acceptor elsewhere.

At this point, though, Gorby and Lovley's teams had only managed to show that the hairs were conductive, not that they actually did ferry electrons all the way down their length. "Think of it like a garden hose," says Nealson. "Even though it's made of waterproof material, it could be full of holes. Until you test it from one end to the other you don't actually know that it'll move water."

Manipulating the fragile proteins that make up the hairs to provide a definitive answer proved a fiddly task. It wasn't until October this year that Mohamed El-Naggar of the University of Southern California, as part of a team that included both Gorby and Nealson, succeeded. He attached a pair of nanoscale platinum electrodes to either end of a hair and found that the flow was up to a billion electrons per second over a length of micrometres, more than enough to drive respiration (*Proceedings of the National Academy of Sciences*, vol 107, p 18127). "It was the conclusive proof that these are indeed 'nanowires', conductive appendages able to transport electrons between the living and the non-living world," says El-Naggar.

It is worth pausing to let this sink in. Not only do these bacteria "breathe" rock, but they do it by plugging themselves into their surroundings with the equivalent of a power cable. Whenever a *Shewanella* or *Geobacter* reaches out with one of its hairy nanowires, delicately taps a flake of iron and makes a connection, it kick-starts respiration within its central cell. Electrons start whizzing down the wire, offloading onto the metal (see diagram). For the bacteria, this point of contact is synonymous with life itself - like a gasp of air for us oxygen-breathers.

It is plausible that these nanowires are leftovers from when respiration first evolved 3 or 4 billion years ago, at a time when oxygen was rare but iron was relatively abundant. Cells with the ability to offload electrons onto iron particles through wires would have had a competitive advantage.

But that might not be all. Ever since Gorby and his colleagues first watched *Shewanella* grow hairs back in 2003, they had photographed colonies cultured in the lab. What they found was that the hairs didn't just connect cells to their environment, but also to each other. If these hairs were indeed nanowires, could they be a form of electrical network to maximise each cell's access to iron-rich particles - less a snorkel, and more a communal lung, or perhaps even more? If so, did such networks exist in nature?

> The tangle of wires was less a snorkel, more a communal lung - and perhaps even more

Even as the Californian team was chewing over those possibilities, news arrived of something rotting in the state of Denmark.There, microbiologist Lars Peter Nielsen of the University of Aarhus and his colleagues were puzzling over the anomalously fast rate at which hydrogen sulphide was breaking down in samples of mud from the nearby sea floor.

Hydrogen sulphide is oxidised by oxygen, which diffuses into the sea-floor sediments from the water above. It came as no surprise, then, that when the team removed the oxygen from the overlying water the reaction centimetres down in the silt stopped. The surprise was how the reaction deep within the mud instantly started up again when they bubbled oxygen back into the water - long before the gas could have reached down by diffusion (*Nature*, vol 463, p 1071).

All of this was easy to understand if a bacterial network existed that extended over centimetres, Nielsen surmised. Then bacteria at the bottom of the mud could break down hydrogen sulphide, extract energy in the shape of electrons, shunt those electrons through their nanowires onto other bacteria living in the oxygenated mud above them, who could then dump them onto oxygen molecules near the surface. Where bacteria could not respire themselves, the flow of electrons through the network would mean they could complete the circle of respiration simply by putting out feelers to their neighbours, powering the process through a sort of universal mains grid.

It was a nice idea, but lacking one thing: evidence. Nielsen simply did not have the tools to say for certain whether a nanowire network existed in his samples. Gorby did. When he got wind of the results, he invited Nielsen and his mud over to California to look at it using STM techniques. It is too early for definitive statements, but Nielsen says that he is very happy with the first results.

Two in a brew

In Amherst, Lovley's team is moving in a similar direction. At the annual conference of the International Society for Microbial Evolution in Seattle, Washington, in August, Lovley's student Zarath Summers reported what had happened when she and her colleagues had grown a mixture of two *Geobacter* species, *G. metallireducens* and *G. sulfurreducens*, in a rich organic brew of ethanol and sodium fumarate. In such an environment, both species are handicapped. *G. metallireducens* can break down ethanol but cannot dump electrons onto fumarate - in other words, it can eat but it cannot respire. For *G. sulfurreducens* the situation is reversed: it can reduce fumarate but cannot metabolise ethanol.

Cultured together, though, both species began to break down ethanol with abandon, suggesting they had some way to pool their resources. Sure enough, micrograph pictures revealed a network of nanowires connecting cells of both species. When Summers knocked out the genes that allow *Geobacter* to produce conductive nanowires, the mixed cultures were unable to break down the ethanol (*Science*, vol 330, p 1413).

In essence, it seems, these two species can live in electrical symbiosis, sharing energy, and life, simply by touching each other with their fine, conducting hairs. Nealson has shown something similar by using a metal wire to connect two colonies of *Shewanella*, one supplied with food and the other in contact with an electron acceptor. Maintain the connection, and both colonies are fine. Cut the wire and both die.

A wired-up world in which life is electricity and electricity is shared across colonies and between species? If such cooperation is possible between bacterial species - and even, as some of Gorby's experiments suggest, between bacteria and archaea, two entirely separate domains of life - maybe we need to reconsider where the boundaries of life lie. "We think of microbes as single cells," says El-Naggar, "but I question what the difference between unicellular and multicellular life is if cells can transfer energy simply by touching each other." Instead, we must start to think of such bacterial colonies as a single "superorganism", he says.

Such networks might be more common than we think. Nanowires have now been observed sticking out of bacteria that live at the bottom of lakes, rivers and the sea. In the lab, they grow out of species that live off iron, oxygen and methane, as well as some of the planet's oldest life forms, cyanobacteria. Gorby and his colleagues are now studying nanowires in bacteria that form the plaque on our teeth, and others that line our gut. They say they see "wire-like things" in everything from soybean roots to hydrothermal vents in the ocean's abyss. Gorby thinks nanowired bacterial networks might extend over hundreds of metres underground.

The existence of networks of bacteria pulsing electrons among themselves suggests other eye-opening parallels. In Nielsen's marine sediments, for example, placing more electron-hungry oxygen at the top would cause more electrons to be sucked up through the network, encouraging the breakdown of hydrogen sulphide by the bacteria at the bottom of the column. "Almost by definition, there is some form of information flow between one part of biosphere to another," says El-Naggar.

Gorby goes further. "I believe that there are electrically coupled biochemical processes going on in these microbial communities that are completely analogous to any brain chemistry that we know," he says. That does not mean an individual bacterium can think, any more than a single neuron can think, he adds. "But hook a few hundred trillion of them in an electrically integrated circuit, and the only limits are those of our imagination."

Superorganisms? Superbrains? Whatever the truth is, the bacterial abilities we are uncovering go beyond anything we credited them with. Time to take a deep breath.

Tapping the nanogrid

If bacteria under our feet are busy passing electrons around willy-nilly, could we use them to our advantage? The idea of microbial fuel cells has been around for a while. In sewage plants, for example, anaerobic microbes fed organic waste respire, producing electrons that can be collected on an electrode and harvested to generate electricity. Yet they are not very efficient.

Might some basic bioengineering build a better bacterial battery? Derek Lovley's team at the University of Massachusetts in Amherst reasoned that their *Geobacter* bacteria strains had evolved over billions of years to optimise iron reduction, not electricity production. But through months of systematically selecting the "fittest" bacteria - those that donated the most electrons to an electrode - they were able to produce a new strain 10 times more efficient at producing electricity (*Biosensors and Bioelectronics*, vol 24, p 3498). This new strain is far hairier, further evidence that the hairs' function is to ferry electrons away from the bacteria (see main story).

Oil from sunlight

Korneel Rabaey of the University of Queensland in Brisbane, Australia, believes the cost of making bacterial fuel cells means they will never be used on a large scale. He is more excited by the prospect of "electrosynthesis". This is essentially a form of artificial photosynthesis that reverses the direction of electron flow that occurs when bacteria respire and break hydrocarbon bonds.

One idea is for electrons generated by solar panels to be fed to a network of electrosynthesising bacteria, which use them and carbon dioxide from the atmosphere to form hydrocarbon bonds. Such a process could produce industrial organic compounds like caustic soda more efficiently and at a reduced cost.

Lovley has a loftier aim: converting carbon dioxide to the hydrocarbon fuel butanol. If the technique works, more complex hydrocarbons might be possible - raising the intriguing prospect of using bacteria and sunlight to make oil.

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