come to us? Why not go to them?" he asked. "We try to do stuff in many different venues and in fact, out on the street sometimes, in street carnivals and street demonstrations and so on. And take the science to where the people are. So I think there is a practical impetus behind festivals. It's all part of trying to integrate science with the wider culture."

The idea quickly spread. When the organizers of a San Diego Science Festival (www.sdsiencefestival.com) sought help and assistance from the Cambridge Festival, it eventually led to the establishment of the Science Festival Alliance. The US National Science Foundation (Arlington, VA) has joined the bandwagon and provides a 3-year US\$3 million grant to promote the science programmes. Festivals in Cambridge, MA, and San Diego, CA, have already received help from the Alliance and two others in San Francisco and Philadelphia, PA, will be held in 2011. Alliance manager Ben Wiehe, who formerly promoted science cafés for WGH's *NOVA* and who has a master's degree in anthropology from the University of Chicago (IL, USA) noted, "The Alliance supports anyone interested in starting a science festival or otherwise getting involved in supporting a science festival and with an initial focus in the United States."

"We're trying to make science part of culture"...

Durant, who helped write the grant to create the Alliance, said he hopes that the festivals will open a dialogue between the public and scientists: "I'm hoping we can encourage more people to see science as having something for them [...] The best events for me in festivals are where scientists and members of the community just end up having a conversation with each other either in the course of a larger event or off to one side of an event. We're trying to break down the barriers."

He added: "Many scientists have their work funded from the taxpayer in one way or another and many feel, I think genuinely, that it's part of their responsibility to pay back a little bit by participating in these events."

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Can bacteria save the planet?

New developments in systems biology and biotechnology to harness bacteria for renewable energy and environmental regeneration

Philip Hunter

Ever since the German physician Robert Koch (1843–1910) demonstrated in 1877 for the first time that a microorganism, *Bacillus anthracis*, could cause sickness, bacteria have gained notoriety as agents of disease and contamination with few saving graces. If anything, the approval rating of microorganisms has deteriorated even further in recent years in the light of the role we now know they have in complex diseases—such as stomach ulcers, heart attacks and strokes—and the intractable problem of bacterial resistance to antibiotics.

Yet, the reputation of bacteria might begin to improve dramatically, as they are likely to have a crucial role in solving a range of problems facing humankind: they might be engineered to provide alternative sources of energy to replace fossil fuels, clean up pollution from heavy metals and toxic chemicals, manufacture new materials from renewable sources and power a new generation of nanoscale machines.

Although few of these developments are close to fruition and significant challenges remain, the underlying science is blooming. "Yes, I think bacteria will be applied in [the] future for many vital applications," said Sang Yup Lee, head of the Metabolic and Biomolecular Engineering National Research Laboratory at the Advanced Institute of Science and Technology in Daejeon, South Korea. "In fact, bacteria have been studied intensively and extensively for the production of valuable and bulk chemicals, materials, and energy from renewable resources by engineering their metabolism."

The greatest value of this work so far has been the knowledge and tools created for the manipulation of bacteria at the level of the whole genome. Indeed, according to Lee, genome-wide optimization is the key to successfully engineering any bacteria for industrial applications. In this context, industrial bacteriology can

be seen as a proving ground for the knowledge generated by the emerging field of systems biology.

In the early days, as Lee noted, the genetic engineering of bacteria for industrial applications emulated evolution: random mutations in key genes were introduced and strains that were better suited to the tasks required—such as synthesizing a new compound—were selected. After several rounds of random mutagenesis and selection, a point of diminishing returns would be reached where further improvement would be increasingly hard to make and the resulting bacterial strain would thus be adopted for the process.

...genome-wide optimization is the key to successfully engineering any bacteria for industrial applications

However, as mutations in even a single gene invariably have multiple consequences for several metabolic pathways, the process of mutagenesis and selection inevitably created undesirable results. This in turn led to the idea of metabolic engineering, whereby the expression of several genes that participate in a given metabolic network would be optimized as a whole, either through suppression or amplification of their expression. Even so, such a technique is still a rather primitive implementation of systems biology. "Despite being systematic, this approach to some extent limits the outcome of strain improvement because the scope for engineering the cell is often local rather than truly system-wide," Lee commented. "[C]onsideration of the overall bioprocess, from strain improvement to its actual cultivation, is often neglected."

This is where systems biology has stepped in: its tools and protocols allow for the engineering of bacteria by making changes to genes or their expression level across the whole genome. This approach is called systems metabolic engineering (Park *et al*, 2008), which Lee defined as “an upgraded version of metabolic engineering with the aid of systems biology tools, not only providing systematic strategies for cellular and metabolic engineering but also elucidating strain-specific problems that are otherwise overlooked.” Such problems can extend to the host organism; for instance, if the aim is to produce a new product, or a product that is produced in higher quantities than occur naturally, the cell might either fail or might initiate feedback mechanisms to slow or shut down synthesis. System-wide approaches identify the genes involved in the feedback mechanisms and, through site-specific mutagenesis, attempt to turn them off. Alternatively, adaptive evolution can be used to expose successive generations of cells with the desired effect to the same level of toxicity to evolve cells with a higher tolerance. This selection process hopefully yields a strain that still produces the desired products in suitable quantities but is more tolerant of the toxicity.

Lee has applied this approach to the widely used model bacteria *Escherichia coli* for the production of biodegradable plastic—which is usually made from fossil

Systems biology will also have an important role in engineering bacteria for the sustainable generation of energy

fuels. The plastic made by Lee’s process, polylactic acid (PLA), which is made by polymerizing lactic acid, has been known for almost a century and is already used for food and compost wrappings. Until now, however, it has not been possible to produce PLA directly by bacterial fermentation. Bacteria do produce lactic acid, but polymerization into PLA requires an additional catalytic step to prevent degradation of the polymer by water molecules formed during the process. Lee has succeeded in engineering *E. coli* to perform this step themselves in a single fermentation process by utilizing polyhydroxyalkanoates (PHAs) as copolymers that are produced naturally by the same bacteria (Jung *et al*, 2010). The result is a potential industrial process for the direct production of PLAs that does not require additional chemical steps outside the fermentation system. This system alone, however, will not provide acceptable alternatives to petroleum-based plastic for all its current applications. Although PLA has desirable properties, such as biodegradability, sustainability and low toxicity, and is suitable for some household, commercial and biomedical products, it is too stiff and brittle for

other purposes. Lee has therefore shown that bacteria can be engineered further to manufacture plastic with different properties by combining PLAs with PHAs and other polymers (Yang *et al*, 2010). “[C]opolymers containing lactate and other monomers were produced again by metabolic engineering to solve some of these problems, and provide more opportunities,” he said.

Systems biology will also have an important role in engineering bacteria for the sustainable generation of energy. The use of bacteria for energy production has a huge advantage over the use of plants, as they do not compete with food crops for arable land and because, in principle, they can yield self-replicating bioreactors with the potential to manufacture fuels in a continuous production line. In fact, bacterial fuel production is already near the product stage: the biotech company Amyris (Emeryville, CA, USA) has pioneered the use of bacteria to produce biofuels from plant biomass, such as sugarcane, and plans to enter the market within the next few years.

However, Amyris’s use of sugarcane as its raw material still competes with food production. An alternative strategy might involve harnessing the photosynthetic capability of one particular bacteria phylum, cyanobacteria, and adapting it to produce storable fuels such as hydrogen, or

hydrocarbons from sunlight, water and carbon dioxide. Modified cyanobacteria could even become the raw material for solar panels to directly generate electricity rather than fuels, according to Halil Berberoglu from the University of Texas at Austin, USA, whose background in the physics of heat transfer has led him to research in photobiological fuel production.

One major challenge of efficiently utilizing cyanobacteria in solar panels is the removal of waste material—given that solar panels will often be practically inaccessible after installation—but a more fundamental challenge for bacterial fuel production concerns the efficiency of energy conversion—that is, the ratio of energy output to input. Berberoglu explained that compared with current photovoltaic cells, which convert up to about 40% of solar radiation into electrical energy, bacteria use only around 8% of the incident radiation to synthesize carbohydrates.

Modified cyanobacteria could even become the raw material for solar panels to directly generate electricity rather than fuels

This poor efficiency is explained in part by the evolutionary history of cyanobacteria: they evolved in relatively dark places without direct access to sunlight and so became efficient at harvesting any available light through dense packing of chlorophyll. Accordingly, they had to evolve mechanisms to avoid photodamage when exposed to full sunlight. “They produce more pigments than necessary in good light to maximize the probability of capturing all the available photons,” Berberoglu said. “Therefore, when they are irradiated with full sunlight, they absorb more light than they can utilize and waste this energy as heat and/or fluorescence. This also causes them to ‘shut down’ temporarily or in some cases [they] can be irreversibly damaged.”

Again, this is where systems biology is able to step in. The bacteria can be engineered with a reduced chlorophyll content to allow exposure to higher radiation levels without inhibiting energy production. There is also the possibility of engineering bacteria to produce hydrogen directly by breaking the chemical bonds in water and cutting out the intermediate step of carbohydrate production, according to

Berberoglu. Although both plants and bacteria already ‘split’ water in this way, they generate separate streams of protons and electrons instead of producing hydrogen. The electrons act as the carriers for energy that is ultimately converted to glucose by reducing carbon dioxide. A main focus of solar energy conversion research is therefore to engineer bacteria that combine protons and electrons into hydrogen after splitting water. Fortunately, the biochemistry to do this already exists in the form of the hydrogenases, some of which are capable of yielding hydrogen from either water or organic compounds. Berberoglu pointed out that, in practice, future bioreactors that produce hydrogen or other fuels might not be made from bacteria after all, but instead might comprise artificial systems that mimic the underlying chemistry, as this would prove more efficient and avoid the problem of waste removal.

Another major sector of applied bacteriology concerns magnetotactic bacteria, which contain magnetic iron crystals—made either of magnetite (Fe_3O_4) or greigite (Fe_3S_4)—inside membrane-bound organelles known as magnetosomes. The organelles are hooked together in chains and traverse the whole cell from pole to pole (Frankel & Bazylinski, 2006), according to Richard Frankel, professor of physics at California Polytechnic State University in San Luis Obispo, USA, whose research focuses on the role of iron in microorganisms.

This internal compass enables the cell to align itself with the earth’s magnetic field when it swims (Lefèvre *et al.*, 2009) and helps it to find optimum concentrations of oxygen and nutrients within a sediment. Most bacteria accomplish this by chemotaxis: swimming almost randomly until they reach a location with an optimum concentration of the desired nutrient. Within a sediment, however, chemotaxis is highly inefficient because the individual cells are buffeted continually by neighbouring molecules in Brownian motion and lose their heading, according to Frankel. Magnetosomes thus enable the bacteria to maintain their alignment by reference to the earth’s magnetic field. “The cell functions as a kind of self-propelled magnetic compass needle,” said Frankel. “Most magnetic bacteria are found at the oxic/anoxic interface (OAI) in their habitat, and swimming along the geomagnetic field increases their ability to find and remain at the OAI, as well

as allowing them to efficiently traverse the interface in order to sequentially encounter higher concentrations of electron donors and receptors.”

Magnetotactic bacteria have potential uses in a range of applications, from waste removal to drug delivery

Magnetotactic bacteria have potential uses in a range of applications, from waste removal to drug delivery. Their natural ability to take up iron from their environment could, in principle, be engineered to soak up other heavy metals, according to Dennis Bazylinski, whose laboratory at the University of New Hampshire in Durham, USA, specializes in magnetotactic bacteria. “We and others have been able to ‘trick’ magnetotactic bacteria into biomineralizing crystals in which iron is replaced by another metal,” he said. “Others have done this with some success for cobalt. We have definitively shown that one species can incorporate manganese into the crystal structure of magnetite.” This was achieved by growing the bacteria in a solution with a high concentration of both metals, which coaxed the bacteria to take up either. “I don’t know if I would actually call it a trick but it worked,” said Bazylinski.

But, as Bazylinski noted, being able to sequester heavy metals would only be useful if the bacteria can be removed easily from the waste water. If the bacteria are still magnetic, they can be removed conveniently by applying a suitable magnetic field. According to Bazylinski, this combination of being magnetic and able to take up heavy metals other than iron can be achieved in two ways. First, the bacteria could be engineered to take up both iron and other metals in similar amounts, so they still have magnetic fields. Second, the same effect could be achieved if the genes that enable bacteria to take up specific metals could be transferred to another species that is magnetic. In those cases, the bacteria could be manipulated with magnetic fields to move into areas of high contamination and then removed afterwards, Bazylinski added.

Research at the Japanese National Institute of Technology and Evaluation (NITE) in Tokyo has indicated that this second method might be feasible. The genes responsible for

biosynthesis of the magnetosomes are clustered, which makes it easier to transfer them between species than if the genes were scattered widely across the genome. "We picked up about 300 candidate genes that would be related to magnetosome synthesis," said Nobuyuki Fujita, Deputy Director General of the Department of Biotechnology at NITE. "These genes were found to be clustered in three genomic regions which are commonly possessed in the genomes of five magnetic bacteria whose genomic sequences have so far been determined." Fujita and his colleagues concluded from these observations that magnetic bacteria have themselves acquired the ability to synthesize magnetosomes through horizontal transfer of these multiple gene clusters.

Magnetotactic bacteria might also be used for targeted drug delivery, Fujita commented, by engineering relevant compounds onto the surface of the magnetosomes, which could then be directed to specific sites. "By genetic engineering targeted to these surface proteins—or by crosslinking to existing proteins—one can display, in principle, any kind of protein on the surface of magnetite," he said.

The ability to quickly find and move to areas that have an optimum concentration of oxygen or crucial nutrients relies on the remarkable swimming ability of bacteria, which can move through fluids at speeds of 40 $\mu\text{m/s}$ —which is equivalent to a human travelling in a Formula One racing car or a slow plane. To achieve

...randomly swimming bacteria could be exploited to drive microscopic assemblies of gears many times their size...

such a speed, bacteria generate immense power for their size by rotating their helical-shaped tails, or flagella, akin to microscopic ships' propellers. Recent research at the Argonne National Laboratory in Princeton, NJ, USA, found that randomly swimming bacteria could be exploited to drive microscopic assemblies of gears many times their size, suggesting they could be harnessed as engines for nanoscale machines (Sokolov *et al*, 2009). "Possible applications include miniature fluid devices, such as microreactors, micromixers and microtransporters," said Igor Aronson, one of the study's authors, himself from Northwestern University in Illinois, USA. "We plan to explore this direction in future."

The structures used by the researchers were made by conventional photolithography, yielding a system of individual gears each about 380 μm in diameter and 50 μm thick, compared with a size of around 1 μm for many bacteria. The gears each weighed approximately 6 μg , about a million times greater than each of the individual bacteria moving them. The study also demonstrated that the speed of the gears could be controlled by altering the amount of oxygen available to the bacteria. In addition, the application of chemotaxis or possibly magnetotaxis to coordinate the direction

of swimming could further increase the efficiency of such a system.

Such uses of bacteria, along with many other potential applications of systems biology, are a long way from realization. However, the results so far demonstrate that there is the real and exciting prospect of using bacteria not only to clean up some of the damage inflicted by humans, but also to open new horizons in environmental technologies, in medicine and in research itself.

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