Microbial Biotechnology (2009) **2**(2), 125–127 doi:10.1111/j.1751-7915.2009.00091.x

Editorial

Bioremediation, a broad perspective

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Introduction

Bioremediation is the utilization of organisms or derivatives from organisms to degrade pollutants. The chief advantage of bioremediation is its reduced cost compared with conventional techniques such as incineration for which the remediation of all contaminated sites in the USA alone is estimated to be $1.7 trillion (Kuiper *et al*., 2004) or $7000 per citizen. In addition, bioremediation is often a permanent solution (providing complete transformation of the pollutant to its molecular constituents like carbon dioxide and water) rather than a remediation method that transfers wastes from one phase to another (Kuiper *et al*., 2004). Biological catalysts have enormous catabolic potential for remediating wastes; however, the interactions between bacteria and pollutants are often complex and suitable remediation does not always take place. Moreover, many man-made compounds lack good biological catalysts [for most of the 10 million organic compounds described biodegradation has not been investigated (Wackett and Hershberger, 2001)], and in many instances good biocatalysts fail to transform pollutants in the environment (Ramos *et al*., 2009). Hence, the field remains a fertile area for the application of new biotechnological methods to facilitate bioremediation such as metabolic engineering, proteomics, reverse genetics, transcriptomics, metabolomics and genome-scale metabolic modeling. In addition, follow-on studies are important for determining why pollutants persist.

Metabolic engineering involves redirecting the cell's metabolism to achieve a particular goal using recombinant engineering (Bailey, 1991). One of the first and finest examples of this approach in bioremediation was the metabolic engineering of *Pseudomonas* sp. B13; five different catabolic pathways from three different bacteria were combined to allow for degradation of methylphenols and methylbenzoates in a single organism (Rojo *et al*., 1987). In this special issue, Ju and Parales (2009) enable for the first time bacteria to utilize chloronitrobenzenes for growth without the addition of co-substrates and create the first strain that grows on 3-chloronitrobenzene; chloronitrobenzenes are manufactured for pesticides, fungicides, dyes and polymers. They accomplish this feat by cleverly introducing an enzyme that removes nitro groups, nitrobenzene 1,2-dioxygenase from *Comamonas* sp. strain JS765, into *Ralstonia* sp. strain JS705, a strain that has an ortho pathway for the degradation of chlorocatechols. The authors carefully show that 3-chloronitrobenzene is converted by the cloned nitrobenzene 1,2-dioxygenase into 4-chlorocatechol (with release of nitrite) which is subsequently degraded by the host *Ralstonia* sp. strain JS705. They also utilize an active-site mutant of the large subunit of the dioxygenase (F293Q) to reduce the doubling time on 3-chloronitrobenzene by 25%.

Related to the degradation of nitroaromatic compounds by microbes is the research article by Fernández and colleagues (2009) which shows that the model bacterium *Pseudomonas putida* KT2440 can grow in the presence of saturating concentrations of the widely used nitroaromatic explosive, 2,4,6-trinitrotoluene. Using DNA microarrays, transposon mutants and isogenic mutants, the authors found the microorganism reacts to the toxic compound via the activation of a series of detoxification functions including nitroreductase, isoquinolone oxidoreductase, dehydrogenase and chaperones to prevent or repair cell damage. The authors also show that multidrug efflux pump genes (*mexEF*/*oprN*) are induced to reduce intracellular trinitrotoluene concentrations. This work is groundbreaking in that few groups have applied transcriptomics to bioremediation, and this technique

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promises to help unravel unforeseen regulatory bottlenecks related to successful remediation. The Ramos group also recently used whole-transcriptome profiling to determine mutualistic interactions in the rhizosphere for strains relevant for bioremediation; for example, 90 rhizosphere upregulated genes were identified for *P. putida* growing on corn roots (Matilla *et al*., 2007).

Although *P. putida* is an important bacterium in the rhizosphere, many other key species for biodegradation exist, which are not as easily amenable to genetic engineering techniques. One of these is rhodococci, Gram positive bacteria which play an important role in degradation of (hetero-) aromatic compounds. Tomás-Gallardo and colleagues (2009) in this issue present the molecular details of tetralin degradation, a widely used organic solvent for fats, resins and waxes. They use a combination of proteomics, reverse genetics and heterologous expression to discover a large operon encoding the tetralin degradation pathway in *Rhodococcus*. In addition to this, they provide strong evidence for the regulatory control of pathway induction by tetralin, and showed that a new two-component regulator system was necessary for this control. All in all, they author a very complete study, which increases dramatically our understanding of *Rhodococcus* metabolic pathways.

Public concern for the environment is especially poignant when confronted with the devastation caused by marine oil spills. The infamous Exxon Valdez oil spill still reverberates in the public mind but also served as a proving ground for bioremediation treatments. One treatment used with success was to apply nitrogen amendments to stimulate degradation by natural microbial populations. Atlas and Bragg (2009) analysed samples taken 18 years after the spill from sites reported to be heavily oiled in previous surveys. They found that the low percentage of samples still contaminated with moderate or high levels of subsurface oil residues are scattered in small patches and contain mostly highly weathered residues. Moreover, the nutrient levels in these samples were higher than in the surrounding seawater. Therefore, the authors suggest that nutrients no longer are a limiting factor for degradation whereby further applications of nitrogen fertilizer would be ineffective for eliminating remaining contamination. As alternative methods would probably be too disruptive to the environment and wildlife, the authors conclude that natural attenuation would be the most preferable treatment at this time.

Much less in the public mind is the threat of subsurface contamination, a type of contamination which is also the least understood. To gain more insights, Parisi and colleagues (2009) undertook metabolomic studies of samples taken from petroleum-contaminated aquifers underlying a former refinery. Their findings suggested that contaminants of regulatory concern (COC) such as benzene are largely recalcitrant to degradation. Instead, the autochthonous microbial community preferentially used other non-COC hydrocarbons even when stimulated with nutrient amendments. These findings reveal that a possible obstacle for effective remediation under anaerobic conditions could be the absence of catalytic microorganisms.

Further in the line of subsurface contamination, Scheibe and colleagues (2009) present a genome-based metabolic model of the metabolism of *Geobacter sulfurreducens*, and coupled this to a hydrological transport model, in order to predict *in situ* uranium bioremediation. As *Geobacter* activity to reduce U(VI) underground is critically dependent on the availability of acetate as an electron donor and Fe(III) as an electron acceptor, plus ammonium as key nutrient, predictive modelling clearly helps to discover the limiting factors and concentrations under environmental conditions. The model accurately predicted the behaviour of *Geobacter* in a field trial with uranium bioremediation, demonstrating the power of coupling genome-scale metabolic models with hydrological models for field-scale behaviour.

Further insights into the rates of intrinsic bioremediation, that of microbial degradation of hydrocarbon subsurface contaminants under anaerobic conditions at two fuel-contaminated sites, are provided in this issue by Gieg and colleagues (2009). Using deuterated compounds and skilful analytical work, they show that the long lag phases (weeks to months) seen in many laboratory experiments may not adequately predict the fate of these fuel contaminates as they measure lags of hours to days for a wide range of compounds; hence, these pollutants may be degraded far more rapidly than predicted. Evidence for the anaerobic bioremediation of a wide range of compounds including toluene, *m*-xylene, ethylbenzene, 1,3,5,-trimethylbenzene and hexane includes identification of degradation intermediates involving fumarate addition as well as other intermediates.

Biosensors based on the bacterial sensing systems are increasingly used in industrial and environmental applications because of their sensitivity, simplicity and robustness (Garmendia *et al*., 2008). In this issue, Kulakova and coworkers applied this method to establish the whole-cell biosensor for phosphonoacetate using LysR-type transcriptional activator PhnR, which controls expression of the phosphonoacetate degradation operon in *Pseudomonas fluorescens* 23F (Kulakova *et al*., 2009). The *phnR* gene, together with the promoter region of the structural genes, was inserted in the broad-host range promoter probe vector. *Escherichia coli* cells having the resultant plasmid exhibited phosphonoacetate-dependent green fluorescent protein fluorescence in response to threshold concentrations of as little as 0.5 μM phosphonoacetate, some 100 times lower than the detection limit of currently

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available non-biological analytical methods. On the other hand, *P. putida* cells having the same plasmid were less sensitive, though with shorter response times.

In the realm of pesticide remediation, Govantes and colleagues (2009) review the regulation of enzyme activities involved in the degradation of the herbicide atrazine. The authors describe recent advances made in understanding how alternative nitrogen sources negatively regulate atrazine degradation genes and how atrazine transport likewise could be a target for nitrogen regulation. The extrapolation of these findings provides an explanation for the poor atrazine degradation observed in soils which had undergone nitrogen (fertilizer) amendments typical to agricultural settings. As a result, the authors propose strategies which should be valuable for improving the bioremediation of this herbicide under 'real world' field conditions.

Also included in this special issue is a review of chitosan and its potential as an antimicrobial and in other applications by Raafat and Sahl (2009). The authors indicate that chitosan, a natural high-molecular-weight biopolymer consisting of *N*-acetyl-*D*-glucosamine and *D*-glucosamine, may be used more frequently given its excellent biocompatibility. Applications range from its use in magnetic nanofactories to eliciting plant defence responses to drug encapsulation. The authors also review the manner in which chitosan acts as an antimicrobial for fungi and bacteria.

These excellent reports demonstrate clearly that research in bioremediation remains robust as it incorporates various approaches from systems biology (e.g. metabolomics, transcriptomics, proteomics and genome-scale metabolic models) to innovative field studies. New biological activity is being generated, important regulatory pathways are being discerned, and the fate of contaminants are being assayed to help us move to a sustainable industrial model, one in which once-polluted areas are remediated and new sites are not created.

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