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BIOTOOL

Biological procedures for diagnosing the status and predicting evolution of polluted environments

Integrating and strengthening the European Research Area

Instrument: Specific Targeted Research Project

Thematic Priority 6 Sustainable development, global change and ecosystems

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Final Report

Period covered: September 2004 to December 2007
Date of preparation: January 2008
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Duration: 40 months

Project coordinator: PD Dr. Dietmar H. Pieper
Project coordinator organization: HZI

Contents

Publishable final activity report

1. Project execution

1.1. Summary of project objectives

The objective of BIOTOOL is the assessment, evaluation and prediction of natural attenuation processes to implement natural attenuation as the accepted key groundwater and soil remediation strategy in Europe. This will require benchmarked monitoring tools for diagnosing biological status and predicting evolution of contaminated soil and groundwater, which have to be rooted in biological processes. The generation and validation of such novel instruments will be materialized through the application of a suite of state-of-the-art genomic, proteomic and analytical technologies to environmental samples and sites themselves. We will exploit the translocation of indicator chemicals from below ground into above-ground vegetation as a cheap and rapid monitoring tool for subsurface contamination. Diagnosis of the biological status and evolution models for polluted environments will be achieved through [i] the design and utilization of DNA and specifically DNA-array technology for examining the catabolic potential of any given particulate sample and [ii] the identification of protein biomarkers as descriptors of soil and groundwater conditions and biological attenuation. The progress in microbial community functional genomics and proteomics will be employed to gain a mechanistic understanding of microbial responses to chemical insults, plant/microbe interactions and microbial community adaptations that determine microbial-driven soil and groundwater attenuation processes. Such mechanistic understanding will add a considerable predictive power to the genomic and proteomic approaches. Determining the links between environmental factors and expression of degradation abilities will be crucial for strategies aiming at an optimal expression of the catalytic power of the indigenous microbial community. The robustness of diagnostic instruments for future normative applications will be validated in microcosms and used for assessment of contaminated sites under study.

The specific objectives of the project were:

- **Establishment of the correlation between soil/groundwater contamination and plant contamination.**

- **The design and utilization of DNA and specifically DNA-array technology for examining the catabolic potential of any given particulate sample.**

- **The access and analysis of the soil/groundwater meta-proteome as biomarker.**

- **The use of lipid biomarkers as general prediction instruments of stress/toxicity on soil and groundwater microorganisms.**

- **Elucidation of the roles of natural and chemical stresses and plant/microbe interactions on the metabolic activities of soil and groundwater microbial catalysts.**

- **The robustness of above diagnostic instruments will be validated in microcosms and used for assessment of contaminated sites under study.**

1.2. Contractors

Partic. Role*	Partic. no.	Participant name	Participant short name	Country	Date enter project	Date exit project
CO	1	German Research Centre for Biotechnology[1]	GBF	D	Month 1	Month 40
CR	2	Consejo Superior de Investigaciones Cientificas, Centro Nacional de Biotecnologia	CSIC	ES	Month 1	Month 40
CR	3	Technical University of Denmark, DK	DTU	DK	Month 1	Month 40
CR	4	Ecole Polytechnique Federale de Lausanne	EPFL	CH	Month 1	Month 40
CR	5	Czech Academy of Sciences Institute of Microbiology	IMIC	CZ	Month 1	Month 40
CR	6	National Environmental Research Institute	NERI	DK	Month 1	Month 40
CR	7	Centre for Environmental Research Leipzig-Halle GmbH	UFZ	D	Month 1	Month 40
CR	8	KAP Ltd[2]	KAP	CZ	Month 1	Month 40
CR	9	BIONOSTRA S.L.	BIONOSTRA	ES	Month 1	Month 40

* CO = Coordinator
CR = Contractor

[1]Since 018.07.06 Helmholtz Centre for Infection Research (short name HZI)

[2]Since 01.01.05 EarthTech CZ Ltd. (short name Earth Tech)

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1.3. Work performed

For the project, experimental sites located in the Czech Republic and in Denmark that are polluted with chlorinated hydrocarbons or petroleum hydrocarbons have been selected. In the Czech Republic, the Hradcany site was used as a military airport from the Second Word War, founded by the German Army and, after an intermediate use by the Czechoslovak Army, the Soviet Army operated it from 1968 until 1990, when it was closed. First investigative and remedial works were commenced in 1986 and full-scale clean-up activities started in 1997. The site is a part of the Bohemian Cretaceous Basin, the most important source of high quality groundwater in the Czech Republic. Contamination limits future use and revitalization of the site. The North Bohemia Carcass Disposal Plant (SAP) Mimoň is one of the largest and most intensive chloroethenes contaminations of soil and groundwater in the Czech Republic. Perchloroethene was used in huge amounts in operation of the SAP Plant since 1963 to 1988. Frequent operational leakages caused a large plum of contamination. The sites have been characterized in detail regarding their geological and hydrogeological characteristics, soil and/or rock composition and global chemical analysis of unsaturated and saturated zone of the rock environment and thus supports background data. During the project, evolution of the experimental sites (soil and groundwater contamination changes, biodegradation activity characteristics) was followed. In addition cone penetrometry and membrane interface probing was used in order to obtain a precise geological cross section description and to evaluate vertical and horizontal distribution of contamination.

In order to exploit the translocation of indicator chemicals from below ground into above-ground vegetation as a cheap and rapid monitoring tool for subsurface contamination, a mathematical model describing uptake, translocation and volatilization of chemicals in / from trees was developed, parameterized and tested with lab and field data. The model was also adapted to simulate herbs and grasses, in order to select the best indicator species.

Generally, more polar, but persistent chemicals are translocated to the upper stem of trees growing on the polluted sites. Possible indicator chemicals would be chlorinated solvents perchloroethene, trichloroethene, dichloroethene, but not vinylchloride) and (chlorinated) phenols. Less applicable are BTEX, due to their low persistence in the root zone, and their high volatility. Not applicable are alkanes, due to their high vapour pressure. Out of the PAH, naphthalene is the only promising indicator chemical.

Laboratory studies on perchloroethene, trichloroethene, *cis*-dichloroethene, heavy metals, mono- and dichlororphenol were undertaken, in order to determine toxicity, calibrate the model, and find the relation between subsurface- and vegetation contamination. Simultaneously, an automated method for measurement of chemical activity of indicator chemicals in tree samples has been developed.

Process studies indicated that neither herbs nor grasses are well suited for monitoring, and that needle trees are better than deciduous trees for several reasons (rooting depth, wood structure, seasonal effects). Herbs and grasses cannot be used as indicator species for volatile compounds (chlorinated ethenes, BTEX), because the chemicals volatilize too fast from stem (and probably roots). Furthermore, these species do not root as deep as trees (in particular coniferes), and grow very fast, which leads to dilution of the chemicals. Heavy metals, such as copper and cadmium, can easily be found in leaves and wood. Thus, vegetation sampling can also be used to find subsurface contamination with several heavy metals.

Data from field campaigns showed that trees can clearly be used as indicators of the extent of subsurface contamination. Even though a correlation between trees and subsurface pollution could

be established in some cases, the correlations are not generally valid, it will not be possible to conclude from tree core measurements alone to the concentration and depth of subsurface pollution. In conclusion, tree core sampling must rather be seen as a way to assess the presence of pollutants than as a technique that allows determining the subsurface concentrations.
The method was applied at the SAP site on a larger scale. A before undetected plume of PCE was found employing the new method, which was confirmed by MIP, an independent method of physical soil exploration. It was proven that monitoring of the tree core chloroethene contamination is in very good relation with direct groundwater sampling in the area of interest. Based upon this result, this method is recommended to all environmental firms as effective, fast and cheep method for monitoring of chloroethene in shallow groundwater aquifers. A practical guide ("*A Guide to Vegetation Sampling for Screening of Subsurface Pollution*") gives guidance for the application of the method and summarizes the existing knowledge about features and limitations of the method. The guideline is distributed primarily via internet.

Initially, all necessary parameters were set for a systematic investigation of the effect of different chemical and physical stresses on growth and fatty acid composition of typical soil bacteria, with the goal to use lipid biomarkers as general prediction instruments of stress/toxicity on soil and groundwater microorganisms and to elucidate the roles of natural and chemical stresses on the metabolic activities of soil and groundwater microbial catalysts. From the data obtained from the PLFA analysis of different experimental systems investigated, it becomes evident that the *trans/cis* ratio of unsaturated fatty acids can be used as an excellent toxicity tool in laboratory experiments. However, this tool cannot be transferred to real bioremediation plants as it is only a real urgent stress response system and other adaptive mechanisms are in use in the case of long term adaptation. In contrast, a direct relation could be observed between the TPH (total petroleum hydrocarbon) concentration present in soils and the degree of saturation of the PLFA, describing the membrane rigidity of the microbial community as a useful stress parameter. Moreover, the analysis of the phospholipid fatty acids (PLFA) profile in contaminated soils is an elegant technique for a qualitative and quantitative assessment of the microbiota present. In the project we developed a method for rapid extraction of lipids from soil samples by the so-called Accelerated Solvent Extraction that allowed the higher throughput of samples. Using this method we were able analyze the status and follow the evolution of the experimental Hradcany site during different lifetime of the air sparging treatment. In detail, the monitoring showed that this method gives valuable information on: (i) the quantity of microorganisms present in the investigated soil (ii) the composition of the microbiota regarding the presence of eukaryotic (fungi, protozoa) and prokaryotic (bacteria) organisms (iii) the composition of the microbiota regarding the type of bacteria (Gram-negative or Gram-positive) that are predominating and (iv) the status of these bacteria with respect to the stress, usually caused by the presence of toxic pollutants. Analyses of respective taxonomical compositions showed a clear increase of bacterial diversity indicating that taxonomical diversity estimations may help to detect effective aerobic bioremediation, as the stimulation of certain members able to survive and degrade the compounds help to induce a shift towards an increasing complexity in the ecosystem under treatment
To further evaluate pollutant stress effects, DNA array technology was used to decipher the interplay between expression of catabolic genes and stress caused in a model soil bacterium. The pollutant toluene can be regarded as a potential nutrient, as a membrane-damaging toxic drug, and as a macromolecule-disrupting agent. Expression profiles suggest that the bulk of the available transcriptional machinery is reassigned to endure general stress, whereas only a small share of the available machinery is redirected to the degradation of the aromatic compounds.

Gene arrays dedicated to achieve a fast monitoring of catabolic gene diversity and abundance and thus the complete catabolic landscape and catabolic potential of microbial communities have successfully been developed and also been validated. By using the current knowledge on

biodegradation pathways of organic compounds in cultured bacteria, catabolic gene families coding for these activities and the molecular targets traced by culture-independent means in soil samples were defined. After an extensive literature mining on biodegradation pathways, an overall of eleven catabolic gene families catalyzing key steps of aerobic and anaerobic catabolic pathways have been selected. The databases to cover the microbial catabolome targeted for the Biotool microarray and other genetic fingerprinting applications comprises 1820 sequences. Validation included analysis of correctness of the array design using sequenced strains and mixtures, as well as verification and correlation with data obtained by catabolic gene fingerprinting (HZI) and metagenome surveys. Also a novel type of array capable to probe not only the catabolic potential for reductive dehalogenation, but also to identify organisms capable of reductive dehalogenation and the bacterial guilds which are potentially involved in the delivery of electron donors in such environments has been developed.
To further fine-tune catabolic gene analysis, various PCR primers for catabolic genes have been optimized and upgraded which allows targeted and detailed analysis of subgroups of important genes in study sites. The purpose of the development of molecular fingerprinting for a full array of catabolic gene families is the capability to rapidly determine the intrafamily diversity and predominance of gene variants in a extensive number of samples. Thereby obtained detailed pictures of the catabolic gene structure and sequence diversity in environmental samples are significantly increasing our knowledge of the functional potential of microbial communities and identification of shifts in catabolic gene structure allow the deduction of the evolutionary fitness of catabolic genes, operons and their respective hosts under changing environmental conditions. The methods have not only been adapted for the use with a capillary electrophoresis gene sequence analyzer, allowing very reproducible and semi-quantitative analyses but also by combination with a reverse transcriptase step to expression and thus catabolic activity studies.

However, PCR as well as hybridization based approaches to survey microbial communities involved in bioremediation and natural attenuation are limited by the number of sequences of genes available. One approach that does not rely on conserved nucleotide sequences is to use genomic libraries to retrieve genes from natural bacterial communities without cultivation. This functional environmental approach was successfully applied for screening of oxygenase enzymatic activities as key activities of aerobic BTEX degradation potential. Analysis of these libraries, which represented a wide fraction of the whole metagenome from two representative sites showed that abundance of key catechol 2,3-dioxygenase activity correlated with pollution level, indicating its usefulness as marker activity. Primer-based screening in coordination with other methods allowed identification of predominant catabolic gene families and indicated key subgroups. Activity-based meta-genome screening was thus validated as a suitable technology to analyze catabolic diversity and to characterize gene landscapes and gave indications on spatial and catabolic profile differences. This activity-based meta-genome screening was also suitable to recover biocatalysts with novel properties that have been selected by environmental pressure. Genetic analysis revealed a highly upgraded picture of catabolic gene diversity in contaminated environments, as subfamilies assumed to be important from culture-based studies and often used as marker for degradative potential were not abundant, but subfamilies expected from fingerprinting and array analysis were observed to be abundant also by the metagenome based approach.
However, one critical factor that limits the exploration of desired catabolic reactions is the recurrent difficulty of efficiently screening or selecting for genes of interest in large libraries of cloned DNA, in case the reactions are phenotypically silent. In this context, we have demonstrated a general approach to translate biotransformations lacking easily observable phenotypes into traits that can be selected for through the combination of evolved transcriptional regulator variants responsive to the product of a desired reaction with a suitable transcriptional response. By combining one *Pseudomonas* derived evolved transcriptional regulator variant responsive to 1,2,4 trichlorobenzene with a suitable transcriptional response, we were able to generate a genetic trap in which the action

of the gene for dehydrochlorination of hexachlorocyclohexane was eventually converted into detectable phenotypes. To allow an even wider applicability of metagenome based screenings, genetic tools to implement genetic traps for surveying metagenomic libraries in a variety of Gram-negative hosts were constructed and a broad host range genetic platform to format orthogonal sensor circuits in the chromosome of Gram-negative engineered as bioindicators for the production of given chemical compounds has been developed.
Microbial communities in bioremediation and natural attenuation scenarios however, do not only rely on appropriate biocatalytic activities but on a complex interaction between members of such communities. Aquifers, for example, are dynamic ecosystems showing complex interactions between physical, chemical and biotic components and can be considered as heterogeneous assemblages of discrete macro- and micro-scale habitats, providing a variety of living conditions, which influence the heterogeneous distribution of the microbial community structures and their inherent activities. Using optimized protocols for high-throughput community analyses and numerical ecology tools on various aquifers contaminated with chlorinated solvents we could shown that population diversity, and more specifically the diversity of the dehalorespiration guild alone, does not drive ecosystem stability and natural attenuation processes. The positive relationship between the presence of multiple pathways towards a product and functional stability parallels theoretical concepts in higher ecological organization. Ecosystem stability is the outcome not of population diversity *per se*, but of functional redundancy, which is ensured by the presence of a reservoir of species able to perform the same ecological function, even in very diverse and fluctuating environmental conditions. Recognizing the diversity and the links within each key functional group of a system can lead to better ways to model diversity and function, as well as helping to improve process stability.

In an approach complementary to molecular diagnostics we were attempting to include the application of state-of-the-art proteomic technologies for the identification of protein biomarkers, which are descriptive of soil status and predictive of its evolution. To explore and categorize proteins present in polluted sites in a fashion independent of the specific host that bears them, we set up a new technology for generation of natural single-chain antibody libraries against predetermined proteins by inoculation of camels (*Camelus dromedarius)*. *Camelidae* produce immunoglobulins that have only one variable domain that could recognize, by itself, the antigen. The amplified variable domain of a heavy-chain antibody of *Camelidae* is the simplest affibody that could recognize an antigen. As it is a smaller molecule the advantages are not only the specialized recognition of antigen but also better stability, easier fusion to other molecules and better recognition of crevices in the three-dimensional structure of the antigen. We have shown the power of camel antibody technology for production of large numbers of antibodies that recognize specifically given catabolic enzymes. The new angle of such a technology is the ability to innoculate the animal with a mixture of proteins and the recovery of active clones against each of them. While these antibodies offer very good opportunities for identification of distinct enzymes in in vitro assays, a suitable protein extraction procedure from soil that can be employed for revealing the biodegradative landscape remains to be established.
The study of the global properties of complex metabolic networks in organisms has prompted us a way to tackle the first models about the organization and evolution of environmental catabolic networks. To organize all the available information in a coherent database, with substantial capacity for the interaction with the experimental biologist working in this domain, MetaRouter, a Bioinformatics system for maintaining heterogeneous information related with Biodegradation had been developed (http://pdg.cnb.uam.es/MetaRouter/). This knowledge on biological systems was now used to develop a machine learning approach for the prediction of the "biodegradability" of new compounds, based on their chemical descriptors. The system allows the interpretation of the chemical characteristics of the compounds that are better related with the capacity of the biological systems to metabolize them, opening the possibilities for their study in the laboratory, and for

predicting the biodegradative fate of a new chemical compound before releasing it in the environment. As the extremely rapid pace of production of novel compounds by the chemical and pharmaceutical industry makes the detailed experimental assessment of their biodegradability virtually impossible, automatic predictive methods, such as the one of the Biodegradation Prediction Server (http://www.pdg.cnb.uam.es/BDPSERVER), are essential to evaluate the potential of new compounds to pollute the environment. The new predictive system may help to implement recent international regulations on the use of new chemicals.

In conclusion, BIOTOOL has established tree core analyses as innovative method, taking use of the qualitative relationship between soil/groundwater contamination and plant contamination, for rapid monitoring of subsurface contamination and subsurface biodegradation processes. The optimization of DNA as well as lipid extraction procedures in order to study the catabolic potential of contaminated environments has been successfully accomplished and a new kit for the efficient DNA extraction from polluted soil was developed. Novel or optimized tools (catabolome arrays, molecular fingerprinting, metagenomics, lipid profiling community diversity measures) were applied to the study sites and the combination of techniques derived from diverse scientific fields opens the way to a consistent strategy for predicting the actual behavior and future evolution of contaminated aquifers.

1.4. Biotool website

The website of Biotool project was launched the 7th of December of 2004, and it can be found at: www.gbf.de/biotools/
The site is intended to serve as a tool to inform general or specialized audiences about Biotool project aims and results, and also it is being used to speed up the communication between the project partners.

1.2. Dissemination and use

Catabolic genes fingerprinting methods

The purpose of the development of molecular fingerprinting for a full array of catabolic gene families is the capability to rapidly determine the intrafamily diversity and predominance of a certain gene polymorphism variant in a extensive number of samples, that otherwise would be very expensive and time consuming using the standard approach of PCR amplify, clone and random sequence. To generate an informative molecular fingerprint, and depending of the technique, there are a number of variables to optimize on every catabolic gene family targeted. The exploitable part of these developments consist in a collection of protocols for molecular fingerprinting generation based on PCR-single-strand conformation polymorphism (SSCP) technique, optimized for the catabolic gene families targeted. This implies the testing and optimizations of samples preparation, primer design, amplification conditions, electrophoretic conditions including between others gel matrix concentration, running temperature, buffering agents, or salt concentration.
Rieske non-heme iron oxygenases of the toluene/biphenyl subfamily are one of the key enzymes responsible for aerobic aromatic degradation The PCR-SSCP technique was optimized to assess their diversity and distribution in soil DNA and bacterial isolates. The central cores of genes encoding the catalytic α-subunits were targeted since they are responsible for substrate specificity of these enzymes.

Possible market applications or how they might be used in further research: Genetic monitoring of degradation potential and degradation processes

Stage of development: ready developed protocols

Collaboration sought or offered: Information exchange, training, consultancy

Results have been published: Witzig R, Junca H, Hecht HJ, Pieper DH, 2006, Assessment of toluene/biphenyl dioxygenase gene diversity in benzene-polluted soils: links between benzene biodegradation and genes similar to those encoding isopropylbenzene dioxygenases. Appl Environ Microbiol. 72:3504-3514.

Vegetation Sampling for Screening of Subsurface Pollution

Tree core sampling provides a rapid, reliable and inexpensive method to investigate the extent of shallow contamination by chlorinated ethenes in soil and groundwater. Tree core sampling can be used to detect and monitor not only plume distribution, but also to qualitatively assess natural attenuation. Tree cores may also be sampled at sites which are difficult to access with heavy equipment, for example private gardens in residential areas or dense forests.

A practical guide was developed to enhance the application of the method. The purpose of this practical guide (deliverable 20) is to encourage problem holders, consulting and and engineering companies to apply this method in order to save money and time, or where traditional methods are not applicable or provide problems (e.g., downtown areas).

Possible market applications or how they might be used in further research: Screening method for subsurface pollution; monitoring of natural attenuation.

Stage of development: developed for some chemical classes (chloroethenes, heavy metals), but large unexploited potential for other chemical classes. Practical utilization of the wood core sampling method developed by Danish BIOTOOL partners was tested on the SAP site by partner Earth Tech. It was proved that monitoring of the tree core chloroethene contamination is in very good relation with direct groundwater sampling in the area of interest. Based upon this result, this method is recommended to all environmental firms as effective, fast and cheep method for monitoring of chloroethene in shallow groundwater aquifers.

Collaboration sought or offered: Application of method in the field; training courses; consultancy; information exchange.

Results have been published: Larsen M, Burken J, Machackova J, Karlson UG, Trapp S (2007): Using tree core samples to monitor natural attenuation and plume distribution after a PCE spill. *Env. Sci. Technol.,* in print.

The tree core monitoring method was presented at several conferences
- DECHEMA 2005, Frankfurt;
- ISEB / ESEB / JSEB 2006 Leipzig;
- 2nd FEMS Congress of European Micorbiologists, 2006, Madrid;
- GdCh Gesellschaft Deutscher Chemiker Jahrestagung der Fachgruppe Umweltchemie und Ökotoxikologie, 2007, Osnabrück

Lipids as diagnostic tools

The fast and easy method of the analysis of phospholipid fatty acids (PLFA) in contaminated soils is an elegant technique for a first qualitative and quantitative assessment of the microflora present in that soil. In detail, the method can be used with respect to:

- a quantitative estimation of the amount of microorganisms present in the investigated soil
- a qualitative and quantitative estimation of the microflora present in the soil regarding the presence of eukaryotic (fungi, protozoa) and prokaryotic (bacteria) organisms
- a qualitative estimation of the type of bacteria (Gram-negative or Gram-positive) that is the predominant group in this soil.
- An expression of the status of these bacteria with respect to the stress, usually caused by the presence of toxic pollutants, present in the certain type of soil. Here, the risk caused by the pollutants as well as successful bioremediation process can be monitored

Possible market applications or how they might be used in further research: Rapid monitoring of microbial communities

Stage of development: ready developed protocols

Collaboration sought or offered: Information exchange, training, consultancy

A scientific article summarising these data was submitted to *Environmental Microbiology* in November 2007. A practical guide (deliverable 15) for the application of lipids as biomarkers was also prepared and will be spread among the interested scientific community.

DNA extraction kit

Optimization of DNA extraction from polluted soil has several advantages among other protocols already commercially available. Among these advantages are the following:

1. The optimized protocol is based on a chemical treatment which permits the extraction of genomic DNA >35 kb in size and the DNA shearing is avoided in comparison with DNA extraction methods based on mechanical procedures.
2. The optimized protocol is fast and requires only two specific buffers for the extraction step.
3. The extraction of DNA from Gram (+), Gram (-), yeast and fungi has been efficiently accomplished
4- The complete eradication of humic acids from the polluted soil allows the use of the DNA in following enzymatic procedures.
5- The protocol could be scaled up and down depending on the need of the final user.

Possible market applications or how they might be used in further research: The DNA extraction kit designed by BIONOSTRA will be exploited by the company. Among the companies interested in the distribution of the kit in Europe, CONDA Laboratories, internationally recognized for supplying key ingredients for research and testing, were selected to be the official distributors. The kit will be on the market during the first semester of the year 2008.

Stage of development: ready developed

Collaboration sought or offered: Commercial application

Genetic traps

The one critical factor that limits the exploration of desired catabolic reactions is the recurrent difficulty of efficiently screening or selecting for genes of interest in large libraries of cloned DNA. Many valuable reactions catalyzed by bacterial genes are phenotypically silent, so the only procedure to test their occurrence is the individual examination of the enzymatic reaction of interest in each of the clones. This hampers the discovery of novel enzymatic reactions or the improvement of existing ones. There is, therefore, a great need for alternative means to find novel variants of selected biocatalysts as well as altogether novel biological transformations, particularly in the absence of DNA sequence information on homologous enzymes.
In this context, CSIC has demonstrated a general approach to translate inconspicuous biotransformations (lacking easily observable phenotypes) into traits that can be selected for or screened with the panoply of genetic assets available for *E. coli*. The pilot construct was based on the properties of the transcriptional regulator XylR. By combining one evolved XylR variant responsive to 1,2,4 trichlorobenzene (TCB) with a suitable transcriptional response, CSIC was able to generate a genetic trap in which the action of a gene for dehydrochlorination of γ-HCH of was eventually converted into detectable lacZ-based phenotypes. This is just one example of the efficacy of natural or mutated effector-responsive transcriptional regulators for revealing the catalytic potential of any cloned DNA segment.
On that basis, CSIC has further improved the genetic tools for construction of genetic trap “a la carte” for specific biotransformations.

Possible market applications or how they might be used in further research: Iddentification of enzymes catalyzing novel reactions

Stage of development: ready developed

Collaboration sought or offered: Information exchange, consultancy

More information is available at: Mohn W, Garmendia J, Galvao T and de Lorenzo V. 2006. Surveying biotransformations with *à la carte* genetic traps: translating dehydrochlorination of lindane (γ-HCH) into *lacZ*-based phenotypes. *Env. Microbiol*. 8: 546-555.

Biodegradation prediction server

The extremely rapid pace of production of novel compounds by the chemical and pharmaceutical industry makes the detailed experimental assessment of their biodegradability virtually impossible. Automatic predictive methods are therefore essential to evaluate the potential of new compounds to pollute the environment. We have developed a machine learning approach for the prediction of the "biodegradability" of new compounds, based on their chemical descriptors.
Biodegradation Prediction Server: http://www.pdg.cnb.uam.es/BDPSERVER/

Possible market applications or how they might be used in further research: The new predictive system may help to implement recent international regulations on the use of new chemicals

Stage of development: ready developed

Collaboration sought or offered: Information exchange, consultancy

More information is available at: Gómez, M.J., Pazos, F., Guijarro, F.J., de Lorenzo, V. and Valencia, A. (2007). The environmental fate of organic pollutants through the global microbial metabolism. *Mol. Sys. Biol.* 3: 114.