Biotechnology

Cell-free biotech will make for better products

A new type of biological engineering should speed up innovation

May 6th 2017

THE stuff of life comes wrapped in tiny bags called cells. Inside are DNA molecules that carry the instructions for how to run the cell, to make it grow, and to cause it, ultimately, to divide into two cells, if that is to be its fate. Messages made of a slightly different molecule, RNA, carry these instructions to molecular machines called ribosomes. A ribosome's job is to read the RNA messages and translate them into proteins, the workhorse molecules of cells. Those proteins then supervise and execute the running, the growing and the dividing.

It is a system that has worked well over the 4bn years that life has existed on Earth. To some biotechnologists, though, the cell is old hat. They approve of the machinery of DNA, RNA, ribosomes and proteins, which can be engineered to make useful chemicals, ranging from drugs to the building-blocks of plastics. But they want to get rid of the bags that contain it, retaining only the part of the protoplasmic "gloop" inside a cell needed to do their bidding.

In this way they hope to control, far more precisely than is possible by conventional genetic engineering (or even by improved methods of gene modification, such as CRISPR-Cas9, that are now being developed) which genes are translated by the ribosomes—and thus what products are churned out. Equally important, cell-free biotechnology of this sort means no biochemical effort is wasted on running, growing or dividing any actual cells. The initial intention is to create a quicker way of finding the best genes for making a particular product. In the end, those working in the field aspire to the idea that cell-free production will equal mass production.

Processing power

A typical recipe for making cell-free protoplasmic gloop is this. Take four litres of culture containing *E. coli* (a gut bacterium favoured by genetic engineers). Split the bacterial cells open by forcing them through a tiny valve at pressure, thus shredding their membranes and DNA, and liberating the ribosomes. Incubate the resulting mixture at 37°C for an hour, to activate enzymes called exonucleases that will eat up the fragmented DNA. Centrifuge, to separate the scraps of cell membrane and other detritus from the gloop that contains ribosomes. Dialyse to remove unwanted ions. Then stir in amino acids (the building blocks of proteins), sugar and an energy-carrying molecule called adenosine triphosphate (ATP) to power the process. Finally, add a pinch of new DNA to taste, to instruct the gloop which proteins it is supposed to produce.

This particular recipe is the one used by Synvitrobio of Berkeley, California, a firm founded by Zachary Sun and Richard Murray of the California Institute of Technology and George Church of Harvard University. Other recipes, with different starting organisms, are possible. Yeast works, as does *Streptomyces*, another bacterium. Cells from tobacco plants or the ovaries of Chinese hamsters are also good places to begin. But all such formulae are variations on the theme of isolating a cell's protein-making machinery in a free-floating suspension.

Synvitrobio's engineers have built a robotic system to mix the final stage of their recipe. This robot parcels the purified protoplasm into an array of 384 tiny test tubes, each with a volume of a few millionths of a litre. It then drops some DNA molecules into each tube and the gloop gets to work on the process of turning the information in those molecules into proteins. Currently, the system can handle eight DNA sequences per test tube, meaning 3,072 proteins can be processed in parallel. The sequences can be up to 10,000 genetic "letters" long—enough to encode almost any protein you care to mention.

At present, Synvitrobio is using its system to test DNA sequences (or, rather, the resulting proteins) to see if they might be worth investigating as antibiotic drugs. Such drugs work by binding to a biologically important molecule and changing that molecule's characteristics in some way that is detrimental to the organism of which it is part. To look for this binding, each mini test tube is also supplied with some of the target molecules, each attached to a "reporter" molecule that emits a flash of light if binding takes place.

Tubes which flash brightly indicate that one or more of the DNA sequences therein are worth a second glance. Synvitrobio's technique is thus able to screen potential drugs at a rate limited only by the availability of new DNA sequences. Since synthesising new sequences on demand is now a routine technology, that means the world's gene libraries can be plundered for likely candidates, and the best of these then tweaked mercilessly until something good enough for the job turns up. Inserting such sequences into the genomes of organisms is far more time-consuming than simply dropping them in some gloop.

At the moment, that is the point when Synvitrobio passes the newly discovered molecule on, for a suitable cut of the proceeds, to someone who can turn it out in bulk by the conventional technique of pasting the relevant gene into appropriate cells, and breeding these cells in fermentation tanks similar to those used for brewing beer. This is because it is expensive to produce cell-free protoplasm in the volumes required to manufacture antibiotics for sale. A few firms are, however, doing so for drugs that can command high prices.

One such is Sutro Biopharma, based near San Francisco. It uses a cell-free system to create antibodies for the treatment of cancer. In April, Sutro announced it had employed its system to make STRO-001, an antibody that inhibits tumour growth. The firm plans to start trials of STRO-001 in 2018. Cell-free production of the antibodies for that trial is about to begin.

Antibodies are specialised proteins, so once Sutro's system has identified the best candidate for the job, all that is required is to seed the gloop with the DNA which encodes that candidate. Other firms, though, hope to go further than this, by devising manufacturing systems that put together entire metabolic pathways for the production of chemicals other than proteins. These, as in a natural metabolic pathway, consist of a series of enzymes (another type of specialised protein) that catalyse a sequence of chemical changes, gradually converting one molecule into another.

Genomatica, an established biotechnology firm based in San Diego, is experimenting with a cell-free system which produces 1,4-butanediol in this way from simple sugars. 1,4-butanediol is a small molecule that is used to make polymers such as Lycra. Generally, it is cheaper to manufacture molecules of this size using chemistry, rather than biology, but 1,4-butanediol is an exception. It is already made for industry with the aid of genetically modified *E. coli*. Genomatica's system churns out

the enzymes involved in this synthesis, creating an entire cell-free metabolic pathway—and one in which all the sugar is devoted to making the target chemical, rather than a percentage of it being creamed off to run a cell's other biochemical processes. The firm has not yet put the system to commercial use, but has high hopes for it.

GreenLight Biosciences, a firm in Medford, Massachusetts, proposes to use its own cell-free system, also based on *E. coli*, to produce industrial quantities of an undigestible analogue of ribose, a naturally occurring sugar, for use in zero-calorie beverages. The company says it has already got its process to the point where it can make thousands of litres of solution of this sugar at a time. GreenLight is also working on cell-free systems that will generate industrial quantities of specially designed RNA molecules that interfere with the development of insect larvae, and can thus be used as pesticides. Currently, such RNA costs $5,000 per kilogram to produce. GreenLight thinks that by scaling the process up it can reduce this to between $50 and $100.

Whether cell-free biotechnology will be able to displace fermentation by genetically modified organisms as a routine way of making chemicals remains to be seen. Fermentation is a tried and trusted technique, used by humans since the invention of beer around 12,000 years ago. But the idea of stripping molecular biology down to its bare essentials has an efficiency about it which suggests that, for some applications at least, the utility of the biological cell may have run its course.

Conservation

Tourists really do seem to help to preserve wild animals

Who best protects megafauna?

May 6th 2017

WHICH countries have the best wildlife-conservation records? That was the question posed by a group of biologists led by Peter Lindsey of the University of Pretoria, in South Africa. Their conclusions, just published in *Global Ecology and Conservation* and summarised in the map below, suggest one determinant is the economic value of wildlife to a country, with nature-tourism destinations in east, central and southern Africa, led by Botswana, dominating the list of high performers.

The team looked at megafauna, defined as terrestrial mammals weighing 15kg or more as adults, if carnivores, or 100kg or more, if herbivores or omnivores. For each of 152 countries examined, they constructed a megafauna-conservation index composed of three elements. The first was, for every relevant species, the fraction of the country it inhabited. The second was the percentage of megafauna habitat which had legal protection. The third was the percentage of GDP a country devoted to conservation.

Besides the safari belt, America, Canada and Scandinavia, excluding Denmark, scored well (though, as the researchers note, "the financial contribution to predator conservation in Norway probably includes funds aimed at keeping predator population as low as possible, which hardly qualifies as conservation"). So did Bhutan, which came fourth. Low scorers included Britain and China (both densely populated, so lacking natural habitat), and, more surprisingly, Australia.

Frat-boy crustaceans

Crayfish may help researchers understand drunkenness

What happens when you get a crayfish wrecked

Apr 22nd 2017

HUMANS are not the only species to enjoy a snifter. Myriad experiments on other animals, from rats and monkeys to bees and fruit flies, show that they also get drunk, will seek out alcohol given the opportunity and may even develop a dependence on the stuff. But alcohol promotes conviviality as well as drunkenness, and that relationship is less well explored. In particular, there are few studies of whether the link is reciprocal—whether conviviality, or at least a sociable environment, affects susceptibility to alcohol. This question has, however, now been looked into. In a paper just published in *Experimental Biology*, Matthew Swierzbinski, Andrew Lazarchik and Jens Herberholz of the University of Maryland have shown that a sociable upbringing does indeed increase sensitivity to alcohol. At least, it does if you are a crayfish.

The three researchers' purpose in studying drunken crayfish is to understand better how alcohol induces behavioural changes. Most recreational drugs, from cocaine and heroin to nicotine and caffeine, have well-understood effects on known receptor molecules in brain cells. That is not, though, true of ethanol, as the type of alcohol which gets people drunk is known to chemists. Ethanol's underlying molecular mechanisms are poorly understood. But one thing which is known is that crayfish are affected by the same concentrations of the stuff as those that affect humans. Since crayfish also have large, easy-to-study nerve cells that can be examined for clues as to ethanol's molecular mechanisms, Mr Swierzbinski, Mr Lazarchik and Dr Herberholz are using them to try to track those mechanisms down.

Their latest experiment involved 102 of the crustaceans that had each been kept for between seven and ten days in the company of several dozen others, and a further 63 that had been raised in isolation for similar amounts of time. Each crayfish was then transferred individually to a tank containing a solution of ethanol in water, and videoed for three hours to record what happened next.

As might be expected, those animals put into the most concentrated solution, 5.8% by volume, the strength of a potent beer, got pretty drunk. First, they started walking around on tiptoes. Then, they began flicking their tails and doing somersaults (see picture). Finally, the most inebriated ended up lying on their backs, kicking their legs in the air—or, rather, in the water.

Crayfish put in weaker solutions, a half or a tenth as concentrated, behaved similarly, but got there more slowly—and, in the case of those in the weakest solution, often managed to avoid the leg-kicking stage altogether. Crayfish, in other words, behave much like a bunch of roisterers out on the town of a Saturday night.

Crucially, though, when the researchers examined the videos in detail, to record what happened when, they found that, regardless of alcohol concentration, animals that had spent the previous few days in company got drunk about 25% faster than those that had been kept in solitary confinement. They therefore suspect that society makes whatever receptor molecules it is that interact with ethanol more plentiful in crayfish nervous systems than they otherwise would be. The next stage is to compare nerve

cells from social and solitary animals, to try to work out what those receptor molecules might be—and then, if they can be so identified, to see if what is true in crayfish is also true in people.

The germ of an idea

Using fluorescent bacteria to find landmines

A new way to clear minefields

Apr 20th 2017

BATTLEFIELDS strewn with mines are one of the nastiest legacies of war. They ensure that, long after a conflict has ceased, people continue to be killed and maimed by its aftermath. In 1999, the year the Ottawa Mine Ban Treaty came into force, there were more than 9,000 such casualties, most of them civilians. Though this number had fallen below 4,000 by 2014 it is, according to the Landmine and Cluster Munition Monitor, an international research group, rising again as a consequence of conflicts in Libya, Syria, Ukraine and Yemen.

These days most mines have cases made from plastic. Only the firing mechanisms include any metal. That means mines are hard to find with metal detectors. Many ingenious ways to locate and destroy them have been developed, ranging from armour-plated machines that flail the land, via robots equipped with ground-penetrating radar, to specially trained rats that can smell the explosives a mine contains. Such methods have, though, met with mixed success—and can also be expensive. Flails, for instance, scatter shrapnel and explosive residue around a minefield, making it hard to confirm that no undetonated devices remain. Minehunting rats, meanwhile, cost around $8,000 each to train. Often, therefore, mine detection boils down to rows of nervous people wearing blast-resistant clothing and creeping laboriously across a field, prodding the ground ahead to check for buried objects.

Shimshon Belkin, Aharon Agranat and Amos Nussinovitch of the Hebrew University of Jerusalem reckon they have a better approach. They have created a form of *Escherichia coli*, a bacterium widely studied by geneticists, that synthesises a fluorescent protein in response to traces of vapour given off by a mine's explosives. As they report in *Nature Biotechnology*, they have now tested their invention's effectiveness as a mine-hunter.

To turn their bacteria into a mine-detection system, they encapsulated them in beads of alginate, a material derived from seaweed that is permeable to vapours from explosives. They then scattered the beads across an area in which real mines had been buried and left them for a day, to give the vapours from the mines time to stimulate fluorescent-protein production in those beads that had landed above mines. That done, they used a laser to scan the field from a distance. The laser beam stimulated any fluorescent protein it hit to light up, indicating the location of a mine.

The result was a qualified success. The mines being sought had been buried either in one of two sorts of sand or in garden soil. The bugs detected all six sand-covered mines, and also places where flakes of explosive had been buried uncased, but were not fooled by an explosive-free dummy buried in the same material. They did not, though, detect either of the mines buried in garden soil, or flakes of explosive so buried. Whether this was because the researchers had not allowed enough time for vapour evaporating from the explosives to penetrate the soil (they had buried the targets only five days before the tests) or because those vapours cannot penetrate such soil well enough for the bacteria to detect them is a subject for a further test.

Even if it can be used only in sand, though, the approach Drs Belkin, Agranat and Nussinovitch have come up with may be useful. They hope to turn it into a working mine-detection system within three years. They think they can improve the bugs' sensitivity to vapours from explosives and plan to test other ways of encapsulating and dispersing them. For safety's sake, the *E. coli* they use are engineered not to be pathogenic. They also require a special nutrient, contained within the bead. Once this is exhausted the bugs die rapidly.

Besides improving their bacteria, the group would also like to speed up the laser-scanning system, so that it can cover the ground faster. What they use at the moment could be operated from a vehicle, but if it were made compact and light enough, it might also be mounted on a light aircraft or drone. If all that can be done, the world may, at last, have a cheap and effective mine-detection system.

Cloning voices

Imitating people's speech patterns precisely could bring trouble

You took the words right out of my mouth

Apr 20th 2017

UTTER 160 or so French or English phrases into a phone app developed by CandyVoice, a new Parisian company, and the app's software will reassemble tiny slices of those sounds to enunciate, in a plausible simulacrum of your own dulcet tones, whatever typed words it is subsequently fed. In effect, the app has cloned your voice. The result still sounds a little synthetic but CandyVoice's boss, Jean-Luc Crébouw, reckons advances in the firm's algorithms will render it increasingly natural. Similar software for English and four widely spoken Indian languages, developed under the name of Festvox, by Carnegie Mellon University's Language Technologies Institute, is also available. And Baidu, a Chinese internet giant, says it has software that needs only 50 sentences to simulate a person's voice.

Until recently, voice cloning—or voice banking, as it was then known—was a bespoke industry which served those at risk of losing the power of speech to cancer or surgery. Creating a synthetic copy of a voice was a lengthy and pricey process. It meant recording many phrases, each spoken many times, with different emotional emphases and in different contexts (statement, question, command and so forth), in order to cover all possible pronunciations. Acapela Group, a Belgian voice-banking company, charges €3,000 ($3,200) for a process that requires eight hours of recording. Other firms charge more and require a speaker to spend days in a sound studio.

Not any more. Software exists that can store slivers of recorded speech a mere five milliseconds long, each annotated with a precise pitch. These can be shuffled together to make new words, and tweaked individually so that they fit harmoniously into their new sonic homes. This is much cheaper than conventional voice banking, and permits novel uses to be developed. With little effort, a wife can lend her voice to her blind husband's screen-reading software. A boss can give his to workplace robots. A Facebook user can listen to a post apparently read aloud by its author. Parents often away on business can personalise their children's wirelessly connected talking toys. And so on. At least, that is the vision of Gershon Silbert, boss of VivoText, a voice-cloning firm in Tel Aviv.

Words to the wise

Next year VivoText plans to release an app that lets users select the emphasis, speed and level of happiness or sadness with which individual words and phrases are produced. Mr Silbert refers to the emotive quality of the human voice as "the ultimate instrument". Yet this power also troubles him. VivoText licenses its software to Hasbro, an American toymaker keen to sell increasingly interactive playthings. Hasbro is aware, Mr Silbert notes, that without safeguards a prankster might, for example, type curses on his mother's smartphone in order to see a younger sibling burst into tears on hearing them spoken by a toy using mum's voice.

More troubling, any voice—including that of a stranger—can be cloned if decent recordings are available on YouTube or elsewhere. Researchers at the University of Alabama, Birmingham, led by Nitesh Saxena, were able to use Festvox to clone voices based on only five minutes of speech retrieved online. When tested against voice-biometrics software like that used by many banks to block unauthorised access to accounts, more than 80% of the fake voices tricked the computer. Alan Black, one of Festvox's developers, reckons systems that rely on voice-ID software are now "deeply, fundamentally insecure".

And, lest people get smug about the inferiority of machines, humans have proved only a little harder to fool than software is. Dr Saxena and his colleagues asked volunteers if a voice sample belonged to a person whose real speech they had just listened to for about 90 seconds. The volunteers recognised cloned speech as such only half the time (ie, no better than chance). The upshot, according to George Papcun, an expert witness paid to detect faked recordings produced as evidence in court, is the emergence of a technology with "enormous potential value for disinformation". Dr Papcun, who previously worked as a speech-synthesis scientist at Los Alamos National Laboratory, a weapons establishment in New Mexico, ponders on things like the ability to clone an enemy leader's voice in wartime.

As might be expected, countermeasures to sniff out such deception are being developed. Nuance Communications, a maker of voice-activated software, is working on algorithms that detect tiny skips in frequency at the points where slices of speech are stuck together. Adobe, best known as the maker of Photoshop, an image-editing software suite, says that it may encode digital watermarks into speech fabricated by a voice-cloning feature called VoCo it is developing. Such wizardry may help computers flag up suspicious speech. Even so, it is easy to imagine the mayhem that might be created in a world which makes it easy to put authentic-sounding words into the mouths of adversaries—be they colleagues or heads of state.

Regulating the internet giants

The world's most valuable resource is no longer oil, but data

The data economy demands a new approach to antitrust rules

May 6th 2017

A NEW commodity spawns a lucrative, fast-growing industry, prompting antitrust regulators to step in to restrain those who control its flow. A century ago, the resource in question was oil. Now similar concerns are being raised by the giants that deal in data, the oil of the digital era. These titans—Alphabet (Google's parent company), Amazon, Apple, Facebook and Microsoft—look unstoppable. They are the five most valuable listed firms in the world. Their profits are surging: they collectively racked up over $25bn in net profit in the first quarter of 2017. Amazon captures half of all dollars spent online in America. Google and Facebook accounted for almost all the revenue growth in digital advertising in America last year.

Such dominance has prompted calls for the tech giants to be broken up, as Standard Oil was in the early 20th century. This newspaper has argued against such drastic action in the past. Size alone is not a crime. The giants' success has benefited consumers. Few want to live without Google's search engine, Amazon's one-day delivery or Facebook's newsfeed. Nor do these firms raise the alarm when standard antitrust tests are applied. Far from gouging consumers, many of their services are free (users pay, in effect, by handing over yet more data). Take account of offline rivals, and their market shares look less worrying. And the emergence of upstarts like Snapchat suggests that new entrants can still make waves.

But there is cause for concern. Internet companies' control of data gives them enormous power. Old ways of thinking about competition, devised in the era of oil, look outdated in what has come to be called the "data economy" (see Briefing). A new approach is needed.

Quantity has a quality all its own

What has changed? Smartphones and the internet have made data abundant, ubiquitous and far more valuable. Whether you are going for a run, watching TV or even just sitting in traffic, virtually every activity creates a digital trace—more raw material for the data distilleries. As devices from watches to cars connect to the internet, the volume is increasing: some estimate that a self-driving car will generate 100 gigabytes per second. Meanwhile, artificial-intelligence (AI) techniques such as machine learning extract more value from data. Algorithms can predict when a customer is ready to buy, a jet-engine needs servicing or a person is at risk of a disease. Industrial giants such as GE and Siemens now sell themselves as data firms.

This abundance of data changes the nature of competition. Technology giants have always benefited from network effects: the more users Facebook signs up, the more attractive signing up becomes for others. With data there are extra network effects. By collecting more data, a firm has more scope to improve its products, which attracts more users, generating even more data, and so on. The more data Tesla gathers from its self-driving cars, the better it can make them at driving themselves—part of the reason the firm, which sold only 25,000 cars in the first quarter, is now worth more than GM, which sold 2.3m. Vast pools of data can thus act as protective moats.

Access to data also protects companies from rivals in another way. The case for being sanguine about competition in the tech industry rests on the potential for incumbents to be blindsided by a startup in a garage or an unexpected technological shift. But both are less likely in the data age. The giants' surveillance systems span the entire economy: Google can see what people search for, Facebook what they share, Amazon what they buy. They own app stores and operating systems, and rent out computing power to startups. They have a "God's eye view" of activities in their own markets and beyond. They can see when a new product or service gains traction, allowing them to copy it or simply buy the upstart before it becomes too great a threat. Many think Facebook's $22bn purchase in 2014 of WhatsApp, a messaging app with fewer than 60 employees, falls into this category of "shoot-out acquisitions" that eliminate potential rivals. By providing barriers to entry and early-warning systems, data can stifle competition.

Who ya gonna call, trustbusters?

The nature of data makes the antitrust remedies of the past less useful. Breaking up a firm like Google into five Googlets would not stop network effects from reasserting themselves: in time, one of them would become dominant again. A radical rethink is required—and as the outlines of a new approach start to become apparent, two ideas stand out.

The first is that antitrust authorities need to move from the industrial era into the 21st century. When considering a merger, for example, they have traditionally used size to determine when to intervene. They now need to take into account the extent of firms' data assets when assessing the impact of deals. The purchase price could also be a signal that an incumbent is buying a nascent threat. On these measures, Facebook's willingness to pay so much for WhatsApp, which had no revenue to speak of, would have raised red flags. Trustbusters must also become more data-savvy in their analysis of market dynamics, for example by using simulations to hunt for algorithms colluding over prices or to determine how best to promote competition (see Free exchange).

The second principle is to loosen the grip that providers of online services have over data and give more control to those who supply them. More transparency would help: companies could be forced to reveal to consumers what information they hold and how much money they make from it. Governments could encourage the emergence of new services by opening up more of their own data vaults or managing crucial parts of the data economy as public infrastructure, as India does with its digital-identity system, Aadhaar. They could also mandate the sharing of certain kinds of data, with users' consent—an approach Europe is taking in financial services by requiring banks to make customers' data accessible to third parties.

Rebooting antitrust for the information age will not be easy. It will entail new risks: more data sharing, for instance, could threaten privacy. But if governments don't want a data economy dominated by a few giants, they will need to act soon.