Bacteria to the rescue

Rekha Seshadri & John Heidelberg

The genome sequence of a dechlorinating bacterium offers new opportunities for bioremediation of chlorinated organic pollutants.

As a consequence of industrialization, humans have developed and released into the environment numerous chemical compounds that would not otherwise be present. Many of these chemicals and their by-products have accumulated in our soil, water and elsewhere, resulting in severe detrimental effects on human health and habitats. The question now arises: how do we eliminate these compounds and reverse their adverse effects on the environment? Bioremediation, which harnesses the catalytic power of microbial species to degrade chemical compounds, offers one promising solution. In this issue, Kube *et al.*[1] profile the genome of one such bacterial bioremediator, *Dehalococcoides* species (spp.) strain CBDB1. By comparing its genome sequence to that of *Dehalococcoides ethenogenes* strain 195 (ref. 2), the authors highlight the remarkable processes by which this microbe has adapted to an environment fashioned by human activities, namely, the release of chlorinated organic pollutants.

Dehalococcoides species are capable of reducing toxic chlorinated organic compounds in the environment. Although other bacteria can sequentially dechlorinate these substrates, they do so only partially, and in some cases generate even more potent toxic intermediates. By contrast, isolated strains of *Dehalococcoides* spp. can carry out complete dechlorination, yielding nontoxic end-products[3,4] (**Fig. 1**). In fact, field tests have confirmed their effectiveness in the dechlorination and remediation of contaminated groundwater[5].

The genome sequence of *Dehalococcoides* strain CBDB1 reveals a heavy investment in anaerobic dehalorespiration. That is, the bacterium is highly specialized to respire chloroorganic compounds instead of oxygen, and uses hydrogen as the sole electron donor for growth. An additional benefit of sequencing the genome of a second *Dehalococcoides* strain is the discovery of an array of new reductive dehalogenases, which perform the hallmark dechlorination steps. Knowledge of these enzymes should facilitate attempts to bioremediate a broader spectrum of chlorinated compounds. Strain CBDB1 was found to have almost twice as many genes encoding reductive dehalogenases compared with strain 195. This difference, together with likely differences in the substrate specificity of these enzymes, accounts for the diverse dehalogenation spectra of the two strains[6–8].

Comparison of the genomes of strains CBDB1 and 195 reveals that their gene content is similar and that the differences mostly involve the reductive dehalogenase genes and their regulators. Both strains show evidence of gene amplification and genetic exchange of the reductive dehalogenase genes, illustrating the dynamic nature of these genes in the genome. Although not all reductive dehalogenase genes in either strain are found in regions of plasticity, many integrated elements described in strain 195 (ref. 2) do contain reductive dehalogenase genes that are absent in CBDB1, and similarly, CBDB1 possesses putative integrated regions bearing reductive dehalogenase genes that are missing from strain 195. These observations suggest that reductive dehalogenase genes have been added recently to each strain's repertoire.

The notion that this soil bacterium has acquired its dechlorinating abilities recently in an adaptive response to anthropogenic activities—namely, the release of large amounts of chlorinated wastes from dry cleaning and manufacturing industries into the environment—is especially intriguing. Although it is possible that the same or similar compounds exist naturally, their release into the environment in large amounts has apparently resulted in the selection of bacterial strains specialized to utilize them. Thus, these genomes represent a snapshot in time of an organism undergoing

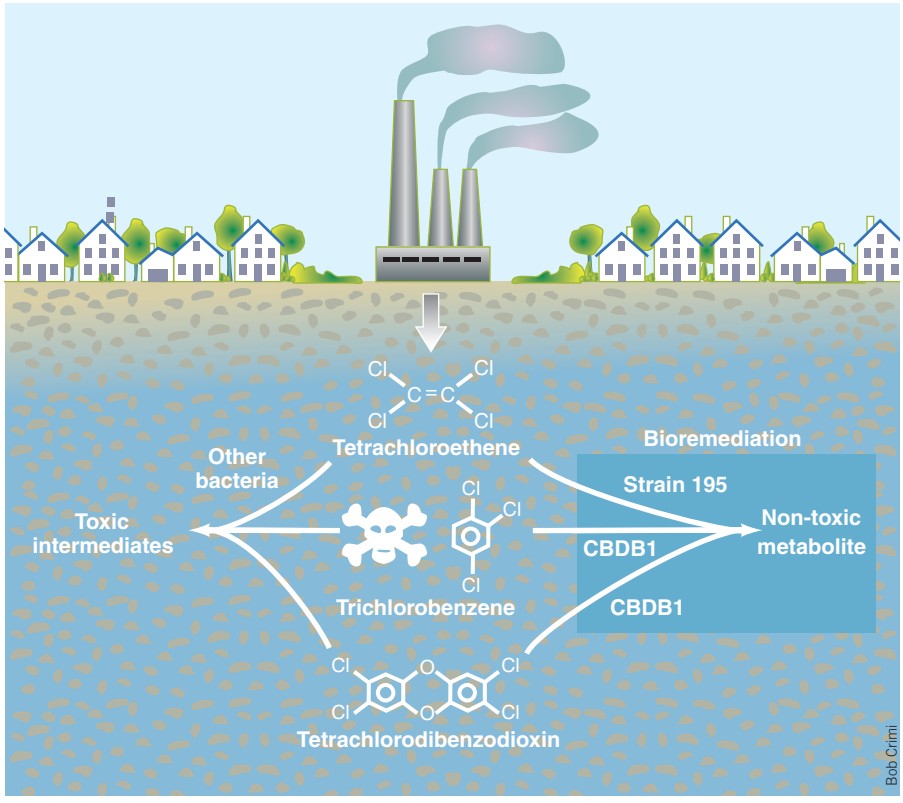

Figure 1 Chlorinated compound effluents from human industry are reductively dechlorinated by several anaerobic microbial species in soil and aquifers, yielding intermediate and terminal metabolites.

Rekha Seshadri and John Heidelberg are in the Institute for Genomic Research, 9712 Medical Center Drive, Rockville, MD, 20850, USA.
e-mail: rekha@tigr.org or jheidel@tigr.org

rapid changes and customizing its physiology to the ambient conditions. However, this is not to imply that nature will always devise a means of clearing obnoxious chemicals in the environment, and the toll on human life and health remains immense.

Horizontal gene transfer and gene expansion are the most likely mechanisms for generating diversity of reductive dehalogenases and dechlorination potential. Nevertheless, the number of independent integration events observed in these genomes is remarkably high given the recent divergence of these two strains (implied by the level of identity of conserved regions). Similar scenarios have been described in clinical isolates of Gram-negative pathogens with regard to capture and spread of antibiotic-resistance gene cassettes or integrons (mobile DNA elements), allowing these pathogens to rapidly adapt to widespread use of antibiotics[9].

Surprisingly, the genome data reveal apparently identical shortcomings in the biosynthetic abilities of both *Dehalococcoides* strains that are not borne out by culture requirements in the lab. Strain 195 requires a complex growth medium containing mixed culture extracts, whereas strain CBDB1 does not. The medium requirements of strain 195 were thought to be explained by this bacterium's lack of enzymes for methionine, glutamate and cofactor synthesis. The new finding that these enzymes are also absent in strain CBDB1, which grows in a simpler medium, presents a conundrum.

The study of Kube *et al.* exemplifies the tremendous value of microbial genomics for unraveling the molecular physiology of organisms and provides insights into the substrate specificities of reductive dehalogenases and into the evolution of dehalorespiration. By capitalizing on what we have learned, we may be able to design more effective approaches for removing chlorinated toxic metabolites from soil and water—a proposal of potentially vast economic and ecological importance. In addition, such genomes undoubtedly serve as a model for studying the evolution of catabolic pathways in bacteria.

1. Kube, M. *et al. Nat. Biotechnol.* **23**, 1269–1273 (2005).
2. Seshadri, R. *et al. Science* **307**, 105–108 (2005).
3. Maymo-Gatell, X., Chien, Y., Gossett, J.M. & Zinder, S.H. *Science* **276**, 1568–1571 (1997).
4. Fennell, D.E., Carroll, A.B., Gossett, J.M. & Zinder, S.H. *Environ. Sci. Technol.* **35**, 1830–1839 (2001).
5. Major, D.W. *et al. Environ. Sci. Technol.* **36**, 5106–5116 (2002).
6. Fennell, D.E., Nijenhuis, I., Wilson, S.F., Zinder, S.H. & Haggblom, M.M. *Environ. Sci. Technol.* **38**, 2075–2081 (2004).
7. Bunge, M. *et al. Nature* **421**, 357–360 (2003).
8. Adrian, L., Szewzyk, U., Wecke, J. & Gorisch, H. *Nature* **408**, 580–583 (2000).
9. Rowe-Magnus, D.A. & Mazel, D. *Int. J. Med. Microbiol.* **292**, 115–125 (2002).

It takes a village to grow a tissue

David L Kaplan, Randall T Moon & Gordana Vunjak-Novakovic

A recent meeting emphasized the importance of integrating concepts from developmental biology and other disciplines into tissue engineering.

Tissue engineering aims to grow tissue replacements by combining cells and matrices under defined laboratory conditions[1]. The shortage of donor tissues and organs and the increasing demand for tissue repair from an aging population have catalyzed research in the field. A US National Institutes of Health–sponsored workshop, 'Tissue Engineering: The Next Generation,' held in Boston on May 2–4, 2005, brought together about 300 participants to explore the future of tissue engineering. A major theme of the meeting was the need to replace the current largely empirical approaches by mechanistic ones and to incorporate approaches from multiple disciplines, in particular, from developmental biology, which offers a wealth of practical and theoretical insights gleaned from hundreds of years of studying embryos.

Current therapeutic approaches with replacement tissues are rather empirical (described by Laura Niklason, Duke University, as "tissue try this"), owing to the enormous complexity of the cells, scaffolds and stimuli that must be screened and the fact that the clinical problem is not always well articulated at the outset. But the field is embracing more systematic studies in which concepts from engineering, developmental biology, genomics, proteomics, cell and molecular biology and systems biology are considered in concert to rapidly identify the most promising strategies. Previous approaches involving inert, nondegradable, nondecorated or homogenous biomaterials are being replaced with more complex designs that direct cell interactions towards tissue assembly and that degrade at rates commensurate with new tissue formation. At the same time, terminally differentiated cells and immortal cell lines are being substituted by progenitor and stem cells to exploit or study cell plasticity.

Most tissue engineering studies have used a single cell type to generate simple tissues *in vitro*. Stem and progenitor cells, which have the capacity to differentiate into multiple cell lineages and form complex tissues, hold great promise, but challenges include access to specific cell lines, control of differentiation and difficulties in handling (according to Mick Bhatia, University of Western Ontario, 40% of these cells die in culture). Workshop participants recognized the need to establish databanks of transfected cells with appropriate reporters to track cell differentiation and tissue development. To exploit stem cells for tissue engineering, one must precisely control their local environmental conditions, including scaffold-derived signals and the levels of nutrients, and molecular and physical regulatory factors. For example, scaffold design can be manipulated to influence the impact of physical stress (Donald Ingber, Harvard University). The selection of culture parameters is one area where developmental biology is likely to provide sound principles.

The use of multiple cell types and combinations of differentiated and progenitor cells will require the development of readouts or reporters linked to genes involved in selective differentiation pathways that are activated by cell-cell and cell-matrix interactions. Here, too, developmental biology should help in determining the right combinations of Hedgehog, Notch, Wnt and other signaling factors needed to direct cell fate and function[3].

Scaffolds are evolving from passive, nondegradable materials to a new generation of designs that actively communicate with surrounding cells. Instead of relying on addition of soluble growth factors, these designs aim to deliver multiple factors with orchestration of temporal and spatial control, recapitulating signaling cascades to optimize tissue outcomes[4].

David L. Kaplan is in the Department of Biomedical Engineering, Tufts University, 4 Colby Street, Medford, Massachusetts 02155, USA; Randall T. Moon is in the Department of Pharmacology, University of Washington School of Medicine and Howard Hughes Medical Institute, Box 357750, Seattle, Washington 98195 USA; and Gordana Vunjak-Novakovic is in the Department of Biomedical Engineering, Columbia University, 351 Engineering Terrace, 1210 Amsterdam Avenue, New York, New York 10025, USA. e-mail: david.kaplan@tufts.edu