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Cleaning up behind us

The potential of genetically modified bacteria to break down toxic pollutants in the environment • *by Víctor de Lorenzo*

These days the terms genome and genomics elicit an instant association with the human genome and the potential benefits for human health that might be squeezed out if it. This is a bit surprising for a microbiologist. Of course, we humans will be the main beneficiaries but what is the actual significance of the human genome in the general context of the biosphere? For instance, in terms of enzymatic diversity, our DNA barely contains enough information to use just a few of the carbon sources that occur in nature. There is no way that we can resist abrupt environmental changes or brusque physico-chemical challenges—let alone nutrient starvation, exposure to heavy metals, lack of oxygen or high/low pressure—with this limited metabolic outfit. How can we deal with the increasingly evident deterioration of the environment brought upon us by our very own actions? It is time to recognise that the main—perhaps the only—chance of counteracting the devastating consequences of our activities on the environment lies in the largely unexplored genetic pool of the microbial world.

Bacteria are the most successful life forms on this planet, being able to colonise the most extreme and diverse biotopes. Many bacteria cope with life with only 1% of the number of genes that a human cell carries within its nucleus. But such a limited amount of genetic information is exploited in the most refined manner, encoding all imaginable devices that are needed at the molecular, physiological and cellular levels to survive and proliferate. Bacteria are particularly good at consuming an amazingly large variety of chemicals as carbon and energy sources. They also employ a large assortment of inorganic compounds as final electron acceptors, making our oxygen-based respiration chain look embarrassingly simple.

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Since bacteria have been around for at least 3 billion years—2 billion years longer than eukaryotic cells—they have had ample time to develop numerous mechanisms with which to draw energy from the environment. We have every reason to believe that virtually every molecular mechanism that supports life was first tested for its performance in a prokaryotic host. Furthermore, exposure of bacteria to a wide variety of chemical compounds created the selective pressure necessary for the evolution of novel catabolic enzymes capable of degrading or modifying these compounds. Millions of years of brutal selective pressure have created catalysts that perform reactions with an efficiency and specificity that organic chemists can only dream of. Indeed, mono- and di-oxygenation of aromatic rings—the initial steps in most metabolic pathways for degradation of hydrocarbons by soil bacteria—are still formidable challenges for chemical engineers. Other pathways and enzymes evolved to degrade quite recalcitrant compounds, such as polycyclic aromatic hydrocarbons. Evolution has generated a wide diversity of bacteria with metabolic enzymes and pathways optimised for breaking down many unpalatable, yet natural, chemicals.

But modern chemistry has released into the environment large amounts of compounds that had never been present in the biosphere in significant amounts. Urban and industrial activities have mobilised otherwise inactive chemical species—typically hydrocarbons, heavy metals or organic compounds with heteroatoms.

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Some obnoxious cases of xenobiotic (from the Greek meaning alien to life) chemicals include chloro-carbon and nitro-carbon bonds, which make them resistant to biodegradation. Polychlorobiphenyls, chlorodioxins, nitroaromatic explosives and the like are just a few examples of emissions that cause environmental damage as well as public panic when they are accidentally released or turn up in food products. These xenobiotics have been in contact with the microbiota only for about 100 years, a mere instant in evolutionary terms. Thus, many of them are still poorly degraded in the soil—if at all.

Of probably even greater environmental concern is the uncontrolled and continuous discharge of bio-active chemicals with a definite, yet poorly understood, impact on the biosphere at local and global levels. Many antibiotics—some of which are extremely difficult to degrade, e.g. the quinolones—are massively used in animal and fish farming and accumulate in soil and aquatic sediments. Hormones, immuno-regulators and all types of medications are simply released into the environment with little care for their downstream repercussions. The ecological consequences of this practice remain to be seen. In the meantime, a challenge for the design of new drugs will certainly be that of their degradability. Again, microbial genetics will be the key, both for the synthesis of the new products and for ensuring their environmental safety.

To deal with these man-made problems, do we have to rely on Mother Nature and simply wait for the evolution of enzymes that can clean up the mess we made, or can we accelerate this process? Although microbes that are able to grow on unusual carbon sources or thrive under extreme conditions have been known for several decades, we have only very recently started to understand the molecular basis of their specific properties. Besides providing beautiful models through which we understand fundamental biological problems (Cases and de Lorenzo, 2001), the genetic analysis of these bacteria also teaches us a lot about new ways to deal with toxic pollutants. Indeed, new metabolic pathways seem to evolve through natural genetic engineering processes. For the most part, these involve mutations that broaden the substrate range of existing enzymes, shuffle sequences, transfer DNA pieces horizontally between members of a microbial community and cut/paste DNA segments to form new hybrid genes and metabolic operons. Finally, most biodegradative gene clusters end up under the control of substrate-responsive promoters. Natural selection provides the mechanisms to accomplish this. Nevertheless, it may take a long time to develop bacteria that can be used as biocatalysts capable of cleaning up modern-day pollution. The challenge, therefore, is to recreate and accelerate these natural processes in the assay tube (Lau and de Lorenzo, 1999).

Hormones, immuno-regulators and all types of medications are simply released into the environment with little care for their downstream repercussions

The challenge now is to recreate and accelerate the natural processes that created enzymes and pathways to break down toxic chemicals in the assay tube

The possibilities of using genetic engineering for improving biodegradation of recalcitrant pollutants had an early boost in the late 1980s with a series of papers published by Ken Timmis and his team from the University of Geneva in Switzerland (Ramos *et al.*, 1987; Rojo *et al.*, 1987). Their results clearly showed that, by judiciously combining genes from different origins, one could produce hybrid metabolic pathways in *Pseudomonas* strains that are able to eliminate recalcitrant compounds, such as mixtures of chloro-benzoates and alkyl-benzoates. Around the same time, the Exxon Valdez oil spill in Alaska added considerable value to the ongoing research on the genetics of microbial biodegradation of hydrocarbons carried out by Al Chatrabarty and his co-workers at the University of Illinois in Chicago (Harvey *et al.*, 1990). This seminal work raised great expectations about designing microorganisms for bioremediation, and raised an enormous interest in microbial ecology, but it also ignited a scientific and public debate about the possible ecological risks of such applications.

Today, things look more complex than had been anticipated during this early excitement. One thing we had to learn is that the use of recombinant organisms for environmental applications strongly differs from contained manipulations in the laboratory (Sayler and Ripp, 2000). Instead of being grown in an optimised environment with nutrients in excess, the recombinant organism is released into a community of diverse organisms. It must establish itself, interact with other members of the community in unknown ways and face a multitude of poorly controllable external factors, some of which place it under considerable stress. In summary, many environmental situations encountered in bioremediation are patently hostile for the recombinant organism. The requirements of biotechnological applications clearly necessitated the development of novel genetic tools and concepts in order to engineer new properties leading to new applications to tackle environmental problems (Timmis and Pieper, 1999). Among others, these included ways to increase the stability of artificial gene constructs, decrease the burden on the cell's metabolism,

minimising lateral transfer of foreign genes to indigenous organisms and the development of non-antibiotic markers. In addition, the combination of genes for biodegradation and production of surfactants might provide a solution to the problem that some pollutants cannot be taken up by certain strains. Clearly, much research will also go into developing improved enzymes and operons, as well as genetic tools in order to construct genetically engineered micro-organisms with an acceptable degree of ecological predictability and whose presence and performance can be monitored within a complex environment.

As often happens with scientific research, the original work intended to increase knowledge has given rise to unexpected developments. One fascinating spin-off is the use of the transcription-regulation machinery found in some hydrocarbon-responsive soil bacteria for the construction of *in situ* bio-monitoring devices and biosensors for environmental control (Sayler and Ripp, 2000). A second benefit is the identification of enzymes in bacterial biodegradation catalysing intricate chemical reactions that can hardly be emulated by organic chemists (Schmid *et al.*, 2001). The respective genes are of great value for the chemical industry when they are spliced into, and expressed in, a suitable heterologous host. Chemists are already using such recombinant strains or enzymes in the production of complex compounds, a method that is increasingly termed 'green chemistry'.

Moreover, the initial questions triggered by the deliberate release of recombinant bacteria for bioremediation have raised fundamental inquiries about microbial ecology and biodiversity. Many of these can now be re-addressed by using DNA chips and the possibility of constructing 'metagenomic libraries' that will be able capture the genetic diversity of a whole given microbial ecosystem (Rondon *et al.*, 2000). In this respect, one intriguing point of intersection between pollution, biodegradation and infection is the growing realisation that many microbial pathogens co-evolve virulence factors together with their ability to degrade unusual carbon sources (Alonso *et al.*, 1999). Genomic science might become the tool with which to tackle such complex phenomena, which involve many levels of interactions between genes and organisms.

Molecular biology tools and concepts can reveal new avenues to address pollution problems closely linked to human health. But how long will it be before genetically modified micro-organisms are actually employed to tackle toxic compounds in the environment? The EU has been funding a considerable number of projects to address the potential environmental risks, and these have yielded two main results. First, there is little scientific basis for considering genetically modified micro-organisms as intrinsically different from their non-recombinant brethren. Indeed, most microbes designed for bioremediation processes have been manipulated in the laboratory to acquire properties that would have evolved naturally over a longer period anyway. Secondly, no evidence has so far been found that the release of such organisms has caused a measurable negative impact on the natural microbial community.

The 1999 Euro-barometer showed that biological research for environmental remediation is the application of genetics that Europeans sympathise with most and whose potential negative consequences they are the least concerned about

Interestingly, the public seems to have anticipated and accepted these conclusions earlier than have the regulatory bodies, who are reluctant to believe that there are very few risks—if any at all—associated with the use of genetic technology in bioremediation. Certainly, these risks are far lower than those created by leaving the pollutants untreated. In fact, the 1999 Eurobarometer on the social perception of biotechnology showed that biological research for environmental remediation is precisely the application of genetics that Europeans sympathise with the most, and whose potential negative consequences they are the least concerned about. Nevertheless, research on bioremediation using genetically modified organisms seems to be tainted by the controversy over transgenic food, and may therefore require more time to regain full gear and broad public support.

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