



ELSEVIER

Biocatalytic degradation of pollutants

Rebecca E Parales^{1*} and John D Haddock²

Microbial reactions play key roles in biocatalysis and biodegradation. The recent genome sequencing of environmentally relevant bacteria has revealed previously unsuspected metabolic potential that could be exploited for useful purposes. For example, oxygenases and other biodegradative enzymes are benign catalysts that can be used for the production of industrially useful compounds. In conjunction with their biodegradative capacities, bacterial chemotaxis towards pollutants might contribute to the ability of bacteria to compete with other organisms in the environment and to be efficient agents for bioremediation. In addition to the bacterial biomineralization of organic pollutants, certain bacteria are also capable of immobilizing toxic heavy metals in contaminated aquifers, further illustrating the potential of microorganisms for the removal of pollutants.

Addresses

¹Section of Microbiology, 266 Briggs Hall, University of California, Davis, California 95616, USA

²Department of Microbiology, 131 Life Sciences II, Southern Illinois University, Carbondale, IL 62901-6508, USA

*e-mail: reparales@ucdavis.edu

Current Opinion in Biotechnology 2004, 15:374–379

This review comes from a themed issue on
Protein technologies and commercial enzymes
Edited by Karl-Erich Jaeger

0958-1669/\$ – see front matter
© 2004 Elsevier Ltd. All rights reserved.

DOI 10.1016/j.copbio.2004.06.003

Abbreviations

PAH polynuclear aromatic hydrocarbon
PCE perchloroethene

Introduction

Wackett and Hershberger define biocatalysis as ‘metabolism for the purposes of making a useful compound’ [1]. The direction of this article, in contrast to most others in this issue, moves away from this idea and focuses more on biodegradation, which is defined by the same authors as ‘a process by which a potentially toxic compound is transformed to a nontoxic one’ [1]. A strong connection remains between the two concepts, because numerous useful biocatalysts have emerged from biodegradation pathways. In this article, we present an overview of recent developments in the field of biodegradation, from single-step biotransformations to the control of biodegradation pathways and the detection of pollutants by chemosen-

sory systems. We also include a section on the genomics of biodegradative organisms, because much information relevant to biocatalysis and biodegradation applications is yet to be mined from this vast wealth of data.

What's new in bacterial biodegradation

Biodegradation research continues to encompass a broad diversity of interesting scientific problems. In the past year, several reports have described progress in basic and applied aspects of biodegradative pathways, the characterization of biodegradation enzymes (Table 1) and their genes, and the use of these enzymes as industrial biocatalysts.

Chlorinated aliphatic and aromatic compounds

Chlorinated solvents are widespread groundwater contaminants, whereas chlorinated dioxins, dibenzofurans and polychlorinated biphenyls (PCBs) are common contaminants of soil and sediment. Some chlorinated hydrocarbons can be employed as terminal electron acceptors for anaerobic microbial respiration, resulting in their reductive dechlorination. This type of metabolism, called dehalorespiration, facilitates the degradation of recalcitrant chlorinated compounds by other microorganisms. *Dehalobacter restrictus* contains perchloroethene (PCE) reductase, which catalyzes the reductive dechlorination of PCE during growth with H₂ as an energy source. Purification of the enzyme from the membrane fraction of *D. restrictus* showed that it contained one molecule of cobalamin and two [4Fe–4S] clusters [2]. The amino acid sequence deduced from the cloned genes encoding the reductase (*pceA*) and a putative membrane-anchoring protein (*pceB*) showed very high identity to PCE reductases in *Desulfotobacterium hafniense*. Unfortunately, dehalorespiration of PCE and trichloroethene produces vinyl chloride, a human carcinogen. This problem can be overcome, however, by the use of *Mycobacterium* strain JS60A, which is able to degrade ethene and vinyl chloride [3•]. Degradation is initiated by a four-component monooxygenase that produces epoxyethane and chlorooxirane. The second reaction of the pathway is unusual for a eubacterium, because it involves coenzyme M — a cofactor present in methanogens [4].

The first demonstration of a pure culture capable of the reductive dechlorination of dioxins has been reported [5••]. Bacteria of the genus *Dehalococcoides* were identified in an anaerobic, dioxin-dechlorinating mixed culture using the polymerase chain reaction (PCR) and 16S ribosomal DNA analysis. Penta-, tetra-, tri- and dichlorinated dioxins were dechlorinated under anaerobic

Table 1

Key biodegradation enzymes.

Enzyme	Source organism	Reference
Tetrachloroethene reductive dehalogenase	<i>Dehalobacter restrictus</i> DSMZ 9455 ^T	[2]
Ethene monooxygenase	<i>Mycobacterium</i> sp. strain JS60	[3*]
Epoxyalkane coenzyme M transferase	<i>Mycobacterium</i> sp. strain JS60	[3*]
Carbazole 1,9a-dioxygenase	<i>Sphingomonas</i> sp. strain KA1	[6,7]
2-Hydroxy-6-oxo-6-(2'-aminophenyl)-hexadienoate hydrolase	<i>Pseudomonas resinovorans</i> CA10 and <i>Janthinobacterium</i> sp. strain J3	[8]
Biphenyl dioxygenase	<i>Burkholderia</i> sp. strain LB400	[9]
Naphthalene dioxygenase	β <i>Proteobacterium</i> strain CJ2	[12*]
1-Hydroxy-2-naphthoic acid dioxygenase	<i>Pseudomonas</i> sp. strain PP2	[13]
Fluorene monooxygenase	<i>Sphingomonas</i> sp. strain LB126	[15]
Cyclohexanone monooxygenase	<i>Acinetobacter</i> sp. strain NCIB 9871, <i>Brevibacterium</i> sp. strains ChnB1 and ChnB2, <i>Acidovorax</i> sp. strain CHX, <i>Acinetobacter</i> sp. strain SE19, <i>Arthrobacter</i> sp. strain BP2, <i>Rhodococcus</i> sp. strains phi1, phi2 and SC1	[17,18]

conditions with H₂ as the electron donor and dioxins as terminal electron acceptor. This observation led researchers to further investigate *Dehalococcoides* sp. strain CBDB1, a known chlorobenzene dehalorespirer. Strain CBDB1 favored removal of chlorine occupying the peripheral positions of dioxin rings. Thus, some chlorinated dioxins were transformed to the highly toxic congener 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, which contains only lateral chlorine substituents; however, further reductive dechlorination converted it to less toxic products.

Bicyclic aromatic compounds: carbazole, dibenzofuran and biphenyl

Recombinant plant symbiotic bacteria might be useful for the rhizoremediation of soil containing persistent organic contaminants. A recombinant strain of *Rhizobium tropici* containing the *Sphingomonas* sp. strain KA1 carbazole terminal oxygenase gene on a multicopy number plasmid was constructed [6]. The enzyme catalyzes oxidation of chlorinated dioxins and dibenzofurans in addition to carbazole [7]. The recombinant strain degraded dibenzofuran and colonized the rhizosphere of sirato, a leguminous plant. The plasmid was stably maintained in nonsterile soil and the strain was moderately competitive with endogenous microflora of most nonsterile soils tested.

The crystal structure of the carbazole degradation pathway enzyme 2-hydroxy-6-oxo-6-(2'-aminophenyl)-hexa-2,4-dienoate hydrolase (CarC) of *Janthinobacterium* sp. strain J3 was determined [8]. The enzyme is a member of the α/β -hydrolase family and has been classified with BphD (biphenyl *meta*-cleavage product hydrolase) as a group I hydrolase, because its substrate is derived from a bicyclic molecule. However, the structure of the enzyme shows greater similarity to the group III hydrolase CumD (cumene *meta*-cleavage product hydrolase), the substrate of which is derived from the degradation of a monocyclic aromatic hydrocarbon containing a short alkyl sidechain.

The structure also suggested that the substrate-binding pocket might be accessed by the 'untying' of an α helix in the lid domain of the enzyme.

Amino acids of the biphenyl dioxygenase of *Burkholderia* sp. strain LB400 involved in substrate interactions with PCBs were identified through site-directed mutagenesis [9]. A model of the enzyme based on the crystal structure of naphthalene dioxygenase [10] was used to locate the amino acids of interest with respect to the substrate-binding site. Three residues present in the substrate-binding pocket had strong effects on the regiospecificity of dioxygenation of chlorinated biphenyls; several neighboring residues not directly involved in substrate binding also showed significant effects. Also of interest in the biodegradation of biphenyl is Tn4371, a catabolic transposon that carries genes involved in the aerobic degradation of biphenyl and 4-chlorobiphenyl. The complete sequence of Tn4371 revealed a mosaic of genes assembled from diverse sources, including plasmids and chromosomes of unrelated bacteria [11]. Tn4371 appears to be a broad host range conjugative transposon that carries genes for plasmid replication and conjugative transfer and might be a member of a family of potentially mobile genomic islands related to IncP and Ti plasmids.

Polynuclear aromatic hydrocarbons

Many microorganisms with catabolic pathways for the degradation of polynuclear aromatic hydrocarbons (PAHs) are known; however, it has been difficult to prove that these organisms actively degrade the pollutants in nature. Madsen and colleagues [12*] used naphthalene labeled with the stable carbon isotope ¹³C as a tracer to link *in situ* biodegradation to a previously unknown group of bacteria. Strain CJ2 was isolated and shown to be closely related to the predominant naphthalene-degrading organisms active *in situ*. CJ2 was most closely related to *Polaromonas vacuolata* and only distantly related to

the naphthalene-degrading *Pseudomonas* spp. commonly isolated from contaminated sites.

The catabolic pathways for three- and four-ring PAHs were examined in several studies. Phenanthrene was degraded by *Pseudomonas* sp. strain PP2 via a dioxygenase-initiated pathway that converged with the naphthalene degradation pathway [13]. Secretion of a surfactant into the medium and increased cell-surface hydrophobicity during growth were postulated to increase uptake of poorly soluble phenanthrene. A new pathway for the degradation of anthracene by *Mycobacterium* sp. strain LB501T was also proposed [14]. Detection of 3-hydroxy-2-naphthoic and *o*-phthalic acid suggested a route that does not proceed through the known pathway, which involves production of 2,3-dihydroxynaphthalene. The known and proposed pathways diverge after the formation of 3-hydroxy-2-naphthoic acid. *o*-Phthalic acid and protocatechuic acid were proposed as intermediates rather than salicylate and catechol, intermediates of naphthalene degradation. Degradation of fluorene through a known pathway involving an initial monooxygenation supported growth of *Sphingomonas* sp. LB126 [15]. This strain also cooxidized anthracene, phenanthrene and fluoranthene without the accumulation of dead-end intermediates. Strain LB126 was unable to cleave the carbon–sulfur bond of dibenzothiophene 5-oxide and dibenzothiophene-5,5-dioxide, however, which accumulated as dead-end cooxidation products.

High temperature increases the bioavailability of hydrophobic pollutants by increasing solubility, diffusion and reaction rates. Feitkenhauer *et al.* [16] showed that a mixture of three- to five-ring PAHs were degraded at 65°C by a mixed culture composed of thermophilic *Bacillus* spp. and *Thermus brockii* when hexadecane was included as a degradable solvent. In addition, *T. brockii* grew rapidly in a bioreactor at 70°C with hexadecane and pyrene as sole carbon sources. These strains could be useful for the rapid remediation of contaminated soils and industrial wastes via composting or in temperature-controlled bioreactors.

Biocatalytic applications

In addition to applications in bioremediation, biodegradative enzymes are useful to the biotechnology industry as biocatalysts for asymmetric organic synthesis. The activities of recombinant Baeyer–Villiger monooxygenases from bacteria isolated from a wastewater treatment plant were investigated [17]. New enzymes were identified that converted substituted cyclohexanones to homochiral ϵ -caprolactones, which are widely used as building blocks in organic synthesis. Overexpression of recombinant proteins often results in the production of inactive aggregates. A high level of expression of soluble cyclohexanone monooxygenase was obtained in recombinant *Escherichia coli* by coexpression of molecular

chaperones and foldases [18]. Expression of the active enzyme was increased 38-fold over that of a strain expressing only the monooxygenase gene. Co-expression of DnaK, DnaJ and GrpE together was the most effective combination of the chaperones and foldases tested.

The investigations cited above are just a few examples of recent advances in biodegradation research that have increased our understanding of microbial diversity and metabolism in the biosphere.

Chemotaxis: a role in biodegradation?

Researchers in the field of biodegradation are starting to seriously address the possibility of a role for chemotaxis in the biodegradation of pollutants, as indicated by recent reviews [19,20]. Several reports of chemotaxis by specific biodegradative strains to various pollutants and man-made chemicals have appeared in the literature in the past 18 months. Park *et al.* demonstrated that the man-made herbicide atrazine elicited a chemotactic response in two motile atrazine-degrading bacteria, *Pseudomonas* sp. ADP and *Agrobacterium radiobacter* J14a [21]. Likewise, *Ralstonia eutropha* JMP134(pJP4), which grows on the herbicide 2,4-dichlorophenoxyacetate (2,4D), showed an inducible chemotactic response to 2,4D [22]. The response required the presence of the TfdK permease, a member of the major facilitator superfamily of transport proteins. These reports are of interest, because they indicate that these bacteria have not only evolved new pathways for the degradation of man-made chemicals, but that they have also evolved appropriate chemosensory systems for their detection. In another study, three *Pseudomonas* strains were found to be chemotactic to nitrobenzoates and aminobenzoates [23]. Results of this study suggest that an inducible broad-substrate benzoate chemotaxis system allows detection of various substituted benzoates, which is particularly useful in nitrobenzoate-degrading bacteria. Chemotaxis by *Ralstonia* sp. SJ98 towards several nitroaromatic compounds that can be co-metabolized has also been demonstrated [24]. A chemotaxis enrichment method was used to isolate an alkane-degrading organism, *Flavimonas oryzihabitans*, which was chemotactic towards oil-gas and hexadecane, as judged by three types of qualitative chemotaxis assays [25]. Finally, the first example of bacterial chemotaxis to an attractant desorbing from a nonaqueous phase liquid (NAPL) has been reported [26••]. In this study, wild-type *Pseudomonas putida* G7, which is chemotactic towards naphthalene, was shown to degrade naphthalene dissolved in the model NAPL 2,2,4,4,6,8,8-heptamethylnonane more efficiently than a nonchemotactic mutant or a nonmotile mutant. The nonchemotactic mutant actually degraded naphthalene more rapidly than the nonmotile mutant, probably because motile cells encountered and adhered to the surface of the NAPL droplet. This study provides direct evidence that motility and chemotaxis stimulate desorption and biodegradation of nonaqueous contaminants.

Genomics and biodegradation

Recent genome sequencing efforts have begun to focus on environmentally relevant bacteria. The genomes of four organisms with potential for biodegradation and bioremediation applications have been completely sequenced and published within the past year and a half. The availability of these genome sequences should accelerate analyses of the metabolic capabilities of these bacteria and reveal their full potential for use in bioremediation applications. Potentially useful enzymes and proteins identified from these genome sequences are listed in Table 2.

The genome sequence of the catabolically versatile *P. putida* KT2440 was recently completed [27]. Overall, the genome of this nonpathogenic organism is quite similar to that of the opportunistic human pathogen *Pseudomonas aeruginosa*, but it lacks many of the virulence genes known to be required for *P. aeruginosa* infection. *P. putida* KT2440 has the ability to grow on a wide variety of aromatic compounds, and this aspect of its metabolism is explored in detail by Jiménez *et al.* [28]. In addition to the expected genes for known aromatic degradation pathways, the genome sequence revealed the presence of 18

dioxygenases, 80 oxidoreductases of unknown function, three enzymes that may be involved in dechlorination reactions, and an unprecedented number of transport systems [27]. Furthermore, this strain has several sets of chemotaxis (*che*) genes as well as 27 genes that appear to encode methyl-accepting chemotaxis proteins, the majority of which have no known function. These findings highlight the enormous amount of information about *P. putida* metabolism that remains unexplored.

Rhodopseudomonas palustris is able to adapt to a wide range of environmental conditions owing to its multiple modes of metabolism, which include photoautotrophy, photoheterotrophy, chemoautotrophy and chemoheterotrophy. It can grow in the presence or absence of oxygen and can use various alternative electron acceptors for respiration. The genome sequence suggests that *R. palustris* can utilize several different nitrogen sources and numerous carbon sources, including fatty acids, dicarboxylic acids and plant-derived aromatic acids, both aerobically and anaerobically [29••]. This organism also has numerous transport and chemosensory systems, which are probably essential for identifying the optimum mode of metabolism under any conditions. In addition, it is capable of

Table 2

Potentially useful enzymes identified in recently sequenced genomes.

Organism	Enzyme/protein	Reference
<i>Pseudomonas putida</i> KT2440	18 Dioxygenases	[27,28]
	15 Monooxygenases	
	80 Oxidoreductases	
	62 Transferases	
	40 Dehydrogenases	
	51 Hydrolases	
	12 Glutathione transferases	
	3 Chlorohydrolases	
	Enzymes for the aerobic degradation of at least 17 aromatic compounds	
<i>Rhodopseudomonas palustris</i> CGA009	Type I ribulose biphosphate carboxylase	[29••]
	Type II ribulose biphosphate carboxylase	
	Iron nitrogenase	
	Molybdenum nitrogenase	
	Vanadium nitrogenase	
	Hydrogenase	
	Anaerobic benzoate and 4-hydroxybenzoate degradation enzymes	
	Phenylacetate degradation enzymes (CoA-mediated)	
	Protocatechuate degradation enzymes (<i>meta</i> pathway)	
	Homoprotocatechuate degradation enzymes	
	Medium-chain fatty acid degradation enzymes	
	Homogentisate degradation enzymes	
	Carotenoid biosynthesis enzymes	
<i>Geobacter sulfurreducens</i>	111 c-type cytochromes	[30]
	3 [NiFe] hydrogenases	
	Aromatic ring cleavage dioxygenase	
	Methylamine catabolic enzymes	
<i>Shewanella oneidensis</i> MR-1	39 c-type cytochromes	[31•]
	Heterodimeric [Fe] hydrogenase	
	[NiFe] hydrogenase	

nitrogen fixation. Interestingly, one of its closest relatives is *Bradyrhizobium japonicum*, a nonphotosynthetic nitrogen-fixing symbiont of soybeans [29**]. *R. palustris* carries two forms of RubisCO for carbon dioxide fixation, has three different nitrogenases and, based on the identification of *kaiB* and *kaiC* homologs, might have a circadian clock.

The genome sequences of two dissimilatory metal-reducing bacterial strains, *Shewanella oneidensis*, MR-1 and *Geobacter sulfurreducens*, were recently completed [30,31*]. These members of the Proteobacteria can respire iron and manganese oxides, as well as more toxic metals such as Cr(VI) and U(VI). The 5 Mb genome sequence of *S. oneidensis* revealed the presence of 39 *c*-type cytochromes, whereas that of *G. sulfurreducens* (3.8 Mb) boasted 111 *c*-type cytochromes. The large numbers of cytochromes are believed to be required for the complex and diverse respiratory capacities of these bacteria, and this information should aid in the analysis of their respiratory systems. An additional bonus obtained from the genome sequence of *S. oneidensis* was the identification of a 162 kb plasmid and λ -like and Mu-type phages, which might prove useful for the development of genetic transfer strategies [31*]. Although *G. sulfurreducens* was previously thought to be a strict anaerobe, the genome sequence suggests a more versatile metabolism; for example, genes encoding various protective proteins such as catalase and superoxide dismutase were identified, as were various oxidases commonly associated with aerobic metabolism [30]. Although these bacteria are only capable of transforming rather than eliminating metal contaminants, they do reduce the soluble forms of chromium and uranium to insoluble precipitates, thus removing them from groundwater. The potential utility of dissimilatory metal reduction as a bioremediation strategy was tested in a uranium-contaminated aquifer [32*]. In this study, acetate was injected to stimulate metal reduction by indigenous bacteria. Initially, U(VI) and Fe(III) reduction occurred simultaneously, resulting in a decrease in dissolved uranium and an increase in the population of *Geobacter*. Once the iron was reduced, however, sulfate reduction became the predominant form of metabolism, *Geobacter* numbers decreased, and uranium concentrations increased in the groundwater. These results emphasize the need to understand the biological reduction process in order to control and optimize the bioremediation of toxic metals.

Conclusions

The potential of biodegradative bacteria in the development of bioremediation applications for biomineralization of organic pollutants and 'bioimmobilization' of inorganic compounds has not yet been fully realized, but examples of both types of strategies have been reported. The role of chemotaxis in biodegradation is becoming more apparent, and might be a factor in choosing strains

for bioaugmentation purposes. It is also expected that microbial biodegradation pathways will continue to yield useful enzymes for biocatalysis and biotechnological applications, and the additional information gained from genome sequences of environmental bacteria could lead to the identification of other novel reactions and pathways.

Acknowledgements

Research activities of RE Parales have been supported by the Strategic Environmental Research and Development Program, project CU1212.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
 - of outstanding interest
1. Wackett LP, Herschberger CD: *Biocatalysis and Biodegradation: Microbial Transformation of Organic Compounds*. Washington, DC: American Society for Microbiology Press; 2001.
 2. Maillard J, Schumacher W, Vazquez F, Regeard C, Hagen WR, Holliger C: **Characterization of the corrinoid iron-sulfur protein tetrachloroethene reductive dehalogenase of *Dehalobacter restrictus***. *Appl Environ Microbiol* 2003, **69**:4628-4638.
 3. Coleman NV, Spain JC: **Epoxyalkane: coenzyme M transferase in the ethene and vinyl chloride biodegradation pathways of *Mycobacterium* strain JS60**. *J Bacteriol* 2003, **185**:5536-5545.
 - CoM is a cofactor of methanogenesis in methanogenic archaea and its involvement in epoxyalkane degradation is the only known occurrence in bacteria.
 4. Allen JR, Clark DD, Krum JG, Ensign SA: **A role for coenzyme M (2-mercaptoethanesulfonic acid) in a bacterial pathway of aliphatic epoxide carboxylation**. *Proc Natl Acad Sci USA* 1999, **96**:8432-8437.
 5. Bunge M, Adrian L, Kraus A, Opel M, Lorenz WG, Andreesen JR, Gorisch H, Lechner U: **Reductive dehalogenation of chlorinated dioxins by an anaerobic bacterium**. *Nature* 2003, **421**:357-360.
 - Reductive dehalogenation is the only reported anaerobic biological process to be active on dioxins. The sediment used to establish the dechlorinating mixed culture appears to contain different populations of bacteria with different preferences for the ring position where dechlorination occurs.
 6. Saiki Y, Habe H, Yuuki T, Ikeda M, Yoshida T, Nojiri H, Omori T: **Rhizoremediation of dioxin-like compounds by a recombinant *Rhizobium tropici* strain expressing carbazole 1,9a-dioxygenase constitutively**. *Biosci Biotechnol Biochem* 2003, **67**:1144-1148.
 7. Habe H, Ashikawa Y, Saiki Y, Yoshida T, Nojiri H, Omori T: ***Spingomonas* sp. strain KA1, carrying a carbazole dioxygenase gene homologue, degrades chlorinated dibenzo-p-dioxins in soil**. *FEMS Microbiol Lett* 2002, **211**:43-49.
 8. Habe H, Morii K, Fushinobu S, Nam J-W, Ayabe Y, Yoshida T, Wakagi T, Yamane H, Nojiri H, Omori T: **Crystal structure of a histidine-tagged serine hydrolase involved in the carbazole degradation (CarC enzyme)**. *Biochem Biophys Res Commun* 2003, **303**:631-639.
 9. Zielinski M, Kahl S, Hecht HJ, Hofer B: **Pinpointing biphenyl dioxygenase residues that are crucial for substrate interaction**. *J Bacteriol* 2003, **185**:6976-6980.
 10. Kauppi B, Lee K, Carredano E, Parales RE, Gibson DT, Eklund H, Ramaswamy S: **Structure of an aromatic ring-hydroxylating dioxygenase-naphthalene 1,2-dioxygenase**. *Structure* 1998, **6**:571-586.
 11. Toussaint A, Merlin C, Monchy S, Benotmane MA, Leplae R, Mergeay M, Springael D: **The biphenyl- and 4-chlorobiphenyl-catabolic transposon Tn4371, a member of a new family of genomic islands related to IncP and Ti plasmids**. *Appl Environ Microbiol* 2003, **69**:4837-4845.

12. Jeon CO, Park W, Padmanabhan P, DeRito C, Snape JR, Madsen EL: **Discovery of a bacterium, with distinctive dioxygenase, that is responsible for *in situ* biodegradation in contaminated sediment.** *Proc Natl Acad Sci USA* 2003, **100**:13591-13596.
- For a variety of reasons, it has been difficult to show that the activities of microorganisms in pure cultures are operable in the field. This article demonstrates that the study of culturable laboratory strains may not always be relevant to what is happening in the field.
13. Prabhu Y, Phale PS: **Biodegradation of phenanthrene by *Pseudomonas* sp. strain PP2: novel metabolic pathway, role of biosurfactant and cell surface hydrophobicity in hydrocarbon assimilation.** *Appl Microbiol Biotechnol* 2003, **61**:342-351.
14. van Herwijnen R, Springael D, Slot P, Govers HA, Parsons JR: **Degradation of anthracene by *Mycobacterium* sp. strain LB501T proceeds via a novel pathway, through *o*-phthalic acid.** *Appl Environ Microbiol* 2003, **69**:186-190.
15. van Herwijnen R, Wattiau P, Bastiaens L, Daal L, Jonker L, Springael D, Grovers HA, Parsons JR: **Elucidation of the metabolic pathway of fluorene and cometabolic pathways of phenanthrene, fluoranthene, anthracene and dibenzothiophene by *Sphingomonas* sp. LB126.** *Res Microbiol* 2003, **154**:199-206.
16. Feitkenhauer H, Muller R, Markl H: **Degradation of polycyclic aromatic hydrocarbons and long chain alkanes at 60–70°C by *Thermus* and *Bacillus* spp.** *Biodegradation* 2003, **14**:367-372.
17. Kyte BG, Rouviere P, Cheng Q, Stewart JD: **Assessing the substrate selectivities and enantioselectivities of eight novel Baeyer-Villiger monooxygenases toward alkyl-substituted cyclohexanones.** *J Org Chem* 2004, **69**:12-17.
18. Lee DH, Kim MD, Lee WH, Kweon DH, Seo JH: **Consortium of fold-catalyzing proteins increases soluble expression of cyclohexanone monooxygenase in recombinant *Escherichia coli*.** *Appl Microbiol Biotechnol* 2004, **63**:549-552.
19. Parales RE, Harwood CS: **Bacterial chemotaxis to pollutants and plant-derived aromatic molecules.** *Curr Opin Microbiol* 2002, **5**:266-273.
20. Pandey G, Jain RK: **Bacterial chemotaxis toward environmental pollutants: role in bioremediation.** *Appl Environ Microbiol* 2002, **68**:5789-5795.
21. Park J-H, Feng Y, Ji P, Voice TC, Boyd SA: **Assessment of bioavailability of soil-sorbed atrazine.** *Appl Environ Microbiol* 2003, **69**:3288-3298.
22. Hawkins AC, Harwood CS: **Chemotaxis of *Ralstonia eutropha* JMP134(pJP4) to the herbicide 2,4-dichlorophenoxyacetate.** *Appl Environ Microbiol* 2002, **68**:968-972.
23. Parales RE: **Nitrobenzoates and aminobenzoates are chemoattractants for *Pseudomonas* strains.** *Appl Environ Microbiol* 2004, **70**:285-292.
24. Pandey G, Chauhan A, Samanta SK, Jain RK: **Chemotaxis of a *Ralstonia* sp. SJ98 toward co-metabolizable nitroaromatic compounds.** *Biochem Biophys Res Commun* 2002, **299**:404-409.
25. Lanfranconi MP, Alvarez HM, Studdert CA: **A strain isolated from gas oil-contaminated soil displays chemotaxis towards gas oil and hexadecane.** *Environ Microbiol* 2003, **5**:1002-1008.
26. Law AM, Aitken MD: **Bacterial chemotaxis to naphthalene desorbing from a nonaqueous liquid.** *Appl Environ Microbiol* 2003, **69**:5968-5973.
- Using a wild-type naphthalene-degrading strain and nonchemotactic and nonmotile mutants, this study reports direct evidence that bacterial motility and chemotaxis stimulate desorption and biodegradation of naphthalene dissolved in a non-aqueous phase liquid.
27. Nelson KE, Weinell C, Paulsen IT, Dodson RJ, Hilbert H, Martins dos Santos VAP, Fouts DE, Gill SR, Pop M, Holmes M *et al.*: **Complete genome sequence and comparative analysis of the metabolically versatile *Pseudomonas putida* KT2440.** *Environ Microbiol* 2002, **4**:799-808.
28. Jiménez JI, Minambres B, Garcia JL, Diaz E: **Genomic analysis of the aromatic catabolic pathways from *Pseudomonas putida* KT2440.** *Environ Microbiol* 2002, **4**:824-841.
29. Larimer FW, Chain P, Hauser L, Lamerdin J, Malfatti S, Do L, Land ML, Pelletier DA, Beatty JT, Lang AS *et al.*: **Complete genome sequence of the metabolically versatile photosynthetic bacterium *Rhodospseudomonas palustris*.** *Nat Biotechnol* 2004, **22**:55-61.
- The genome sequence of this metabolically interesting organism revealed numerous aspects of its varied lifestyle, including the presence of five aromatic ring cleavage pathways, three nitrogenases, two forms of RubisCo, the predicted presence of a circadian clock, and a wealth of transport and sensory systems.
30. Methé BA, Nelson KE, Eisen JA, Paulsen IT, Nelson W, Heidelberg JF, Wu D, Wu M, Ward N, Beanan MJ *et al.*: **Genome of *Geobacter sulfurreducens*: metal reduction in subsurface environments.** *Science* 2003, **302**:1967-1969.
31. Heidelberg JF, Paulsen IT, Nelson KE, Gaidos EJ, Nelson WC, Read TD, Eisen JA, Seshadri R, Ward N, Methe BA *et al.*: **Genome sequence of the dissimilatory metal ion-reducing bacterium *Shewanella oneidensis*.** *Nat Biotechnol* 2002, **20**:1118-1123.
- The genome sequence of this metal-reducing bacterium provides the basis for a detailed analysis of the organism's diverse respiratory abilities and metal-reducing potential. Numerous cytochromes and a [Fe] hydrogenase are predicted to participate in the metal reduction and electron transport processes.
32. Anderson RT, Vrionis HA, Ortiz-Bernad I, Resch CT, Long PE, Dayvault R, Karp K, Marutzky S, Metzler DR, Peacock A *et al.*: **Stimulating the *in situ* activity of *Geobacter* species to remove uranium from the groundwater of a uranium-contaminated aquifer.** *Appl Environ Microbiol* 2003, **69**:5884-5891.
- This study demonstrates the use of acetate to stimulate metal-reducing bacteria to precipitate uranium in a contaminated aquifer. The undesired reversibility of this beneficial process was also shown, highlighting the need for a detailed understanding of the process and controlled conditions for its use.