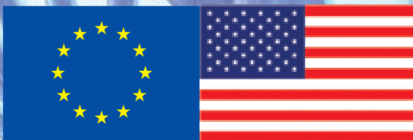

EC-US Task Force on Biotechnology Research

Workshop on Metabolomics and environmental Biotechnology

16-17 June 2008
Mallorca, Spain

CONFERENCE PROCEEDINGS

Interested in European research?

Research*eu is our monthly magazine keeping you in touch with main developments (results, programmes, events, etc.). It is available in English, French, German and Spanish. A free sample copy or free subscription can be obtained from:

European Commission
Directorate-General for Research
Communication Unit
B-1049 Brussels
Fax (32-2) 29-58220
E-mail: research-eu@ec.europa.eu
Internet: http://ec.europa.eu/research/research-eu

EUROPEAN COMMISSION

Directorate-General for Research
Directorate Directorate E "Biotechnology, Agriculture and Food"
Unit E2 — Biotechnologies

Contact: Ioannis ECONOMIDIS

European Commission
Office SDME 08/34
B-1049 Brussels
E-mail: Ioannis.Economidis@ec.europa.eu

European Commission

EC-US TASK FORCE ON BIOTECHNOLOGY RESEARCH

Metabolomics and Environmental Biotechnology

A Workshop of the Environmental Biotechnology Working Group of the EC-US Task Force
on Biotechnology Research - 16-17 June 2008 Palma de Mallorca, Spain

Luxembourg: Office for Official Publications of the European Communities, 2008

How to obtain EU publications

Our priced publications are available from EU Bookshop (http://bookshop.europa.eu), where you can place an order with the sales agent of your choice.

The Publications Office has a worldwide network of sales agents. You can obtain their contact details by sending a fax to (352) 29 29-42758.

EUROPEAN COMMISSION

EC-US TASK FORCE ON BIOTECHNOLOGY RESEARCH

Metabolomics and Environmental Biotechnology

A Workshop of the Environmental Biotechnology Working Group of the EC-US Task Force on Biotechnology Research

16-17 June 2008
Palma de Mallorca, Spain

edited by
Dr Balbina Nogales, Prof. Lily Young,
Dr Anna Palmisano and Dr Ioannis Economidis

Directorate-General for Research
2008 EC-US TASKFORCE ON BIOTECHNOLOGY RESEARCH EUR 23589 EN

Sponsored by:

European Commission (EC)

National Science Foundation (NSF)

US Department of Agriculture (USDA)

US Department of Energy (DOE)

Strategic Environment Research & Development Program (SERDP)

Office of Naval Research (ONR)

Office of Naval Research Global (ONRG)

Universitat de les Illes Balears (UIB)

Consejo Superior de Investigaciones Científicas (CSIC)

Societat Catalana de Biologia (SCB)

FOREWORD

Since 1990, the EC-US Task Force on Biotechnology Research has been coordinating transatlantic efforts to guide and exploit the ongoing revolution in biotechnology and the life sciences. The Task Force was established in June 1990 by the European Commission and the Office of Science and Technology Policy. The Task Force has acted as an effective forum for discussion, coordination, and development of new ideas for the last 18 years.

Task Force members are European Commission and US Government science and technology administrators who meet annually to enhance communication across the Atlantic, and to encourage collaborative research. Through sponsoring workshops, and other activities, the Task Force also brings together scientific leaders and early career researchers from both sides of the Atlantic to forecast research challenges and opportunities and to promote better links between researchers. Over the years, by keeping a focus on the future of science, the Task Force has played a key role in promoting a diverse range of emerging scientific fields, including biodiversity research, neuroinformatics, genomics, nanobiotechnology, neonatal immunology, biologically-based fuels, and environmental biotechnology.

The EC-US Task Force has sponsored a number of Working Groups on topics of mutual transatlantic interest. The idea to create a Working Group on Environmental Biotechnology research was discussed in the Task Force meeting of October 1993. The EC-US Working Group on Environmental Biotechnology set as its mission "To train the next generation of leaders in environmental biotechnology in the United States and the European Union to work collaboratively across the Atlantic." Since 1995, the Working Group supported three kinds of activities, all of which focus one early career scientists: 1) Workshops on the use of molecular methods and genomics in environmental biotechnology; 2) Short courses with theoretical, laboratory and field elements; and 3) Short term exchange fellowships. The short term exchange fellowships were created to enable young scientists to develop collaborations with colleagues across the Atlantic and to learn a new skill or expertise in the area of environmental biotechnology.

Working Group history

1994	First EC-US Workshop on Environmental Biotechnology held in Brussels. Working Group established.
1995	First Meeting of Working Group. Established mission and goals.
1996	EC-US Workshop on "Molecular and Biochemical Bases of Biodegradation" held in Granada, ES.
1998	US-EC Short Course on "Molecular Approaches for *in situ* Biodegradation" held at Rutgers University, NJ, USA.
2003	EC-US hands on short course on "Molecular Biology for the Environment" held at the Autonomus University of Madrid, ES.
2004	US-EC Workshop on "Genomics and Environmental Biotechnology" held at The Institute of Genomic Research (TIGR), MD, USA.
2005	EC-US Workshop on "A Celebration of a Decade of Environmental Biotechnology Exchange Activities" held at the European Commission, Brussels, BE.
2008	EC-US Workshop on "Metabolomics and Environmental Biotechnology," Mallorca, ES.

Fifteen years after its conception, the Working Group on Environmental Biotechnology continues its activities by organising a workshop on the use of metabolomics to advance the science of environmental biotechnology. The goal of the workshop, "Metabolomics and Environmental Biotechnology," is to bring together internationally-renowned senior scientists as well as 20 promising young scientists identified by the senior scientist participants.

On behalf of the EC-US Task Force on Biotechnology Research, we would like to thank the EU organisers of this workshop Dr. Balbina Nogales (U. de les Illes Balears, Palma de Mallorca, ES) and Dr. Juan Ramos (CSIC, Granada, ES). We also thank Dr. Lily Young (Rutgers, USA) for organizing the participation of the US scientists in the workshop. We are grateful for the many contributions of the other Working Group members and advisors including Drs. Spiros Agathos (U. Louvain, BE), Michael Bramucci (Dupont, USA), Victor de Lorenzo (CSIC, Madrid, ES), Jerome Kukor (Rutgers U., USA), Barbara Methè (JCVI, USA), Ivonne Nijenhuis (UFZ, DE), Joe Suflita (U. Oklahoma, USA), Judy Wall (U. Missouri, USA), and Gerben Zylstra (Rutgers U., USA). Finally, the coordinating efforts of the WG's executive secretaries Dr Anna Palmisano (Office of Science/US DoE) and Dr Ioannis Economidis (EC/ DG Research) are highly appreciated.

The Chairs of the EC-US Task Force on Biotechnology Research

Timothy Hall, Acting Director
European Commission

Kathie L. Olsen, Deputy Director
US National Science Foundation

The views expressed in this document are those of the workshop participants and do not necessarily reflect the views of the sponsors or governments.

WORKSHOP ON METABOLOMICS AND ENVIRONMENTAL BIOTECHNOLOGY

Agenda

Sunday, June 15, 2008. Arrival of participants. Registration.

Monday, June 16, 2008

8:30-9:00.	Registration and poster set-up
9:10-9:30.	Welcome and Opening Remarks
9:30-10:00	Presentation of the EC-US Working Group on Environmental Biotechnology (Dr. Anna Palmisano and Dr. Ioannis Economidis)
10:00-10:30	***Prof. Uwe Sauer.*** Quantitative fluxomics and metabolomics of microbial central metabolism
10:30-11:00	***Prof. Helena Santos***. Dynamics of intracellular metabolite pools in bacteria by NMR: input for systems biology
11:00-11:15	Coffee break
11:15-11:45	***Prof. Caroline S. Harwood.*** Development of a model bacterial system for hydrogen production
11:45-12:15	***Prof. Judy Wall.*** Metabolomic approaches to pathway discovery in *Desulfovibrio* strains
12:15-12:45	***Prof. Joseph M. Suflita.*** Metabolite profiling as a key tool for understanding the functioning of microbial communities in biocorroding pipelines on the North Slope of Alaska
13:00-14:15	Lunch
14:15-14:45	***Dr. Philippe Schmitt-Kopplin.*** Application of high resolution technologies for metabolomic approaches in environmental microbiology
14.45-15:15	***Prof. Ron S. Tjeerdema***. Application of NMR-Based techniques in aquatic toxicology
15:15-15:45	***Prof. J. Colin Murrell.*** DNA stable isotope probing, whole genome amplification and metagenomics
15:45-16:00	Coffee break
16:00-16:30	***Prof. Jizhong Zhou***. GeoChip-based metagenomic analysis of microbial communities in response to elevated CO_2, warming and contaminants.
16:30-17:00	***Dr. Wei E. Huang***. Application of confocal Raman microspectroscopy to the study of non-culturable microorganisms.
17:30-19:30	Poster discussion session.

20:30 Dinner

Tuesday, June 17, 2008

9:00-9:30 ***Prof. Victor de Lorenzo.*** The global microbial biodegradation network

9:30-10:00 ***Prof. Maia Kivisaar.*** Role of oxidative damage on evolution of *Pseudomonas putida* under conditions of nutrient starvation

10:00-10:30 ***Dr. Balazs Papp.*** Evolution of microbial metabolic networks: a systems biology approach

10:30-10:45 Coffee break

10:45-11:15 ***Dr. Barbara Methé.*** Identification of small non-coding RNAs in members of the Geobacteraceae

11:15-11:45 ***Prof. Dave Stahl.*** A novel electron transfer system determines methane production in a biogeochemically significant microbial mutualism.

11:45-12:15 ***Prof. Bess B. Ward.*** Microbial N cycling in estuarine sediments – Ecosystem and consortial scale patterns in microbial diversity and net nitrogen fluxes

13:00-14:15 Lunch

14:15-14:45 ***Prof. Nikolai Ravin.*** Revealing the biocatalytic potential of hyperthermophilic archaea from genome sequence data

14.45-15:15 ***Dr. Mary S. Lipton.*** Proteomic characterization of microbial communities: strategies and challenges

15:15-15:45 ***Prof. Kenneth N. Timmis.*** *Ferroplasma acidiphilum*: a unique microbe with a Fe-protein-dominated proteome

15.45-16:00 Coffee break

16:00-16:45 Discussion. Future perspectives. Conducted by Prof. Judy Wall and Prof. Victor de Lorenzo.

16:45-17:00 Closing remarks

21:00 Workshop dinner

Wednesday, June 18, 2008

Participants’ departure.

LIST OF POSTER PRESENTATIONS

P1. *Zamboni, N., J. Büscher, J. Ewald, D. Czernik, A. Kümmel, M. Heinemann, and U. Sauer.* Targeted metabolomics.

P2. *McKinlay, J., and C.S. Harwood.* Understanding and engineering metabolic pathways that participate in hydrogen production by photosynthetic *Rhodopseudomonas palustris.*

P3. *Fonseca, L.L., C. Sanchez, E. Voit, and H. Santos.* In vivo ^{13}C-NMR spectroscopy to monitor the kinetics of intracellular metabolite pools in *Saccharomyces cerevisiae* during adaptation to heat shock: input data for a multi-level model of the trehalose cycle.

P4. *Lucio, M., A. Fekete, and P. Schmitt-Kopplin.* Data driven systems biology based on a time dependent metabolomic approach using ultrahigh resolution mass spectrometry (ICR-FT/MS).

P5. *Van Scoy, A.R., C.Y. Lin, B.S. Anderson, B.M. Philips, M.R. Viant and R.S. Tjeerdema.* Impacts of crude versus dispersed oil in salmon as characterized by NMR-based metabolomics.

P6. *Parisi, V.A., LM. Gieg, R.C. Prince, and J.M. Suflita*. Metabolite profiling to characterize the functioning of microbial communities in a petroleum-contaminated subsurface ecosystem.

P7. *Chen, Y., M.G. Dumont, J.D. Neufeld, L. Bodrossy, N. Stralis-Pavese, N.P. McNamara, N. Ostle, M.J.I. Briones, and J. Colin Murrell.* Combining stable isotope probing and metagenomics, a powerful tool for ecologists.

P8. *Turse, J.E., S.J. Callister, N. Jaitly, L. McCue, M.J. Marshall, R.D. Smith, J.K. Frederickson, and M.S. Lipton.* Impact of comparative proteomics on analysis of microbial communities.

P9. *Huang, W., A. Ferguson, I.P. Thompson, R.M. Kalin, M. Larkin, M.J. Bailey, A.S. Whiteley and Y. Wang*. Linking in-situ microbial identity and functionality without the bias.

P10. *Bhadury, P. and B.B. Ward*. Molecular diversity of marine phytoplankton communities based on key functional genes.

P11. *Waldron, P.J., J.D. Van Nostrand, W-M. Wu, C.W. Schadt, D.B. Watson, Z. He, L.Y. Wu, C.S. Criddle, P.M. Jardine, T.C. Hazen, and J.Z. Zhou*. GeoChip applications to analyzing microbial communities in uranium contaminated sites.

P12. *Beloqui, A., D. Reyes Duarte, R. Torres, J.M. Vieites, J. Polaina, K.N. Timmis, P. Golyshin and M. Ferrer.* Access to unique dehalogenases: environmental metagenomic versus protein engineering.

P13. *Mardanov, A., E. Bonch-Osmolovskaya, and N. Ravin.* Molecular analysis of microbial communities from hydrothermal environments of Kamchatka volcanic area in Russia.

P14. *Pflüger, K., M. Chavarria, R.J. Kleijn, U. Sauer, and V. de Lorenzo.* Metabolic Master and Commander: the phosphotransferase system (PTS^{Ntr}) of *Pseudomonas putida.*

P15. *Keller, K.L.*. Developing inframe/markerless deletion techniques in *Desulfovibrio vulgaris* Hildenborough to study metabolic pathways.

P16. *Putrinš, M., H. Ilves, M. Kivisaar and R. Hõrak.* Relationship between membrane functions-regulating ColRS system and glucose flux in *Pseudomonas putida.*

P17. *Pósfa, G., T. Fehér, G. Plunkett III, D. Frisch, K. Umenhoffer, I. Karcagi, G. Balikó, Z. Győrfy, J. Campbell, F.R. Blattner.* Reduced genome *Escherichia coli*: a platform for genomic and metabolic engineering.

P18. *Hillesland, K.L. and D.A Stahl.* Evolution in a microbial mutualism affects community stability and productivity.

P19. *Ní Chadhain, S.M. and G.J. Zylstra*. Profiling the microbial community response to hydrocarbon challenges.

P20. *Martinez, P.M., A-B. Dohrmann, C.C. Tebbe, S. Weber, N. Stelzer, H-H. Richnow, and I. Nijenhuis*. Investigation of anaerobic degradation of monochlorobenzene using stable isotope tracers.

P21. *George, I., M.R. Liles, M. Hartmann, W. Ludwig, R.M. Goodman, and S.N. Agathos.* Changes in soil *Acidobacteria* communities after 2,4,6-trinitrotoluene contamination.

P22. *Lanfranconi, M.P., V. Capó, R. Bosch, and B. Nogales*. Changes in diversity and gene expression of marine bacterial communities under experimental hydrocarbon pollution.

ABSTRACTS

ORAL PRESENTATIONS

QUANTITATIVE FLUXOMICS AND METABOLOMICS OF MICROBIAL CENTRAL METABOLISM

Uwe Sauer, Stephanie Heux, Sarah-Maria Fendt, Roelco Kleijn and Nicola Zamboni

Institute for Molecular Systems Biology, ETH Zurich

The direction and rate of molecular fluxes through metabolic networks depend on thermodynamics, kinetic properties of the participating enzyme(s), and a complicated regulatory network that includes transcriptional and allosteric regulation. Hence, intracellular fluxes are the functional output of integrated biochemical and genetic interactions within complex metabolic networks that are pivotal for understanding of network operation (1). In contrast to the directly measurable concentrations of metabolites and proteins, however, fluxes are per se non measurable and must be inferred from other quantities. For this reason, quantification of intracellular fluxes has long lagged behind our capability to track global metabolite, mRNA or protein concentration changes.

With recent advances in ^{13}C-labeling methods, large-scale experimental analysis of intracellular fluxes is now feasible (2). Larger scale flux analysis now allows us to investigate general principles of how microbes distribute and manage their intracellular carbon traffic. A key focal area is quantitative analysis of how the flux through metabolic networks is controlled by the large regulation network. When is which regulation mechanism active, and which flux does it actually control? The growing body of available flux data allows also testing whether the detected flux distributions arise from general principles of network organization and operation (3).

While the generation of metabolomics data does not require mathematical models per se, it is rather difficult to infer biological meaning from metabolomics data without thorough computational analysis or mathematical modelling. A second challenge is to minimize systematic errors in quantitative metabolomics due to the multiple required processing steps and the large chemical diversity of metabolites. Here we use Network-embedded thermodynamics analysis (4) to i) assess the consistency of metabolomics data with thermodynamic principles and ii) to infer flux directionality for ^{13}C-flux analysis.

References:

1. Sauer, U. Mol. Sys. Biol. 2, 62-68 (2006).
2. Fischer, E. & Sauer, U. Nat. Genet. 37, 636-640 (2005).
3. Schütz, R., Küpfer, L & Sauer, U. Mol Sys. Biol. 3, 119 (2007).
4. Kümmer, A., Panke, S., Heinemann, M. Mol. Sys. Biol. 2, 34 (2006).

DYNAMICS OF INTRACELLULAR METABOLITE POOLS IN BACTERIA BY NMR: INPUT FOR SYSTEMS BIOLOGY

Helena Santos, Ana R. Neves, Paula Gaspar, Rute Castro and Ana L. Carvalho

Instituto de Tecnologia Química e Biológica, Rua da Quinta Grande, 6, Apt. 127, Oeiras, 2780-156, Portugal santos@itqb.unl.pt

Lactic acid bacteria (LAB) are used worldwide in the production of fermented dairy products. *Lactococcus lactis*, a model organism for LAB, is a suitable object for metabolic engineering strategies aiming at the improvement of food quality and human health. However, a deep understanding of the metabolic network as well as of the interdependence relationships at a system level is essential to a rational design of strains. To achieve this ambitious goal, powerful techniques

involving global transcriptome and proteome analysis need to be complemented with the analysis of the ensemble of metabolites in the cell, desirably as a function of time.

During the last decade our team directed a considerable effort to the development of in vivo NMR techniques with the goal to characterize and quantify metabolite pools directly in living cells [1-5]. It has become possible to monitor the dynamics of those pools in response to well-defined stimuli, such as a pulse of substrate, a switch in the gas atmosphere (anaerobic/aerobic) or pH, as well as the effect of a specific mutation. ^{13}C-NMR was used for the determination of the dynamic pools of intracellular metabolites with a time resolution of about 30 seconds, since NMR can measure continuously and non-invasively the actual amounts of ^{13}C-label incorporation into specific carbon atoms of different metabolic intermediates. ^{31}P-NMR was used to follow phosphorylated intermediates and the energetic status of the cells, giving a detailed picture of the metabolizing cell.

Examples of our work on the application of ^{13}C-NMR and ^{31}P-NMR to direct metabolic engineering strategies will be presented.

References:

1. R. Neves, R. Ventura, N. Mansour, C. Shearman, M. J. Gasson, C. Maycock, A. Ramos & H. Santos (2002) Is the glycolytic flux in *Lactococcus lactis* primarily controlled by the redox charge? Kinetics of NAD^+ and NADH pools determined *in vivo* by ^{13}C-NMR. J. Biol. Chem. 277, 28088-28098
2. P. Gaspar, A. R. Neves, A. Ramos, M. J. Gasson, C. A. Shearman & H. Santos (2004) Engineering *Lactococcus lactis* for the production of mannitol: high yields from food-grade strains deficient in lactate dehydrogenase and the mannitol transport system. Appl. Environ. Microbiol. 70, 1466-74
3. R. Neves, W. A. Pool, J. Kok, O. P. Kuipers & H. Santos (2005) Insights into sugar metabolism and its control in Lactic Acid Bacteria - The input from *in vivo* NMR studies. FEMS Rev. Microbiol. 29, 531-554
4. P. C. Lemos, Y. Dai, Z. Yuan, J. Keller, H. Santos & A. M. Reis (2007) Elucidation of metabolic pathways in glycogen-accumulating organisms with *in vivo* ^{13}C-NMR. Environ. Microbiol. 9, 2694-2706
5. E. O. Voit, A. R. Neves and H. Santos (2006) The intricate side of Systems Biology. PNAS, U.S.A., 103, 9452-9457

DEVELOPMENT OF A MODEL PHOTOSYNTHETIC BACTERIUM FOR HYDROGEN PRODUCTION

Caroline S. Harwood

Microbiology, University of Washington, 1959 NE Pacific Street, Mail stop 357242, Seattle, WA 98195-7242

Photosynthetic microbes can produce the clean-burning fuel hydrogen using one of nature's most plentiful resources, sunlight. Anoxygenic photosynthetic bacteria generate hydrogen and ammonia during biological nitrogen fixation. This reaction is catalyzed by the enzyme nitrogenase and consumes nitrogen gas, ATP and electrons. One bacterium, *Rhodopseudomonas palustris*, has a remarkable ability to obtain electrons from green plant-derived material and to efficiently absorb both high and low intensity light energy to form ATP. Manipulating *R. palustris*, or a similar organism, to produce hydrogen commercially will require us to identify all its genes that contribute to hydrogen production and to understand how this process is regulated in cells. We have obtained mutant strains of this bacterium for which growth depends on hydrogen production. In these mutants metabolism is redirected such that cells use nitrogenase as an electron sink and hydrogen-producing enzyme, and not as a catalyst for ammonia synthesis. We have used the mutants to show that, in addition to nitrogenase genes, 18 genes outside of the nitrogenase gene cluster may contribute to hydrogen production. Our results demonstrate that photosynthetic bacteria can be genetically manipulated for sustained production of pure hydrogen in a variety of cultivation

conditions in the absence of oxygen, nitrogen or other gases as long as light and an electron donor are supplied.

METABOLOMIC APPROACHES TO PATHWAY DISCOVERY IN *Desulfovibrio* STRAINS

Judy D. Wall

Biochemistry Department and Molecular Microbiology & Immunology, University of Missouri, Columbia, MO 65211 USA, Virtual Institute for Microbial Stress and Survival, http://vimss.lbl.gov

Genome sequences of anaerobes able to obtain energy for growth from sulfate reduction have been available since 1997 with the publication of that from the hyperthermophilic, sulphate-reducing archaeon *Archaeoglobus fulgidus* (Nature 390:364-70). Over half a dozen complete genome sequences are now available for delta-Proteobacteria that are sulfate reducers yet an understanding of the fundamental mechanisms of energy generation and carbon metabolism remain obscure. This knowledge is critical to the application of these bacteria to bioremediation processes or to the limitation of their apparent ability to corrode metals in anaerobic environments. An approach to the elucidation of incomplete metabolite pathways or to mapping the electrical circuitry used for energy generation is to follow the metabolites, determine their concentrations and fluxes when subjected to perturbations. This is the goal of metabolomics in its various forms. Multiple metabolomics approaches are now being used to identify the nature and function of the TCA cycle in the sulfate-reducer, *Desulfovibrio vulgaris* Hildenborough. ^{13}C tracer studies have confirmed the functioning of an unusual citrate synthase. In addition, the exometabolome of fermenting *Desulfovibrio desulfuricans* G20 is being examined in wild type and in a cytochrome mutant to elucidate the electron flow operational in the bacterium. These approaches and others will be discussed.

METABOLOMICS AS A TOOL TO UNDERSTAND BIOCORROSION IN NORTH SLOPE OIL PIPELINES

Joseph M. Suflita

University of Oklahoma

Biocorrosion remains a chronic problem in the oil and gas industry of major economic importance. Unfortunately, the underlying mechanisms and the requisite organisms responsible for biocorrosion are still poorly understood. We combined metabolomic, molecular, and cultivation procedures to investigate microbial communities in oil production facilities on the North Slope of Alaska. Samples were collected mostly from central processing facilities and from producing well heads. Universal and targeted PCR primers were used to obtain DNA sequences for the identification of organisms, GC-MS was used to interrogate over 70 putative metabolites associated with anaerobic hydrocarbon biodegradation, and enrichment procedures were used to quantify and isolate numerically dominant bacteria. Thermophilic *Archaea*, including sulfate-reducing *Archaeoglobus* species and methane-producing *Methanothermobacter thermautotrophicus* were abundant in the hot ($\geq 60^{o}C$) processing facility and well samples. A thermophilic *Anaerobaculum* sp. was the numerically dominant heterotroph in an oil-water separator. The described members of this genus reduce thiosulfate, sulfur, and cysteine to hydrogen sulfide and are thus also implicated in biocorrosion processes. However, all culturable bacteria screened (sulfate-reducing, anaerobic/facultative heterotrophs, hydrogen oxidizers) were found in low numbers ($\leq$ 5 cells/mL)

suggesting that these organisms would likely be missed in routine screening procedures. In contrast, DNA sequencing of PCR products amplified directly from the samples revealed 6 different species of thermophilic sulfate-reducing bacteria. Metabolomic analysis revealed that light hydrocarbons (C_1 to C_4) were being anaerobically metabolized and allowed us to hypothesize on an alternate mechanism for the anaerobic oxidation of methane. Collectively, these findings suggest that North Slope facilities harbor a thermophilic microbial community quite different from the mesophilic sulfate-reducing and acid-producing bacteria targeted by standard monitoring procedures. In addition, the recycling of low molecular weight hydrocarbons during normal oil production operations may help provide the carbon and energy necessary for pipeline microbial communities.

APPLICATION OF HIGH RESOLUTION TECHNOLOGIES FOR METABOLOMIC APPROACHES IN ENVIRONMENTAL MICROBIOLOGY

Schmitt-Kopplin, Ph., Lucio, M., Fekete, A., Kanavati, B., Hertkorn, N., Gaspar, A., Harir, M. and Gebefugi, I.

HMGU – German Research Centre for Environmental Health, Institute of Ecological Chemistry, Neuherberg, Germany

Recently we embarked on novel and significant joint projects which aim to understand biological systems and processes with molecular resolution. Interdisciplinary research actually operates in several networks at *Helmholtz Zentrum Muenchen* in which biologists, chemists as well as researchers from bioinformatics and other disciplines collaborate. In particular, the joint project *Molecular Interactions in the Rhizosphere* deals with the characterization of the plant-microbe interface and of microbe-microbe interactions in the rhizosphere and its implication for plant performance (health and quality), using the tools of functional genomics, proteomics and transcriptomics, together with metabolomics. Here, molecular-level analysis is aimed at an understanding of biological processes and discovery of new small molecule markers involved in the modulation, activation/deactivation of important known or yet unknown pathways in both involved partners: the microbes and the plant.

Complementary high resolution analytical approaches are used to characterize samples and describe molecular markers:

- targeted analysis - quantitative evaluation of concentrations of chemical (organic and inorganic, natural and anthropogenic, ambient to trace amounts) from various matrices after precise and adapted sample preparation.
- non-targeted analysis - new technologies are developed and optimized for a qualitative/semi-quantitative evaluation of the presence of chemical classes in complex mixtures – molecular inventory needed to allow process descriptions or discovery of new biomarkers.

In this presentation these possibilities will be presented in various metabolomic approaches for the biomarker research involving bacterial and plant extracts, biological fluids, and environmental samples.

References:

1. Rosselló-Mora, R., M. Lucio, A. Peña, J. Brito-Echeverría, A. López-López, M. Valens-Vadell, M. Frommberger, J. Antón, Ph. Schmitt-Kopplin, Metabolic evidences of biogeographic isolation of the extremophilic bacterium *Salinibacter ruber*. *Nature – ISME, ,* (2008), 2, 242-253.
2. Li, X., A Fekete, M. Englmann, M. Frommberger; S., Lv, G. Chen, Ph. Schmitt-Kopplin, At-line coupling of UPLC to chip-electrospray-FT/ICR-MS for the reliable structural confirmation of N-acylhomoserine lactones. *Anal. Bioanal. Chem.* (2007), 389, 1439-1446.

3. Hertkorn N., M. Meringer, R. Gugisch, C. Ruecker, M. Frommberger, E. M. Perdue, M. Witt , Ph. Schmitt-Kopplin, High-precision frequency measurements: indispensable tools at the core of molecular-level analysis of complex systems. *Analytical Bioanalytical Chemsitry*, (2007), 389, 1311-1327.

APPLICATION OF NMR-BASED TECHNIQUES IN AQUATIC TOXICOLOGY

R S Tjeerdema[1], **M R Viant**[2] **and C Y Lin**[3]

[1]Department of Environmental Toxicology, University of California, Davis, CA 95616, USA; [2]School of Biosciences, The University of Birmingham, B15 2TT, Edgbaston, Birmingham, UK; [3]Institute of Environmental Health, School of Public Health, National Taiwan University, Taipei, Taiwan

NMR has traditionally been used for chemical structure elucidation. However, in recent years it has been more broadly applied to the areas of biochemistry and toxicology. During the past 20 years we have applied *in vivo* ^{31}P NMR to characterize the sublethal biochemical actions of toxic chemicals in live, intact aquatic organisms under simulated environmental conditions. For example, using surface-probe techniques we have described the actions of both uncouplers of mitochondrial oxidative phosphorylation and inhibitors of electron transport in adult abalone under both optimal conditions and those which impose additional stress (altered temperature, salinity, etc.; 1, 2). All aquatic organisms live in areas where natural stresses may be numerous; they can potentially influence the actions of toxic chemicals. Thus, *in vivo* NMR can provide an accurate assessment of such actions under realistic conditions – and in real time. More recently, we have also applied NMR-based metabolomic techniques to elucidate the metabolic actions of toxic chemicals or other stresses (3-5). In all organisms, environmental and chemical stresses can cause impacts that can be characterized at many levels – via genomics, transcriptomics, proteomics or metabolomics. However, metabolic actions have the advantage in that they represent the sum total of actions at other levels. Unlike the *in vivo* approach, metabolomics involves the use of immediate cryogenic sample preservation and traditional high-field ^{3}H-NMR analysis. A key advantage is that numerous metabolic intermediates, osmolytes, amino acids and other biomolecules may be simultaneously monitored – providing a whole-organism assessment of metabolic health. We have used the approach to characterize the actions of a pathogen in abalone and pesticides or hydrocarbons in fishes. The metabolomic approach provides an assessment of sublethal actions under simulated environmental conditions. Such actions, while not directly lethal, can potentially produce enough energetic stress to alter or reduce other key functions, such as reproduction, which may serve to reduce populations over time.

References:

1. Viant, M. R., J. H. Walton, and R. S. Tjeerdema, 2001. Comparative toxic actions of 3-trifluoro-4-nitrophenol (TFM) in marine mollsucs as characterized by in vivo ^{31}P-NMR. *Pestic. Biochem. Physiol.* 71, 40–47.
2. Viant, M. R., J. H. Walton, P. L. TenBrook and R. S. Tjeerdema, 2002. Sublethal actions of copper in abalone (*Haliotis rufescens*) as characterized by in vivo ^{31}P-NMR. *Aquat. Toxicol.* 57, 139–151.
3. Viant, M. R., C. A. Pincetich, D. E. Hinton and R. S. Tjeerdema, 2006. Toxic actions of dinoseb in medaka (*Oryzias latipes*) embryos as determined by *in vivo* ^{31}P NMR, HPLC, and ^{1}H NMR metabolomics. *Aquat. Toxicol*. 76, 329–342.
4. Viant, M. R., C. A. Pincetich and R. S. Tjeerdema, 2006. Metabolic effects of dinoseb, diazinon, and esfenvalerate in eyed eggs and alevins of Chinook salmon (*Oncorhynchus tshawytscha*) as determined by ^{1}H NMR metabolomics. *Aquat. Toxicol.* 77, 359–371.
5. Rosenblum, E. S., M. R. Viant and R. S. Tjeerdema, 2006. Effects of the local environment on host-pathogen-drug interactions in red abalone determined by ^{1}H NMR metabolomics. *Environ. Sci. Technol.* 40, 7077–7084.

DNA STABLE ISOTOPE PROBING, WHOLE GENOME AMPLIFICATION AND METAGENOMICS

Prof. J Colin Murrell

Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, UK

We have developed DNA stable isotope probing (DNA-SIP) methods as tools to study the active microorganisms directly in the environment without the need for cultivation. This enables us to define "who does what" in the environment and link the phylogeny of microorganisms with their function. These techniques have been developed using methylotrophic bacteria which are involved in biogeochemical cycling of one carbon (C1) compounds such as methane and methanol in the environment. We have developed phylogenetic (16S rRNA) and functional gene probes in order to identify methanotrophs and methylotrophs directly in the environment. DNA stable isotope probing allows access to the genomes of these organisms without the need for cultivation in the laboratory. We have demonstrated "proof-of-principle" that DNA SIP can be combined with metagenomics in order to screen for genes involved in specific metabolic processes. A metagenomic library was constructed from ^{13}C- labelled DNA retrieved from forest soil incubated with ^{13}C-methane. A clone containing an entire methane monooxygenase gene cluster was retrieved and analysed.

One of the potential drawbacks of DNA stable isotope probing is that relatively long incubation times and relatively high concentrations of ^{13}C-labelled substrates are sometimes needed in order to obtain sufficient ^{13}C-labelled target DNA for metagenomic analysis. We have recently combined DNA-SIP with whole genome amplification techniques using Phi29 polymerase in multiple displacement amplification (MDA) of "heavy" ^{13}C-DNA retrieved from DNA-SIP experiments with environmental concentrations of C1 compounds. Subsequent construction and screening of fosmid libraries with this MDA method has allowed the retrieval of large genome fragments of key methanotrophs and methylotrophs in experiments conducted on marine and terrestrial samples using DNA-SIP experiments with short incubation times (hours-days) and micromolar concentrations of target substrates. The merits and limitations of these approaches will be discussed in the context of metagenomics of key groups of bacteria involved in C1-cycling in the environment and gene mining technologies.

References:

See also http://go.warwick.ac.uk/murrell/

1. Radajewski, S., Ineson, P., Parekh, NR and Murrell, J.C. (2000) Stable isotope probing as a tool in microbial ecology. Nature, 403, 646-649.
2. Dumont, C.G. and Murrell, J.C. (2005) Stable Isotope Probing – linking microbial identity to function. Nature Reviews Microbiology, 3, 499-504.
3. Dumont, MG, Radajewski, S.M., Miguez, C., McDonald, I.R. and Murrell, J.C. (2006) Identification of a complete methane monooxygenase operon from soil by combining stable isotope probing and metagenomic analysis. Environmental Microbiology, 8, 1240-1250.
4. Neufeld, J.D., Wagner, M and Murrell, J.C. (2007). Who eats what, where and when? Isotope-labelling experiments of complex microbial communities are coming of age. ISME Journal, 1, 103-110.
5. Neufeld, J.D., Chen, Y., Dumont, M.G. and Murrell, J.C. (2008) Marine methylotrophs revealed by DNA stable-isotope probing, multiple displacement amplification and metagenomics. Environmental Microbiology (online-early).

RECENT DEVELOPMENT AND APPLICATIONS OF GeoChip FOR MICROBIAL COMMUNITY ANALYSIS

Jizhong Zhou

*Institute for Environmental Genomics and Department of Botany and Microbiology, University of Oklahoma, Norman, OK; (*jzhou@ou.edu); Phone: 405-325-6073; Fax: 405-325-7552)*

Microarray technology provides the opportunity to identify thousands of microbial genes or populations simultaneously. The objective of this study is to further develop and apply a comprehensive functional gene array (GeoChip) to detect and monitor microbial communities at DOE ERSP sites during bioremediation processes. Based on GeoChip 2.0, a new generation, GeoChip 3.0 has been developed, which has several new features. First, GeoChip 3.0 contains ~25,000 probes and covers ~47,000 sequences for 292 gene families. Second, the homology of automatically retrieved sequences by key words is verified by HUMMER using seed sequences so that unrelated sequences are removed. Third, a universal standard has been implemented so that data normalization and comparison of different microbial communities can be conducted. Fourth, a genomic standard is used to quantitatively analyze gene abundance. In addition, GeoChip 3.0 includes phylogenic markers, such as *gyrB*. Finally, a software package has been developed to facilitate the management of such a complicated array, especially for data analysis and future update. Here, we have used GeoChips to successfully analyze microbial functional structure from a variety of environments such as: (1) Microbial communities within a pilot-scale test system established for biostimulation of U(VI) reduction in the subsurface by ethanol injection were examined during the long-term U(VI) reduction phase. The microarray data indicated that both FeRB and SRB populations reached their highest levels during U(VI) reduction, and that U concentrations in the groundwater were significantly correlated with the total abundance of c-cytochrome and *dsrAB* genes. Mantel test analysis of microarray and chemical data indicated a significant correlation between the U concentration and total c-cytochrome or *dsrAB* gene abundance. (2) In a different study of the same system, the effects of dissolved oxygen (DO) and ethanol on the stability of the bioreduced area were examined. Detrended correspondence analysis (DCA) of detected genes showed a shift towards a different community structure after ethanol injections resumed compared to the periods of starvation and exposure to DO. The overall results indicated that ethanol was the main factor affecting community structure. (3) In the third study, groundwater monitoring wells along a contamination gradient were examined. Diversity of nitrate-fixation genes decreased in NO_3^--contaminated wells while signal intensities of metal resistance and reduction genes increased in heavily contaminated wells. Sulfate-reduction genes had greater diversity and greater signal intensity in more contaminated wells. CCA analysis showed pH was an important variable, while NO_3^- and U correlated with the highest contaminated well. (4) We have also used GeoChip to examine a UMTRA site (Rifle, CO). Cluster analysis showed samples in the same locations grouped together, regardless of geochemistry. CCA analysis of environmental parameters and functional genes indicated Fe^{2+} was the most significant geochemical variable for community structure. These studies demonstrate the analytical power of the GeoChip in examining microbial communities and its ability to provide direct linkages between microbial genes/populations and ecosystem processes and functions.

APPLICATION OF CONFOCAL RAMAN MICROSPECTROSCOPY TO THE STUDY OF NON-CULTURABLE MICROORGANISMS

Wei E. Huang

Kroto Research Institute, University of Sheffield, Broad Lane, S3 7HQ, Sheffield, UK

Raman Microspectroscopy is a non-invasive technology to acquire characteristic chemical information from <1 micron area of a material. It has been applied to classify different bacterial species and characterise bacteria states, all at a single cell level. Combined with other techniques (e.g. optical tweezers, fluorescent in situ hybridisation and stable isotope probing), Raman microspectroscopy is now developed to a valuable tool of the study and manipulation of unculturable microorganisms which account for more than 99% microbial species in natural environment. It opens a new frontier for better understanding biodegradation of contaminants, biogeochemical cycles and discovery new enzymes and medicines.

SYSTEMS BIOLOGY OF BIODEGRADATION: THE GLOBAL MICROBIAL CATALYTIC NETWORK

Víctor de Lorenzo

Centro Nacional de Biotecnología, CSIC Madrid 28049 (Spain) vdlorenzo@cnb.csic.es

The increasing amount of information on strains, compounds, enzymes and reactions implicated in microbial biodegradation of toxic pollutants provides us with the building blocks for formulating a global *biodegradation network.* This issue is directly connected to *Systems Biology,* which complements the traditional study of genes and proteins as isolated entities with a perspective that regards biological systems as consisting of components in a network of complex relationships. In these systems, the whole is more than the sum of the parts, and some of the properties of the system cannot be understood from the properties of its individual components, thus requiring the study of the network as a whole. We have studied the structure of the microbial catalytic network that results from pooling all know biodegradation reactions irrespective of the strain where singular biotransformations were found. The topology and connectivity resulting from this analysis indicate that the network architecture is highly organized as thus likely to have been subject to evolutionary selection. Furthermore, the distribution of the different catabolic steps suggests that microbial communities acquire a superior ability to degrade many compounds that cannot be metabolized otherwise by single strains. This is brought about by integration of catalytic steps contributed by distinct members of the consortium in a sort of super-metabolism. We have exploited such properties to train a rule-based classification system for detecting the association between certain chemical compound descriptors and environmental fates. Such descriptors are based on the deconstruction of chemical structures in atomic triads (also referred to as *chemotopes*). A machine learning system was used to identify explicit rules that associate compound vectors to environmental fates as inferred from the analysis of the metabolic network (that represents the global biodegradative potential of microorganisms). Finally, a scheme to predict the fate of new chemical compounds, using the previously identified rules, was implemented as a web server. The results obtained include the evaluation of the prediction capacity of the system and its application to several sets of compounds provided by the European Chemicals Bureau or obtained from the database PubChem Compound -for most of which there are no data on their biological fate. We argue that the frequency of atomic triad presets the susceptibility of the compounds to the global biodegradation network. Our analyses suggest that enzymatic activities of catabolic pathways co-evolve to target discrete molecular motifs which can be shared by many chemicals, rather than

adapting to deal with specific molecules, with obvious consequences for the evolution of the substrate recognition sites of the enzyme pool. It is thus plausible that confrontation of a diverse microbial community with a mix of chemical compounds (i.e., the most frequent environmental pollution scenario) results in the encounter of a multi-species biodegradation network with a landscape of *chemotopes* rather than dealings of single types of bacteria with unique chemical species.

References:

1. 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.
2. Pazos, F., Guijas, D., Valencia, A., de Lorenzo, V. (2005) MetaRouter: Bioinformatics for Bioremediation. *Nucl. Acids Res.* 33: D588-D592
3. Pazos, F., Valencia, A., and de Lorenzo, V. (2003) The organization of the microbial biodegradation network from a systems-Biology perspective. *EMBO Reports* 4: 994-999.

ROLE OF OXIDATIVE DAMAGE ON EVOLUTION OF *Pseudomonas putida* UNDER CONDITIONS OF NUTRIENT STARVATION

Andres Tover, Radi Tegova, Signe Saumaa, Kairi Tarassova, and Maia Kivisaar

Institute of Molecular and Cell Biology, Tartu University and Estonian Biocentre, 23 Riia Street, 51010 Tartu, Estonia. E-mail maiak@ebc.ee

RpoS is a bacterial sigma factor expressed in response to starvation, pH, osmolarity and temperature stresses. Several lines of evidence indicate that expression of RpoS can influence frequency of point mutations and transposition. For example, error-prone DNA polymerase Pol IV is up-regulated by RpoS in *E. coli* starving cells (1). In contrary to that, we found that expression Pol IV gene *dinB* in *P. putida* is not dependent on RpoS (2) and the frequency of occurrence of Pol IV-dependent 1-bp deletions is the same in wild type strain and in its *rpoS*-defective derivative. At the same time, the frequency of emergence of base substitution mutants (base substitutions occur independently from Pol IV) is increased up to 10-fold in long-term-starved populations of *rpoS*-deficient *P. putida* cells. Reducing cellular amount of free oxygen radicals by over-expressing superoxide dismutase or catalase in RpoS-defective strain resulted in decline of mutation frequency. This indicates that reactive oxygen species accumulate during starvation of RpoS-defective bacteria.

Oxidative damage of DNA is an important source of mutation in living cells. It is known that guanine oxidation product 8-oxoG (or GO) can give rise to mutations in starving bacteria. The GO repair system composed of the DNA glycosylases MutY and MutM, and the nucleotide hydrolase MutT, is involved in limiting the mutagenic effect of 8-oxoG. Our results demonstrate that all enzymes of GO repair system are involved in the prevention of base substitution mutations in starved *P. putida* (3). The absence of GO repair enzymes in *P. putida* leads to accumulation of oxidative DNA damage, increase in mutation frequency and characteristic spectrum of mutations. At the same time, the spectrum of base substitution mutations characterized in RpoS-defective starving bacteria is different from that identified in bacteria lacking GO repair system and almost similar to that of wild-type bacteria. These results indicate that elevation of mutation frequency in long-term-starved RpoS-defective *P. putida* is not caused by saturation of GO repair system due to accumulation of oxidative damage of DNA. Importantly, we have observed that viability of RpoS bacteria is declined up to one order of magnitude in long-term-starved populations. It has been argued that the increased mutation frequency of stationary-phase cells does not appear to limit cellular life span and DNA does not seem to be the primary target for oxidative damage in these cells. Bacterial stasis results in an increase and differential oxidation of target proteins (4). Hence,

we hypothesize that accumulation of oxidatively damaged proteins may affect DNA replication fidelity and thereby increase mutation frequency in starving RpoS-defective *P. putida*.

References:
1. Layton, J.C., and Foster, P.L. (2003) Mol. Microbiol., 50:549-561.
2. Tegova, R., Tover, A., Tarassova, K., Tark, M., and Kivisaar, M. (2004) J. Bacteriol. 186:2735-2744.
3. Saumaa, S., Tover, A., Tark, M., Tegova, R., and Kivisaar M. (2007) J. Bacteriol., 189:5504-5514.
4. Nyström, T. (2004). Annu. Rev. Microbiol., 58:161-181.

EVOLUTION OF MICROBIAL METABOLIC NETWORKS: A SYSTEMS BIOLOGY APPROACH

Balázs Papp[1], Csaba Pál[1] and Martin Lercher[2]

[1] *Evolutionary Systems Biology Group, Institiute of Biochemistry, Biological Research Center, Szeged, Hungary*
[2] *Institut für Informatik, Heinrich-Heine-Universität, Düsseldorf, Germany*

How metabolic networks evolve and adapt to ever-changing environmental conditions? Besides altering the kinetic properties or regulation of already existing enzymes, successful adaptation might often hinge on changing the organism's enzyme repertoire by gene gain and loss events.

We integrated comparative genomics with systems biology modelling to examine the recent expansive and reductive evolution of the *E. coli* metabolic network. First, we ask how new metabolic genes are added to the network. What is the contribution of gene duplication and horizontal gene transfer to recent network evolution? What are the selective forces driving network evolution and how new enzymes are integrated into the network?

Second, we ask how evolution can lead to a minimal set of genes sufficient to sustain cellular life by studying the reductive genome evolution of an endosymbiotic bacterium *Buchnera*, a close relative of *E. coli*. Is it possible to predict the enzyme content of *Buchnera* with knowledge of its ancestor and its current lifestyle? And what is the role of chance and necessity in reductive network evolution? Are evolutionary outcomes contingent on prior gene loss events? We investigated these issues by simulating enzyme deletions in an in silico representation of the *E. coli* metabolism.

IDENTIFICATION OF SMALL NON-CODING RNAs IN MEMBERS OF THE GEOBACTERACEAE

Barbara Methé

The J. Craig Venter Institute, Rockville, MD 20850, (bmethe@jcvi.org)

Members of the Geobacteraceae are the subject of comprehensive studies related to their ability to degrade carbon compounds including many contaminants with the reduction of Fe(III). Further interest in these organisms stems from the practical biotechnological roles that they can play as agents of bioremediation and in energy production. We have begun several analyses that leverage information available from the genome sequences of multiple members of the Geobacteraceae to obtain new insights into their regulation.

In order to better understand the regulatory network of the model organism, *G. sulfurreducens*, as well as other members of the Geobacteraceae, we have initiated a study of chromosomally located small non-coding (sRNAs). Increasing evidence suggests that sRNAs exist in numerous organisms where they play important regulatory roles including responses and adaptations to different stresses. We have generated predictions of sRNA candidates through use of the program, sRNAPredict, along with custom written scripts, to compare the *G. sulfurreducens* genome to a total of seven members of the Geobacteraceae.

From the current searches, 88 predictions were generated for *G. sulfurreducens*: None of the predictions from *G. sulfurreducens* were found in all of the other seven genomes tested although 17 were found in six of the seven. However, it cannot be ruled out that the distributions of sRNAs could change as three of the test genomes used in these searches are still in a draft phase. The majority of the predictions (34) were found in only one of the other seven genomes. Of the 88 predictions, seven have matches to covariance models to non-coding RNAs in the Rfam database. This includes a match to a 6S RNA which is believed to associate with the RNA polymerase holoenzyme containing the sigma70 factor and repress expression from a sigma70-dependent promoter. The presence of this sRNA has been experimentally verified through the use of Northern blot hybridizations and sequence specific primers designed to amplify the sRNA from a population of cDNAs.

Additional analyses are currently underway to examine whether or not the physical location of a particular family of sRNAs in a query genome is a random event when compared to a family of genomes, or if syntenic relationships are maintained. This question has relevant implications in terms of genome evolution and in enhancing the identification of sRNA predictions. Further, we have initiated a sequenced-based screening of cDNA libraries made from the small molecular weight fraction of total RNA representing different physiological conditions.

A NOVEL ELECTRON TRANSFER SYSTEM DETERMINES METHANE PRODUCTION IN A BIOGEOCHEMICALLY SIGNIFICANT MICROBIAL MUTUALISM

David A. Stahl[1], Christopher B. Walker[1], Sergey Stolyar[1], Zhili He[2], Zamin K. Yang[2], Janet Jacobsen[2], Joseph A. Ringbauer, Jr. [2], Qiang He[2], Jizhong Zhou[2], Gerrit Voordouw[2], Judy D. Wall[2], Adam P. Arkin[2] and Terry C. Hazen[2]

[1] *Department of Civil and Environmental Engineering, University of Washington, Seattle WA, 98195, USA*
[2] *Virtual Institute for Stress and Survival*

The cycling of key biogenic elements (primarily C, N and S) is the foundation of the biosphere, yet relatively little is known about metabolic interactions that both sustain nutrient cycles and partition available free energy among participating microbial populations. The sharing of available energy is particularly important in anaerobic systems, where limited energy is divided among highly specialized and metabolically interdependent populations. A key interdependence, often at the thermodynamic threshold for cell growth, is between those populations producing and those consuming hydrogen. A significant fraction of biogenic methane depends upon this type of interspecies hydrogen transfer. Methanogens require hydrogen produced by fermenting populations, which in turn depend upon methanogenic hydrogen removal for sustained fermentation. We have used a combination of flux balance modeling, and transcriptional and mutant analyses to examine the metabolism of a model community of two, composed of a *Desulfovibrio* species in syntrophic pairing with a hydrogenotrophic methanogen. These studies showed that the desulfovibrio up-regulates a broad suite of electron transfer enzymes during

syntrophic growth. Mutants in four of these enzymes (Coo, Hmc, Hyd and Hyn) impaired syntrophic growth but had little or no affect on sulfate-respiration, suggesting that a separate and dedicated electron transfer pathway is required for syntrophy. More generally, these results show the importance of moving beyond "the pure culture" for more complete understanding of metabolic systems sustaining key biogeochemical cycles.

MICROBIAL N CYCLING IN ESTUARINE SEDIMENTS – ECOSYSTEM AND CONSORTIAL SCALE PATTERNS IN MICROBIAL DIVERSITY AND NET NITROGEN FLUXES

Bess B. Ward

Department of Geosciences, Guyot Hall, Princeton University, NJ08544, USA
Email: bbw@princeton.edu

Ecosystem scale patterns in nitrogen fluxes characterize the land-margin interface, such as exemplified by Chesapeake Bay, the largest estuary on the east coast of the USA. In the upper freshwater end members of the bay, large lacustrine inputs, dominated by anthropogenic land use, produce net inputs of fixed nitrogen that drive eutrophication and overwhelm the magnitude of natural sediment-water exchanges. By contrast, large ammonium fluxes into the water column from anoxic sediments in the mid and lower bay make an important contribution to local primary production, and are partly balanced by large oxidized nitrogen fluxes into the sediments. Net fluxes of these microbial metabolites result from complex consortial interactions among bacteria and archaea involved in N cycling. The degree to which we remain ignorant of the nature of these interactions is highlighted by the fact that two very important groups, the anammox bacteria and ammonia-oxidizing archaea, were discovered in nature only in the last few years. We suggest that one reason for our long ignorance is the inability of conventional microbial ecology to detect and evaluate consortia, and our failure to recognize the extent to which biogeochemical cycles depend on close consortial relationships. The ecosystem scale patterns in net N fluxes likely result from variations in consortial assemblages and functional guild composition. We will report on our previous work on the distribution and diversity of several groups of bacteria involved in N cycling in Chesapeake Bay, using information derived from functional gene microarrays and Q-PCR to quantify functional groups, and biogeochemical rate measurements, in the context of multivariate analysis of environmental factors. These findings suggest that different kinds of microbial interactions dominate at different places in the estuarine gradient and yield hypotheses about the composition of microbial consortia in both water column and sediment environments.

REVEALING THE BIOCATALYTIC POTENTIAL OF HYPERTHERMOPHILIC ARCHAEA FROM GENOME SEQUENCE DATA

Nikolai Ravin[1], Andrey Mardanov[1], Elisaveta Bonch-Osmolovskaya[2] and Konstantin Skryabin[1]

[1]Centre “Bioengineering” RAS, Moscow, Russia; [2]Institute of Microbiology RAS, Moscow, Russia

Thermophilic prokaryotes are regarded as the important source of industrially valuable genes because of high rates of biochemical processes they perform and high stability of enzymes involved. The full enzyme set of selected prokaryotes may be revealed by complete sequencing of their genomes. Recently we determined genome sequences of five hyperthermophilic archaea. One

of them, crenarchaeote *Desulfurococcus kamchatkensis*, was isolated from the hydrothermal spring (Kamchatka volcanic area, Russia) with temperature 82°C and pH 6.8. The strain is able to grow anaerobically on peptone, meat extract, yeast extract, α-keratin (pork hair), albumin, gelatin, chitin, agarose, sucrose, arabinose being used as the energy and carbon source. The genome project combined traditional ABI-Sanger sequencing and 454 pyrosequencing approaches. We found that *D. kamchatkensis* genome comprises one circular chromosome of 1366949 bp and harbour total 16 mobile elements including 8 copies of ISC1913-like transposon, 7 copies of MITE derived for it and a single copy of a transposon of IS200/IS605 family. Two large clusters of regularly interspaced repeats (CRISPRs) are present, associated with a crenarchaeal-type *cas* gene superoperon. We identified a set of tRNA and rRNA genes as well as 1489 potential protein-coding genes. Many of the predicted metabolic gene products are associated with the fermentation of peptide mixtures including several peptidases with diverse specificities. New genes encoding thermostable proteases, amylases, pullulanases, alpha and beta-glucosidases have been identified. Particularly interesting for biotechnological applications in food industry is heat-stable subtilisin-like protease able to degrade different hardly degradable proteins like keratins.

PROTEOMIC CHARACTERIZATION OF MICROBIAL COMMUNITIES: STRATEGIES AND CHALLENGES

Mary S. Lipton, Stephen J. Callister, Joshua E. Turse, Angela D. Norbeck, and Carrie D. Nicora

Biological Sciences Division; Environmental Molecular Sciences Laboratory; Pacific Northwest National Laboratory. E-mail: mary.lipton@pnl.gov

In nature, microbes rarely exist in pure culture form, but as populations within communities of organisms acting in both a competitive and synergistic manner. Microbially mediated environmental processes depend on the functional activities of the individual organism, populations, and at the level of the community. While function is predicted to be conferred to individual organisms by genes encoded in its genome, each gene must be expressed into a functioning product (i.e. a protein) in order for the microbe to perform its function in nature. Integral to understanding the function and activity of the microbial community is the in-depth characterization of the components of the communities, the activity and function of each of the components and how they interact with each other. However, the complexity of the community and in many cases the absence of a sequenced meta-genome present challenges to this type of characterization.

Central to proteomic analysis is the availability of a genome sequence, however, for many communities, sequence information is either unfinished or absent altogether. To address this challenge in the lack of genomic information, we are pursuing the characterization of a community using sequences closely related to the species identified in the community from the 16S rDNA methodology. The community was extracted from a uranium contaminated flood plain (Rifle, CO) groundwater sample and evaluated in the absence of a metagenome sequence. Preliminary findings suggest that the manner in which the genome sequences were used (a concatenated *Geobacter* sequence, a condensed *Geobacter* sequence, etc.) to generate the database of observed peptides played an important role in evaluating this response.

Other challenges rest in the ability to link functional characteristics with protein identifications. In efforts to characterize the open ocean community in relation to *Peligabacter ubique,* we have found significant strain heterogeneity in the species present and how significant expression of the proteins involved in transport of metabolites and metals are indiciative of the environmental metabolic requirements of this organism.

The proteome characterization of these microbial communities presents a challenging application, and we are in the early stages of seeking to understand the ecology of these communities at the protein expression level and how this protein expression relates to the interaction of microbe with the environment and within the community. These initial studies demonstrated that proteomic characterization of microbial communities is feasible, but requires a significant integrated effort involving experts in the areas of microbiology, proteomics technology development, and bioinformatics.

Acknowledgements:
This research is supported by the Office of Biological and Environmental Research of the U.S. Department of Energy. Pacific Northwest National Laboratory is operated for the U.S. Department of Energy by Battelle Memorial Institute through Contract No. DE-AC05-76RLO 1830.

***Ferroplasma acidiphilum*: A UNIQUE MICROBE WITH A FE-PROTEIN-DOMINATED PROTEOME**

M. Ferrer[1,2], O. V. Golyshina[2], A. Beloqui[1], P. N. Golyshin[2] and K. N. Timmis[2]

[1]Laboratory of Enzyme Discovery, CSIC - Institute of Catalysis, Marie Curie 2, Madrid; [2]Environmental Microbiology Laboratory, HZI, Braunschweig

Ferroplasma[1] is a genus of the Archaea, one of the three branches of the tree of life, and belongs to the order Thermoplasmatales (Euryarchaeota), which contains the most acidophilic microbes yet known. *Ferroplasma* species live in acid mine drainage, acidic pools and environments containing sulphidic ores such as pyrite and characterized by pH values of 0–2 and high concentrations of ferrous iron and other heavy metals[2]. *F. acidiphilum* strain YT (Fa) is a chemoautotroph that grows optimally at pH 1.7 and gains energy by oxidizing ferrous iron and carbon by the fixation of carbon dioxide. Analysis of its “metalloproteome” unexpectedly revealed that 86% of 189 unambiguously-identified cellular proteins of Fa are iron-metalloproteins[3]. These include proteins with deduced structural, chaperone and catalytic roles, not described as iron-metalloproteins in any other organism so far investigated. Removal of the iron atoms results in protein unfolding, so they seem to organize and stabilize the three-dimensional structures of polypeptides, to act as ‘iron rivets’. Analysis of the phylogenetic neighbour *P. torridus* and of the habitat neighbour *A. ferrooxidans* revealed far fewer and only typical metalloproteins.

Purification and characterization of several intracellular Fa proteins has revealed that they have pH activity optima 2-3 pH units below the reported cytoplasmic pH[4,5], a feature we have termed “the pH optimum anomaly”. One of the most extraordinary iron proteins, purple DNA ligase (LigFa), unlike any other DNA ligase studied to date, contains two Fe^{3+}-tyrosinate centres, is deep purple in colour, lacks any requirement for either Mg^{2+} or K^{+} for activity, and has an extremely low pH activity optimum[6]. DNA ligases from closest phylogenetic and ecophysiological relatives have normal pH optima (6.0-7.5), lack iron, and require Mg^{2+}/K^{+} for activity. Ferric iron retention is pH dependent, with release resulting in partial protein unfolding and loss of activity. Reduction of the Fe^{3+} to Fe^{2+} results in an 80% decrease in DNA substrate binding and an increase in the pH activity optimum to 5.0. Amino acid residues determining the pH activity optimum and iron binding have been identified.

Fa has a unique iron-protein-dominated cellular machinery, in which the iron plays the role of a structure organizer, hinting that Fa may have a unique evolutionary trajectory. The possibility that life originated in pyritic environments suggests that this evolutionary trajectory may have occurred in such environments, isolated from the radiative colonization of other environments that characterized early evolution.

References:

1. Golyshina, O. V.,et.al. *Ferriplasma acidiphilum* gen. nov., sp. nov., an acidophilc, autotrophic, ferrous-iron-oxidizing, cell-wall-lacking, mesophilic member of the *Ferroplasmaceae* fam. nov., comprising a distinct lineage of the Archaea. 2000. Int. J. Syst. Evol. Microbiol., 50: 997-1006.
2. Golyshina, O. V. and K. N. Timmis. *Ferroplasma* and relatives, recently-discovered cell wall-lacking archaea making a living in extremely acid, heavy metal-rich environments. 2005. Environ. Microbiol., 7: 1277-1288.
3. Ferrer, M., et al. The cellular machinery of *Ferroplasma acidiphilum* is iron-protein-dominated. 2007. Nature, 445: 91-94.
4. Golyshina, O. V. et al. 2006. The 'pH optimum anomaly' of intracellular enzymes of *Ferroplasma acidiphilum.* Environm. Microbiol., 8: 416-425.
5. Ferrer M et al. .A novel α-glucosidase from the acidophilic archaeon, *Ferroplasma acidiphilum* strain Y with high transglycosylation activity and an unusual catalytic nucleophile. 2005. Biochem. J., 391:269-276
6. Ferrer, M., O. V. Golyshina, A. Beloqui, L. H. Böttger, J. M. Andreu, J. Polaina, A. Lopez de Lacey, A. X. Trautwein, K. N. Timmis and P. N. Golyshin. 2008. Purple ligase: a novel acidophilic di-ferric DNA ligase from *Ferroplasma*. Proc. Natn. Acad. Sci. USA, in press.

DISCUSSION SESSION SUMMARY

Discussion Objective: To identify realistic research objectives for the next decade for Microbial Environmental Biotechnology

Since the first workshop over ten years ago, there has been a revolution in microbiology. The dramatic advance in scientific inquiry has been led by recognition of the vast uncultured microbial diversity present in the environment and by the ever-more rapid generation of whole genome sequences of microbes. Other technological developments have provided remarkable improvements in quantification of microbial functions in both laboratory culture and environmental settings. The participants of the *EU-US Workshop* were asked to consider the progress of environmental microbial biotechnology and to propose areas of inquiry for the next decade. This discussion was lead by Profs. Víctor de Lorenzo and Judy D. Wall. Dr. Balbina Nogales recorded impressions during the session.

The implicit background of the debate was [i] the growing penetration of systems biology, with all its technological developments (*omics* and the like) in every field of biological research (including environmental microbiology), [ii] the shift of the emphasis of the field from environmental *cleanup* to environmental *sustainability* e.g. clean energy and CO_2-neutral processes, and [iii] the promise of synthetic biology to address longstanding environmental problems on the basis of synthetic genomes and engineering non-natural biological catalysts.

The following is a listing of opportunities that were discussed. No priority is intended in the presentation.

- Discovery science is as fundamental to Environmental Biotechnology as is application-directed research.

- A well-trained, creative research force is essential. Because of the rapid pace of data accumulation and technological improvements, it is essential that microbiologists remain flexible in their outlook and expertise.

- A current challenge is the need to integrate vast amounts of information. The quantity of ever-increasing sequencing information has resulted in large sets of *omics* data that need reliable storage, accessibility, and trained personnel for exploration and exploitation.

- Partnerships among disciplines are needed to address the complexity of environmental microbial processes. Computational experts, engineers, hydrologists, geologists, ecologists, and microbiologists trained in physiology and biochemistry will all contribute.

- While sequences of well-studied microbes are now available, large percentages of the predicted open reading frames remain un-annotated or poorly annotated. A concerted biochemical effort should be made to decrease this list and reveal the true array of functional activities.

- The recognition of microbial diversity underscores the need of improved culturing techniques for previously uncultured organisms. Some of these bacteria may be syntrophs or live in consortia. Techniques for culture and analysis of robust consortia and defined communities are needed.

- Much of the microbial biomass in the environment is now known to exist as biofilms. Tools for the analysis of biofilms at multiple levels, including development, structure, and function, should be perfected. Are all biofilms alike? Can biofilms be infected? Can biofilms die? A model system for biofilms allowing extrapolation of results from one research group to another might facilitate the rate of elucidation of this life style.

- Nanobiology tools should be pursued that will open the examination of single cells at the molecular, metabolic and biochemical levels.

- A systems biology approach should be used for integrating all aspects of the fate of chemicals in the environment, including global modeling and predictive methods that could guide further experimentation and decision-making.

- *Omics* tools, i.e., the methods of determining cellular transcripts, proteins, and metabolite concentrations and fluxes, are currently being perfected and applied. However, the relationships among these data sets are not understood. The use of these various sources of information for prediction of function is still rather poor. Modeling efforts are needed to integrate the data so that meaningful predictions from variable inputs can be approached.

- The effects of microbial predation by eukaryotes, bacteria, or viruses require research attention.

- The environmental impacts of bacteriophages on microbes and the functioning of microbial communities is currently a mystery, one that needs solving.

- The variety and flexibility of microbial metabolism in extreme environments continue to surprise. These extremophiles should be aggressively examined if we are to understand the total physiological space of life.

- Questions of evolution of life and the derivation of an evolutionary frame for microbes are now being approached. There is now a possibility for exploring evolution with simple systems (two or a small number of partners), including co-evolution experiments with non-microbial partners, such as *Arabidopsis*.

- There is a realistic danger of niche loss for microbes, especially for plant endosymbionts. There should be a special effort to take a closer look at this issue to avoid the loss of biochemical diversity.

- Synthetic biology offers a splendid opportunity to take up again the notion of designer microorganisms for environmental cleanup. The emphasis in robust design concepts, e.g. modularity, orthogonality (i.e. context independency) and definition of systems boundaries –along with a growing easiness for massive DNA synthesis- open new scenarios for environmental interventions that could not materialize before.

This list of challenges for microbial biotechnology represents opportunities for increased understanding and for the elucidation of solutions compatible with our fragile environment. To meet these challenges EU-US collaboration at all career levels will be required. Establishing strong ties across the Atlantic through efforts such as this Workshop is one step on this critical journey.

This discussion session was conducted and coordinated by Prof. Judy D. Wall and Prof. Victor de Lorenzo with the involvement of the participants of the workshop. The summary reflects the views of the whole group who have agreed on the text.

ABSTRACTS

POSTER PRESENTATIONS

P1. TARGETED METABOLOMICS

<u>Nicola Zamboni</u>, Jörg Büscher, Jennifer Ewald, Dominika Czernik, Anne Kümmel, Matthias Heinemann, and Uwe Sauer

Institute of Molecular Systems Biology, ETH Zürich, Switzerland

From bacteria to higher cells, the pathways that constitute the central carbon metabolism play a fundamental role during adaptation of microorganisms to changing environmental conditions or genetic alterations. For example, they mediate the link of catabolism to anabolism on a variety of substrates, they bring about redox balancing, and primarily contribute energy equivalents to optimally fulfill biosynthetic demand. In biotechnology and metabolic engineering, knowledge on concentration and fluxes in central carbon metabolism is thus compulsory to reveal network operation, the mechanism governing its activity, and putative targets for rational engineering.

Here we demonstrate the application of targeted metabolomics in the investigation of unicellular metabolism. In contrast to broad-scope profiling techniques that aim at capturing a possibly larger number of analytes, we tailor the analytics to quantify the intermediates of central carbon metabolism, amino acids and precursors, nucleotides, and redox cofactors. Owing to the chemical heterogeneity of the analytes of interest, different platforms have to be employed to ensure coverage. In our lab we largely rely on mass spectrometry coupled on-line to gas, liquid chromatography or capillary electrophoresis. In a cross-platform comparison, we systematically tested 10 different methods for the quantification of primary metabolites in cell extracts. To date, routine analysis is performed by GC-TOF and RP-IP-LC-MS-MS and enables to typically detect 80-100 key metabolites in <1 mg of dry biomass.

In combination with carefully validated extraction protocols and standardization, targeted metabolomics enables to quantify intracellular pools in absolute terms. Precise concentrations, in turn, are a prerequisite for integrated analysis by kinetic or thermodynamic modeling. Here we present an example of thermodynamics-based, integrated analysis of quantitative metabolomics and flux data. This approach can be used to (i) test quantitative data sets for thermodynamic consistency and hence verify quality of the measurements, (ii) predict metabolite concentrations beyond the actually measured data, and (iii) identify putative sites of active regulation in the metabolic reaction network. To perform these analyses with any metabolic network, we recently released a generalized software that is freely available for academic purpose.

P2. UNDERSTANDING AND ENGINEERING METABOLIC PATHWAYS THAT PARTICIPATE IN HYDROGEN PRODUCTION BY PHOTOSYNTHETIC *Rhodopseudomonas palustris*

<u>James 'Jake' McKinlay</u> and Caroline S. Harwood

Department of Microbiology, University of Washington

Rising energy demands and the imperative to reduce greenhouse gas emissions are stimulating research on the development of bio-based fuels. H_2 is one of the most promising biofuels, having about three times the energy content of gasoline. The bacterium *Rhodopseudomonas palustris* naturally uses energy from sunlight and electrons from organic waste to produce H_2 and ammonia using any of its three nitrogenases. Normally, the presence of ammonia shuts down nitrogenase activity, presenting a challenge for producing H_2 in ammonia-containing wastewater. We have created *R. palustris* mutants that express nitrogenase at all times, allowing us to use this enzyme to

produce pure H_2 without accompanying ammonia production (2). While these mutants represent a significant step for biofuel production by *R. palustris*, H_2 yield and rate can still be improved further. In order to effectively engineer *R. palustris* for improved H_2 production, we need to better understand the pathways and proteins that are involved in transferring electrons from waste substrates (e.g., acetate) to nitrogenase; the site of H_2 production. To identify which metabolic reactions provide electrons for H_2 production we are conducting ^{13}C-labeling experiments to compare carbon flux distributions between non-H_2-producing wild-type and constitutively H_2-producing mutant strains. *R. palustris* strains are grown on ^{13}C-labeled acetate to allow ^{13}C-labeling patterns to be imprinted on metabolic products, namely proteinaceous amino acids. Labeled amino acids are analyzed by GC-MS and used to estimate central metabolic fluxes using the software *13C-Flux* (3). Electron balances indicate that the best H_2-producing mutants only divert 10% of the electrons consumed to H_2 while growing. The rest of the electrons are incorporated into biomass. H_2 yields can be improved to over 75% of the theoretical maximum by using nitrogen-starved, non-growing *R. palustris* cells. Non-growing *R. palustris* can produce H_2 for at least several months while illuminated (1). Therefore, we also want to understand the metabolic fluxes involved in H_2 production in non-growing cells. Accumulation of label in protein by non-growing cells is likely to be a gradual process at best. Thus, we are exploring methods to analyze labeling patterns in the central metabolites. These methods involve rapid quenching and sensitive LC-MS-MS analysis to contend with the rapid turnover rates and low concentrations of central metabolites.

References:

1. Gosse, J. L., B. J. Engel, F. E. Rey, C. S. Harwood, L. E. Scriven, and M. C. Flickinger. 2007. Hydrogen production by photoreactive nanoporous latex coatings of nongrowing *Rhodopseudomonas palustris* CGA009. Biotechnol Prog 23:124-130.
2. Rey, F. E., E. K. Heiniger, and C. S. Harwood. 2007. Redirection of metabolism for biological hydrogen production. Appl Environ Microbiol 73:1665-71.
3. Wiechert, W., M. Mollney, S. Petersen, and A. A. de Graaf. 2001. A universal framework for 13C metabolic flux analysis. Metab Eng 3:265-83.

P3. *IN VIVO* ^{13}C-NMR SPECTROSCOPY TO MONITOR THE KINETICS OF INTRACELLULAR METABOLITE POOLS IN *Saccharomyces cerevisiae* DURING ADAPTATION TO HEAT SHOCK: INPUT DATA FOR A MULTI-LEVEL MODEL OF THE TREHALOSE CYCLE

L.L. Fonseca[1], C. Sanchez[2], E. Voit[2], and H. Santos[1]

1. Instituto de Tecnologia Química e Biologica, Rua da Quinta Grande, 6, Apt. 127, 2780-156 Oeiras, Portugal
2. The Wallace H. Coulter Department of Biomedical Engineering at Georgia Institute of Technology and Emory University, USA

Trehalose is widely distributed in living cells where it plays a variety of roles generally associated with protection against stress. This disaccharide is frequently found in yeast, fungi, and plants, but also occurs in many bacteria and hyperthermophilic archaea. In *Saccharomyces cerevisiae*, the intracellular concentration of trehalose increases rapidly in response to many environmental stresses, including heat shock. These high trehalose levels have been correlated with tolerance to adverse conditions and led to the notion that trehalose functions as a chemical chaperone. The objective of the present work is to understand the design principles and operation of the trehalose cycle in *S. cerevisiae* through a combination of experimental and computational approaches. Herein, we focus on obtaining real-time metabolic data using NMR of living cells as the main analytical method. A circulatory system was used to pump the yeast cell suspension between a mini-reactor and the NMR tube in a 500 MHz spectrometer. Temperature, pH, and pO2 were

controlled in the bioreactor. A pulse of [1-^{13}C]glucose was added and the time courses of labeled metabolites were monitored under control conditions and also during heat stress (39ºC). Cells accumulated small amounts of trehalose (2-4 mM) under control conditions, while FBP reached 18 mM. Under heat stress (10 min 39ºC), trehalose accumulation reached 8 mM following a pulse of 65 mM glucose. When the duration of the stress was increased to one hour and 3 pulses of glucose were supplied, trehalose increased to 25 mM. This system also allowed monitoring end-product formation (ethanol, glycerol and acetate). The time courses of FBP and trehalose build-up combined with data at the transcriptional and transductional level of relevant genes of the trehalose cycle are expected to be useful input data for computational modeling of this small system.

P4. DATA DRIVEN SYSTEMS BIOLOGY BASED ON A TIME DEPENDENT METABOLOMIC APPROACH USING ULTRAHIGH RESOLUTION MASS SPECTROMETRY (ICR-FT/MS)

Marianna Lucio, Agnes Fekete, and Philippe Schmitt-Kopplin

Helmholtz Zentrum München - Research Center for Environment and Health, Institute of Ecological Chemistry, Neuherberg, Germany. marianna.lucio@helmholtz-muenchen.de schmitt-kopplin@helmholtz-muenchen.de

Genomics, transcriptomics and proteomics have already made significant strides in technology development; instead the tools for comprehensive examination of the metabolome are still emerging; mass spectrometry plays here an essential key role. The importance and the crucial value of Ion Cyclotron Resonance Fourier Transform Mass Spectrometer (ICR-FT/MS) is consolidated because of its high resolving power, mass accuracy, sensitivity, and flexibility, in the metabolomic fields. Accurate mass measurement capability of ICR-FT/MS has been proven as a significant tool for improving level of confidence in metabolomic identification in bottom-up approach. However, all of the advantages of ICR-FT/MS could not be fully demonstrated jet because of the existing gap between the complexity of the mass spectra and capability of interpreting the information by existing software. It is essential to develop integrated software applicable for interpretation of mass spectra obtained from a high-end ICR-FT/MS in order to handle the large amounts of data that are generated in metabolomics studies. The advancement in information technologies, data processing and analytical instrumentation has been paving ways of resolving the issues of bioanalytic with very high precision and accuracy. The major challenge in the post-genome era is also to understand how interactions among the molecules in a cell determine its form and function. Information is achieved through multivariate display and statistical analysis tools, graph modeling and visualization, classification and storage metabolites in database. The statistical tools start from unsupervised method to lead to supervised methods, such as principal component analysis, partial least square regression, discriminate analysis and a variety of clustering techniques; PCA and hierarchical cluster analysis are now widespread. They provide an appropriate starting point for further analysis in the interpretation of metabolite profiling. Common to a great part of these methods is that they build up interdependencies between metabolites, relationships between the abundances of the metabolites as revealed by correlation, covariance or distance matrix.

We present here an example of application of ICR-FT/MS in order to follow the metabolome of *Pseudomonas putida* during growth conditions. Metabolite annotation from the ICR-FT/MS exact mass data was followed with the MassTRIX database (www.masstrix.org). This dynamic metabolome analysis sets the basis of a data-driven systems biology approach.

P5. IMPACTS OF CRUDE VERSUS DISPERSED OIL IN SALMON AS CHARACTERIZED BY NMR-BASED METABOLOMICS

A. R. Van Scoy[1], C. Y. Lin[3], B. S. Anderson[1], B. M. Philips[1], M. R. Viant[2] and R. S. Tjeerdema[1]

[1]*Department of Environmental Toxicology, University of California, Davis, CA 95616, USA;*
[2]*School of Biosciences, The University of Birmingham, B15 2TT, Edgbaston, Birmingham, UK;*
[3]*Institute of Environmental Health, School of Public Health, National Taiwan University, Taipei, Taiwan*

The large maritime transport of crude oil from Alaska to California, poses the potential for catastrophic spills, which could seriously impact salmon populations during their migration from rivers and streams into the ocean. This study examined the toxic actions of the water-accommodated fraction (WAF) and chemically-enhanced water accommodated fraction (CEWAF) of Prudhoe Bay Crude Oil (PBCO) towards the pre-smolt and smolt stages of Chinook salmon (*Onchorhyncus tshawytscha*). After being exposed to various concentrations of oil or dispersed oil in seawater for 96 hours, muscle and liver samples from pre-smolts and smolts were removed and flash frozen. The low molecular weight metabolites were extracted using methanol/water and then analyzed using high resolution one-dimensional ^{1}H and projections of two-dimensional ^{1}H, ^{1}H J-resolved (p-JRES) nuclear magnetic resonance (NMR) spectroscopy. The p-JRES approach reduced peak congestion and subsequently increased the likelihood to integrate peaks accurately. Following spectral processing, the metabolites were examined using principal component analysis (PCA) followed by analysis of variance to identify the metabolic changes. The levels of metabolites including glycolytic and citric acid cycle intermediates (i.e. succinate), amino acids, as well as high energy phosphorous containing compounds (i.e. ATP and phosphocreatine), were altered in both life stages exposed to oil or dispersed oil. These alterations are dose and organ specific, and may potentially serve as biomarkers for toxicity exposure. We conclude that NMR-based metabolomics is a rapid, sensitive, less expensive, and high throughput approach capable of identifying metabolic fingerprints and developing biomarkers in response to environmental contaminants.

References:

1. Carls, M. G., R. A.Heintz, G. D. Marty, and S. D. Rice. (2005). Cytochrome P4501A induction in oil-exposed pink salmon *Oncorhynchus gorbuscha* embryos predicts reduced survival potential. *Mar. Ecol. Prog. Ser.* 301, 253-265
2. Lin, C. Y., H. Wu, R. S. Tjeerdema, and M. R. Viant (2007). Evaluation of metabolite extraction strategies from tissue samples using NMR metabolomics. *Metabolomics*, 3(1), 55-67.
3. Singer, M. M., D. Aurand, G. E. Bragin, J. R. Clark, G. M. Coehlo, M. L. Sowby and R. S. Tjeerdema. (2000). Standardization of preparation and quantification of water-accommodated fractions of oil and their use in aquatic toxicity testing. *Mar. Pollut. Bull*. 40, 1007-1016.
4. Viant, M. R. (2003). Improved methods for the acquisition and interpretation of NMR metabolomic data. *Biochem. Biophys. Res. Comm.*, 310(3), 943-948.

P6. METABOLITE PROFILING TO CHARACTERIZE THE FUNCTIONING OF MICROBIAL COMMUNITIES IN A PETROLEUM-CONTAMINATED SUBSURFACE ECOSYSTEM.

V.A. Parisi[1,2], L.M. Gieg[1,2], R.C. Prince[3], and J.M. Suflita[1,2]

[1] *Department of Botany and Microbiology and* [2] *Institute for Energy and the Environment, Univ. Oklahoma, Norman, OK 73019;* [3] *Exxon Mobil Biomedical Sciences Inc., Annandale, NJ 08801*

Metabolomics can arguably be called the, "…the single best window into the cellular state discovered to date" since it reveals the consequence of enzymatic activity. Since metabolomic measurements directly reflect the current physiological state of a cell, it yields different, and often more interpretationally useful information than the other 'omics' sciences. Metabolic profiling can give an instantaneous snapshot of the physiology of that cell and is usually used to compare genetic variants or to assess the metabolic bases of abnormal phenotypes. However, rarely has metabolic profiling been used to assess the functioning of microbial communities in an entire ecosystem. We investigated a former refinery site in Capser, WY, that was subject to long-term releases of complex residual oil mixtures into the terrestrial subsurface. Oxygen was limited in the aquifer and laboratory incubations of contaminated sediment and groundwater exhibited an average sulfate reduction rate of 55.0 ± 8.0 µM/d. Field metabolic profiling using GC-MS analysis revealed that many classes of hydrocarbons were undergoing endogenous anaerobic biodegradation in petroleum-contaminated areas only. The detection of benzylsuccinate, methylbenzylsuccinate and dimethylbenzylsuccinates in the groundwater attested to the endogenous metabolism of toluene, xylenes and C3-benzenes, respectively. A comparison to synthesized authentic standards revealed that 1,2,4-, 1,3,5trimethylbenzene and 1-ethyl-3-methlybenzene were degraded by the resident microflora at the site. Several naphthoic acids were detected, corresponding to the degradation of naphthalene and/or 2-methynaphthalene. Alkylsuccinates were numerous throughout the site, suggesting the loss of saturated alkanes (C5 and C6) and unsaturated alkanes in the range of C5 to C9. The field metabolic profiling was corroborated by losses of most of the expected hydrocarbons in endogenous laboratory incubations but not in corresponding negative controls. We conclude that metabolomics is a powerful tool for assessing the *in situ* functioning of microbial communities in hydrocarbon-impacted environments.

P7. COMBINING STABLE ISOTOPE PROBING AND METAGENOMICS, A POWERFUL TOOL FOR ECOLOGIST

Yin Chen[1], Marc G. Dumont[1], Josh D. Neufeld[1], Levente Bodrossy[2], Nancy Stralis-Pavese[2], Niall P. McNamara[3], Nick Ostle[3], Maria J.I. Briones[4], and J. Colin Murrell[1*]

[1] *Dept. Biological Sciences, Univ. Warwick, Coventry, CV4 7AL, UK;* [2] *Dept.Bioresources, Austrian Research Centre, GmbH, A-2444 Seibersdorf, Austria;* [3] *Centre for Ecology and Hydrology, Lancaster Environment Centre, Lancaster, LA1 4AP, UK;* [4] *Dept. Ecologia y Biologia Animal, Facultad de Ciencias, Universidad de Vigo, 36200 Vigo, Spain.*

A unique combination of stable isotope probing and metagenomics was applied to study uncultivated methanotrophs in peatlands. Peatlands represent an enormous carbon reservoir and have a potential impact on the global climate due to active methanogenesis and methanotrophy in these soils. Uncultivated methanotrophs from seven European peatlands were studied using a combination of molecular methods. Screening for methanotroph diversity using a particulate

methane monooxygenase-based diagnostic gene array revealed that *Methylocystis*-related species were dominant in six of the seven peatlands studied. The abundance and methane oxidation activity of *Methylocystis* spp. was further confirmed by DNA stable isotope probing analysis of a sample taken from the Moor House peatland (England). After ultracentrifugation, ^{13}C labeled DNA, containing mainly genomic DNA of these *Methylocystis* spp., was separated from ^{12}C DNA and subjected to multiple displacement amplification (MDA) to generate sufficient DNA for the preparation of a fosmid metagenomic library. Potential bias of MDA was detected by fingerprint analysis of 16S rRNA using denaturing gradient gel electrophoresis for low-template amplification (0.01 ng template). Sufficient template (1-5 ng) was used in MDA to circumvent this bias and chimeric artifacts were minimized by using an enzymatic treatment of MDA-generated DNA with S1 nuclease and DNA polymerase I. Screening of the metagenomic library revealed one fosmid containing methanol dehydrogenase and two fosmids containing 16S rRNA genes from theses *Methylocystis*-related species. Sequencing of the 14-kb methanol dehydrogenase-containing fosmid allowed the assembly of a gene cluster encoding polypeptides involved in bacterial methanol utilization (*mxaFJGIRSAC*). This combination of DNA stable isotope probing, MDA and metagenomics provided access to genomic information of a relatively large DNA fragment of these thus-far uncultivated, predominant and active methanotrophs in peatland soil.

P8. IMPACT OF COMPARATIVE PROTEOMICS ON ANALYSIS OF MICROBIAL COMMUNITIES

J. E. Turse, S. J. Callister, N. Jaitly, L. McCue, M. J. Marshall, R. D. Smith, J. K. Frederickson, and M. S. Lipton

Biological Separations & Mass Spectrometry, Biological Sciences Division, Computational Biology & Bioinformatics; Pacific Northwest National Laboratory, Richland, WA.

Understanding the synergism of microbial communities is paramount to the Department of Energy's goals for development of bioremediation and carbon sequestration technologies. Current research on these applications has been directed towards single microbial species and their potential for use in either role. However, microbially mediated environmental processes seldom occur due to the action of a single species. Current attempts to understand the composition of microbial communities have been limited to transcriptomic studies or studies that include few species. These studies have largely relied on genetic information, rather than the expressed proteins to explore the relationships and abilities of the bacteria that make up these communities. Traditional phylogenetic classification of organisms relies solely on nucleotide alignments of either whole genomes or subsets of genes – defining microbial relationships based heavily on computational analyses rather than verification of protein expression. In contrast, we have combined comparative proteomic analysis and genomics to investigate microbial proteomes. Phyloproteomics capitalizes on the data from bottom up proteomics experiments to assess relationships between bacteria on the basis of expressed protein. Further, phyloproteomics can use the sequenced genome and derived proteome of a characterized organism to investigate the proteomes of unknown organisms. The more closely related the organism, the more meaningful the protein identification, providing a better phyloproteomic relationship.

As a trial, we performed phyloproteomic analysis of 11 *Shewanella* species, *Deinococcus radiodurans* R1, *Salmonella enterica* sv. typhimurium LT2 and 4 novel *Shewanella*-like isolates grown under similar conditions. High-throughput, bottom-up proteomics were used to characterize the proteome of each organism. Using the proteomic data obtained from the *Shewanella*e in combination with the two distantly related organisms, *Deinococcus* and *Salmonella*, we explored the abilities to make functional predictions regarding expressed proteins, define a core proteome with conserved functionalities across all organisms in the study, and derive systematic relationships

among the known species. Furthermore, we exploited the ability of bottom-up proteomics to make cross-strain and cross-species protein identification in the absence of a genome: the proteomes of the four environmental isolates of a *Shewanella*-like microbe were analyzed. The result is quantitative, functional protein assignments, providing biological insight to the lifestyle of these previously uncharacterized organisms.

P9. LINKING IN-SITU MICROBIAL IDENTITY AND FUNCTIONALITY WITHOUT THE BIAS

Wei Huang[1], Andrew Ferguson[2], Ian P. Thompson[3], Robert M. Kalin[2], Mike Larkin[2], Mark J. Bailey[4], Andrew S. Whiteley[4] and Yun Wang[1]

[1] Kroto Research Institute, University of Sheffield, Broad Lane, S3 7HQ, Sheffield, UK;
[2] QUESTOR Centre and School of Biological Sciences, The Queen's University of Belfast, Belfast BT7 1NN, UK; [3] Department of Engineering, University of Oxford, UK; 4. Centre for Ecology & Hydrology—Oxford, Mansfield Road, Oxford OX1 3SR, UK

Prokaryotes represent half of the biomass of our planet and are foundation of our biosphere and biogeochemical cycles. However, the vast majority of prokaryotes still remain recalcitrant to culture and subsequent investigation as pure isolates within the laboratory. As a result we lack understanding of the functions many species perform in nature. To circumvent this issue we demonstrate stable isotope (^{13}C) linked analyses which recover population phylogenetic and functional sequences and the subsequent functional analysis of the microbial taxa in situ. We demonstrate the use of rRNA/mRNA stable isotope recovery of key phylogentic and functional RNA's followed by whole cell analyses of these key populations to confirm and quantify in situ functions within a groundwater microbial community. Using these culture independent approaches we identified three species of prokaryote capable of naphthalene biodegradation in a groundwater system. Within these species groups, two taxa could be isolated into the laboratory (*Pseudomonas fluorescens* and *Pseudomonas putida*) whereas, the third eluded culture (*Acidovorax sp*). Despite this, using complementary population and whole cell stable isotope technologies, we were able to resolve the degradation pathways used by both the culturable and non-culturable degraders and assess the rates of nutrient uptake of all populations in situ, ultimately allowing us to resolve their competitive interactions under different naphthalene conditions in situ. This study demonstrates the combined RNA SIP-Raman-FISH approach will be a central tool in resolving the complex roles of uncultured microbes in their natural environment by linking microbial species, their functional pathways and their metabolic functions in natural ecosystems without the need for culturing approaches.

P10. MOLECULAR DIVERSITY OF MARINE PHYTOPLANKTON COMMUNITIES BASED ON KEY FUNCTIONAL GENES

Punyasloke Bhadury and Bess B. Ward

Dept. Geosciences, Guyot Hall, Princeton Univ., NJ 08544, USA, Email: pbhadury@princeton.edu

The phylogeny and diversity of two key functional genes were investigated as the basis for improved understanding of the community structure of natural phytoplankton assemblages across marine environments. New partial *NR* (encoding eukaryotic assimilatory nitrate reductase) and *rbcL* (encoding the large subunit of the ribulose-1, 5- bisphosphate carboxylase/oxygenase)

sequences from cultured phytoplankton strains are reported. Phytoplankton community composition from Monterey Bay (MB), a coastal upwelling site, and the Western English Channel (WEC), a North Atlantic spring bloom environment, was elucidated based on the analysis of *NR* and *rbcL* sequences. Phytoplankton communities were also investigated from the exponential phase of a simulated bloom experiment using waters from MB. Diatoms were by far the most frequently detected phytoplankton group in both environments, consistent with their importance as a major bloom-forming group. Both *NR* and *rbcL* libraries contained sequences with biogeographical patterns typical of geographical restriction and 'cosmopolitan' distributions. The *NR* libraries also contained members of the Cryptophyceae, Chlorarachniophyceae, Dinophyceae and Prymnesiophyceae. The *rbcL* libraries from WEC and MB included sequences from all the major chromophytic algal groups including Bacillariophyceae, Bolidophyceae, Cryptophyceae, Dictyophyceae, Dinophyceae, Pelagophyceae, Prymnesiophyceae and Raphidophyceae. Sequences showing identity with key bloom forming organisms such as *Emiliania huxleyi*, *Phaeocystis pouchetii*, *Pseudo-nitzschia* sp. and *Thalassiosira* sp. were found in the *rbcL* libraries, confirming previous studies from these environments based on taxonomic and chemotaxonomic approaches. Several diversity/pattern analyses detected significant compositional differences among the libraries, which were consistent with patterns in the OTUs that could be identified by phylogenetic analysis. Many environmental sequences could not be identified because they or their relatives have been previously sequenced from culture.

P11. GeoChip APPLICATIONS TO ANALYZING MICROBIAL COMMUNITIES IN URANIUM CONTAMINATED SITES

<u>P.J. Waldron</u>[1], J. D. Van Nostrand[1], W-M. Wu[2], C.W. Schadt[3], D. B. Watson[3], Z. He[1], L. Y. Wu[1], C.S. Criddle[2], P.M. Jardine[3], T. C. Hazen[4], and J. Z. Zhou[1].

[1]University of Oklahoma, Norman, OK,; [2]Stanford University, Stanford, CA; [3]Oak Ridge National Lab, Oak Ridge, TN; [4]Lawrence Berkeley National Laboratory, Berkeley, CA

The objective of this study is to examine the bacterial community structure in wells of varying heavy metal and acid contamination to determine how each contaminant affects bacterial diversity and abundance. Five monitoring wells and an uncontaminated background well from the Field Research Center (FRC) site of the U.S. DOE ERSP (Environmental Remediation Science Program) at Oak Ridge, Tennessee, were sampled to provide a gradient of groundwater nitrate, pH and uranium concentrations. DNA from these samples was analyzed with a comprehensive functional gene microarray containing 24,243 probes for >10,000 genes involved in carbon, sulfur, nitrogen, and metal cycling. Dectected genes with the highest signal intensities from each sample were correlated with the groundwater geochemistry of that well. Wells with similar geochemical profiles had greater gene overlap than dissimilar wells. A higher percentage of nitrogen fixation genes were detected in groundwater with lower nitrate concentrations, while the percentage of nitrate reduction genes generally decreased with decreasing nitrate. Wells with elevated sulfate concentrations had a greater percentage of genes dedicated to sulfate reduction, and higher signal intensities for *dsrAB* genes than the background, indicating a greater abundance of those genes. Contaminated wells did not have a higher percentage of metal reduction and resistance genes than the background, but the total signal intensity of those genes was 1.4- to 2.3-fold greater than the background, indicating that metal-related genes were more prevalent in the contaminated wells. Uranium, nitrate and sulfate were identified by CCA as important factors in determining community structure. This study provides an overall view of the functional genes present in a highly contaminated environment, and shows the differences in functional populations between wells with varying contamination. As indicated by this work, the geochemical profile of the groundwater has significant effects on bacterial community structure, the knowledge of which may be important in planning and implementing successful bioremediation strategies in the future. Currently at the FRC, i*n situ*

bioremediation of a highly contaminated aquifer containing nitrate and uranium, is being conducted. Groundwater flow is controlled and microbial growth and uranium-reduction is stimulated by weekly ethanol injections. Analysis of bacterial communities in groundwater undergoing bioremediation, and their response to elevated nitrate, is also in progress. A comprehensive understanding of bacterial community structure and its response to specific contaminants will be key in applying long-term bioremediation solutions to environmental problems.

P12. ACCESS TO UNIQUE DEHALOGENASES: ENVIRONMENTAL METAGENOMIC *VERSUS* PROTEIN ENGINEERING

Ana Beloqui,[1] Dolores Reyes Duarte,[2] Rodrigo Torres,[3] José M. Vieites,[1] Julio Polaina,[4] Kenneth N. Timmis,[5,6] Peter Golyshin[7,8] and Manuel Ferrer[1]

[1]CSIC, Institute of Catalysis, 28049 Madrid, Spain; [2]Dept. Procesos y Tecnología, Univ. Autónoma Metropolitana, Unidad Cuajimalpa, D.F. Mexico, Mexico; [3]Universidad Industrial de Santander, Fac. Ciencias, Dept. Bioquímica, Bucaramanga, Santander, Colombia; [4]CSIC, Instituto de Agroquímica y Tecnología de Alimentos, 46980 Valencia, Spain; [5]Environmental Microbiology Laboratory, HZI-Helmholtz Centre for Infection Research, 38124 Braunschweig, Germany; [6]Institute of Microbiology, Carolo-Wilhelmina Technical University of Braunschweig, 38106 Braunschweig, Germany; [7]School of Biological Sciences, Bangor University, Gwynedd, LL57 2UW, UK; [8]Centre for Integrated Research in the Rural Environment, Aberystwyth University-Bangor University Partnership (CIRRE).

The majority of current biotechnological applications are of microbial origin, and it is widely appreciated that the microbial world contains by far the greatest fraction of biodiversity in the biosphere, so it is the microbes that will deliver the majority of new applications in the future. Numerous efforts are being undertaken worldwide, with an ultimate goal to deliver new usable substances of microbial origin to the marketplace or provide new toolbox necessary for the initial step in degradation of pollutants. However, the number of isolated microbes is up to twenty four orders of magnitude lower than those estimated Worldwide since the majority of them are not amenable to the culturing and no representatives for many major microbial phyla have been thus far characterized. Therefore, knowledge on new microbes and/or genomic information thereof, or from their communities, will pose an enormous potential for environmental interventions or other *a-la-carte* conversions. The present study highlights the advances in the exploration of metagenomic and protein engineering strategies for creating new "natural" and "artificial" dehalogenases giving an outlook for future trends and possibilities in this enzyme family.

Acknowledgements

A.B thanks Spanish MEC for a FPU fellowship.

P13. MOLECULAR ANALYSIS OF MICROBIAL COMMUNITIES FROM HYDROTHERMAL ENVIRONMENTS OF KAMCHATKA VOLCANIC AREA IN RUSSIA

Andrey Mardanov[1], Elisaveta Bonch-Osmolovskaya[2], and Nikolai Ravin[1]

[1] Centre "Bioengineering" RAS, Moscow, Russia; [2] Institute of microbiology RAS, Moscow, Russia

Kamchatka volcanic region (Russia), is a unique environment where several hundreds of hot springs and thermal pools are characterized by extremely diverse combinations of pH and temperature values and water chemistry. The phylogenetic diversity was determined for microbial communities of different geysers and thermal pools. The "metagenomic" DNA was extracted from microbial biomass and the small-subunit rRNA genes (16S rDNA) were amplified by PCR using primers specific for the *Archaea* or *Bacteria* domain, cloned and sequenced. Many representatives of *Archaea* appear to represents the deep lineages including *Korarchaeaota*. The detail data on diversity of different microbial communities will be presented.
Besides being an object of molecular ecological and evolution studies, microbial communities of Kamchatka are attractive source for isolation of new biotechnologically relevant enzymes. During last twenty years Kamchatka hot environments were used as a source for new archaea and bacteria, now represented in our strain collections. Several thermostable enzymes were isolated from this collection using PCR-screening including new DNA polymerase, reverse transcriptase and three DNA ligases. Another approach implies isolation of strains able to grow on a particular substrate, and isolation of *in situ* enrichment cultures using thermal fluid as the background but supplemented with the substrate(s) of interest. This approach allows enriching the maximal diversity of prokaryotes possessing target genes (encoding proteases, hydrogenases, alkane oxygenases, etc.) and was used to isolate two new alkaline thermostable subtilisin-like proteases able to digest collagens.

P14. METABOLIC MASTER AND COMMANDER: THE PHOSPHOTRANSFERASE SYSTEM (PTS^{Ntr}) OF *Pseudomonas putida*

Katharina Pflüger[1], Max Chavarria[1], Roelco J. Kleijn[2], Uwe Sauer[2], and Víctor de Lorenzo[1]

[1]Centro Nacional de Biotecnología CSIC Cantoblanco-Madrid, 28049 Spain and [2]Institute of Molecular Systems Biology, ETH Zurich, 8093 Switzerland

The activity of most individual bacterial promoters is submitted to various layers of regulatory controls, so that expression of specific genes is coordinated to the overall physiology of the cells. One prevalent physiological sensor to this end is the phosphoenolpyruvate-carbohydrate phosphotransferase transport system (PTS). The genome of *Pseudomonas putida* KT2440 encodes only 5 recognizable proteins belonging to two distinct classes of PTS systems: (i) a classical system for fructose intake (PTS^{Fru}) formed by FruA and FruB, (ii) and a nitrogen metabolic PTS^{Ntr} encoded by *ptsP* (EI^{Ntr}), *ptsO* (NPr), and *ptsN* (EI^{Ntr}). However, under certain metabolic conditions an *in vivo* cross talk between the two systems can be observed [1-4]. In order to explore the gross functional depth of PTS^{Ntr} in the metabolism of this bacterium, we analyzed the metabolic fluxes of isogenic strains bearing non-polar directed mutations in each of the corresponding *pts* genes. This fluxomic analysis revealed that the PTS^{Ntr} controls the connection of pyruvate to the TCA cycle by downregulating the pyruvate shunt, which bypasses malate dehydrogenase in the TCA cycle. It is believed that excess C is removed *via* this shunt in *P. putida*. On the contrary, the distinct breakdown of the carbon flow between the competing Entner-Doudoroff route, the pentose phosphate pathway and the ordinary Embden-Meyerhof-Parnas glycolysis was not affected in any

mutant. All examined metabolic effects of the PTS^{Ntr} could be traced to the sole presence/absence of PtsN ($EIIA^{Ntr}$), regardless of its phosphorylation state. Therefore, we looked for direct interaction partners of PtsN ($EIIA^{Ntr}$) by co-immunoprecipitation and detected a strong protein-protein interaction with the E1 enzyme of the pyruvate dehydrogenase (PDH) complex. This interaction leads to downregulation of the PDH activity, and thereby to alterations in the acetyl-CoA pool. These results, together with the observation that the PTS^{Ntr} influences expression of the TOL biodegradation pathway, biofilm formation, and the intracellular accumulation of polyhydroxyalkanoates, suggest a role of the PTS^{Ntr} as a sort of *metabolic divider* of diverse regulatory duties within the cells.

References:

1. Pflüger, K., and de Lorenzo, V. (2008) *J Bacteriol*
2. Velázquez, F., Pflüger, K., Cases, I., De Eugenio, L. I., and de Lorenzo, V. (2007) *J Bacteriol* 189, 4529-4533.
3. Pflüger, K., di Bartolo, I., Velázquez, F., and de Lorenzo, V. (2007) *J Microbiol Methods* 71, 179-185.
4. Pflüger, K., and de Lorenzo, V. (2007) *J Biol Chem* 282, 18206-18211.

P15. DEVELOPING INFRAME/MARKERLESS DELETION TECHNIQUES IN *Desulfovibrio vulgaris* Hildenborough TO STUDY METABOLIC PATHWAYS

Kimberly L. Keller

Biochemistry Department, University of Missouri, Columbia, MO, USA (kellerkl@missouri.edu)

The anaerobic sulfate-reducing bacteria (SRB) are found throughout nature. During respiratory growth, they have the ability to use sulfate as a terminal electron acceptor where sulfate-reduction is coupled to electron transport, thus linking dehydrogenases to terminal reductases. These bacteria merit research attention because of their potential role in toxic metal bioremediation as well as the recently recognized DNA damaging effects of their metabolic end-product, hydrogen sulfide. To fully understand the potential uses and problems associated with the SRB, it would be most useful to create several pivotal deletions in genes that may have compensatory activities in various metabolic pathways. We are pursuing genetic tools that can possibly be applied to these anaerobic strains, some with very limited genetic accessibility. Tools are being developed in *Desulfovibrio* and include an inframe, markerless deletion procedure and plasmid modification in extracts to facilitate genetic exchange processes.

Our model system, the sulfate-reducing bacterium *Desulfovibrio vulgaris* Hildenborough has seen enormous progress in genetic manipulation; however, the current deletion method of marker exchange mutagenesis does not allow for easy selection of multiple sequential gene deletions because of the low number of selectable markers available in *D. vulgaris*. To broaden the repertoire of genetic tools available for manipulation in *D. vulgaris*, an in-frame markerless deletion system is being developed based on the *upp*-encoded uracil phosphoribosyltransferase as an element for a counterselection strategy. In wild-type *D. vulgaris,* growth is inhibited by the toxic pyrimidine analog 5-fluorouracil (5-FU), whereas a mutant bearing a deletion of the *upp* gene has been shown to be resistant to 5-FU. The introduction of a plasmid containing the wild-type *upp* gene expressed constitutively from the *aph*(5')–III promoter (the promoter for the kanamycin resistance gene in Tn*5*) into the *upp* deletion strain restored sensitivity to 5-FU. This observation is the basis for the establishment of a two-step integration and excision strategy for the deletion of genes of interest. Since the constructed deletions do not contain an antibiotic cassette, multiple gene deletions can be generated in a single strain using this method.

In *D. vulgaris* there are three formate deyhydrogenases, and because their role in electron transport is unresolved, having the ability to sequentially delete these genes in one strain would provide great insight into the actual flow of electrons in *D. vulgaris.* The markerless deletion method is currently being utilized to delete the putative formate dehydrogenase alpha- and beta- subunits, DVU0587 and DVU0588 in *D. vulgaris*. In addition, Gateway Technology methods have been developed that will expedite the process of generating the required deletion vectors by the construction of a destination vector containing the constitutively expressed wild-type *upp* gene. This new method is also being tested for the generation of a deletion for the R-subunit (DVU1703) of a type I restriction-modification system.

P16. RELATIONSHIP BETWEEN MEMBRANE FUNCTIONS-REGULATING COLRS SYSTEM AND GLUCOSE FLUX IN *Pseudomonas putida*

Marta Putrinš, Heili Ilves, Maia Kivisaar and Rita Hõrak

Estonian Biocentre and Institute of Molecular and Cell Biology, Tartu University, 51010 Tartu, Estonia

Two-component system ColRS is well conserved in pseudomonads, but so far its role has been shown only in some unrelated processes. First it was reported, that a *colS*-deficient mutant of *P. fluorescence* was unable to compete with wild-type bacteria in plant root colonization (1). Additionally the ColRS system was shown to be implicated in mutagenesis in *P. putida*: different mutational processes such as point mutations and transposition of Tn4652 were repressed in starving *colS*- and *colR*-knockout strains in a phenol concentration dependent manner (2; 3).

In the current study we show that *colR*-deficient bacteria experience glucose-specific stress. We observed that on a solid medium *colR*-mutant becomes sticky and produces a Congo Red binding substance (CBS). Notably, this phenotype was specific to glucose-grown *colR*-deficient *P. putida* remaining undetectable on the citrate medium. As the presence of phenol in the growth medium enhanced abovementioned phenotype we suspected that the production of CBS is a consequence of membrane stress. Measurement of β-galactosidase activity from non-permeabilised cells demonstrated that compared to wild-type the membrane of *colR*-deficient cells is highly permeable. Moreover, increased amount of cytoplasmic β-galactosidase as well as chromosomal DNA was detectable in the growth medium of *colR*-mutant indicating that some cells undergo lysis in the absence of ColR. Analysis of bacteria at a single cell level by flow cytometry showed that the population of *colR*-deficient strain is heterogeneous. In addition to cells looking like wild-type ones there is a subpopulation of cells with damaged membrane permeable by propidium iodide. Investigation of colony morphology of *colR*-deficient *P. putida* revealed no defect in the early stages of colony formation. However, with time of incubation concavity developed in the centre of the colony, suggesting that glucose-caused stress is experienced by *colR*-deficient *P. putida* specifically in the stationary phase. Interestingly, inactivation of glucose porin encoding *oprB1* eliminated cell lysis as well as CBS production in *colR*-deficient strain indicating that glucose flux may be responsible for membrane stress in the absence of ColRS system.

References:

1. Dekkers LC, Bloemendaal CJ, de Weger LA, Wijffelman CA, Spaink HP, Lugtenberg BJ. (1998) A two-component system plays an important role in the root-colonizing ability of *Pseudomonas fluorescens* strain WCS365. Mol. Plant. Microbe. Interact. 11, 45-56.
2. Hõrak R, Ilves H, Pruunsild P, Kuljus M, Kivisaar M. (2004) The ColR-ColS two-component signal transduction system is involved in regulation of Tn4652 transposition in *Pseudomonas putida* under starvation conditions. Mol. Microbiol. 54, 795-807.
3. Kivistik PA, Putrinš M, Püvi K, Ilves H, Kivisaar M, Hõrak R. (2006) The ColRS two-component system regulates membrane functions and protects *Pseudomonas putida* against phenol. J. Bacteriol. 188, 8109-8117

P17. REDUCED GENOME *Escherichia coli*: A PLATFORM FOR GENOMIC AND METABOLIC ENGINEERING

György Pósfai[1,2], Tamás Fehér[1], Guy Plunkett III[2,3,4], , David Frisch[2,4], Kinga Umenhoffer[1], Ildikó Karcagi[1,2], Gabriella Balikó[1], Zsuzsa Győrfy[1,2], John Campbell[2], and Frederick R. Blattner[2,3,4]

[1]Institute of Biochemistry, Biological Research Center, Szeged, Hungary; [2]Scarab Genomics LLC, Madison, WI, USA; [3]Department of Genetics, University of Wisconsin, Madison, WI, USA; [4]Genome Center of Wisconsin, University of Wisconsin, Madison, WI, USA

In an attempt to engineer a simplified, core-genome *Escherichia coli*, we have reduced the K-12 MG1655 genome by making surgically precise scarless deletions. Genome reduction was achieved without compromising metabolic efficiency of the cells under standard laboratory conditions. The new strains, with genomes up to 24% smaller, were designed by bioinformatic comparative genomics of four *E. coli* strains to identify non-essential genes and recombinogenic, mobile or cryptic virulence sequences, as well as genes with unknown functions for elimination. Besides genome reduction from 4,639,675 to 3,512,873 bp, certain metabolic deficiencies of the parent strain, e.g. pyrimidine or isoleucine biosynthesis were also corrected. Other modifications include elimination of DNA-modification systems, reduction of the immunogenic properties, and changes enabling blue/white screening and inducible expression of clones. Despite the slower growth of some intermediate strains, the multiple deletion strain harboring 69 deletions (MDS69) displays no decrease in growth-rate measured in rich or minimal media. Removal of all mobile genetic elements resulted in an increased genomic and plasmid stability. MDS cells display a decreased mutation rate, especially under stressful conditions due to protein over-expression. The effect of the deletion of error prone DNA polymerase genes on mutational rate is currently being tested. Additional useful features of the strains include efficient transformability and high yield of plasmid production. In the case of several proteins, MDS not only meets, but exceeds the overexpression performance of BL21. These "clean-genome" strains can serve as simplified model systems, can have practical benefits as genetic tools, and can serve as platforms for further metabolic engineering.

P.18. EVOLUTION IN A MICROBIAL MUTUALISM AFFECTS COMMUNITY STABILITY AND PRODUCTIVITY

Kristina L Hillesland and David A Stahl

Department of Civil and Environmental Engineering, University of Washington, Seattle WA, 98195, USA and Virtual Institute for Stress and Survival (VIMSS)

In the natural world, metabolic networks are not stable entities, but are constantly evolving in response to selection imposed by changes in the abiotic environment and the composition of communities. This evolution can occur rapidly and the resulting phenotypic changes can alter community structure. Thus, it is important not only to understand the interconnections between metabolites within a given clone, but also to develop an appreciation about how they are likely to change as organisms adapt by natural selection to diverse ecological challenges. Here, we investigated the effects of evolution on the functioning of a community of two species that must exchange metabolites in order to grow. *Desulfovibrio vulgaris* Hildenborough can grow by fermenting organic acids and transferring electrons in the form of hydrogen to hydrogen consuming species, such as the methanogen *Methanococcus maripaludis*. Hydrogen consumption by another species is essential for growth of *D. vulgaris* because the fermentation reaction cannot provide

enough energy to support growth unless the concentration of hydrogen is very low. To explore how these species might adapt in the context of this mutualistic interaction, we founded 24 genetically identical communities of *D. vulgaris* and *M. maripaludis* growing with lactate as the electron donor, and allowed the communities to evolve independently. We tested whether environmental heterogeneity affected the outcome of evolution by subjecting half of these populations to constant vigorous shaking to maintain a chemically uniform environment. In the other 12 populations (static incubation), chemical gradients could form over the course of the seven-day incubation. The stability of communities changed over the course of 300 generations. After several transfers, all of the evolved communities exhibited signs of instability, causing two communities to go extinct. After several months of evolution, bouts of instability ceased. After 300 generations of evolution, we compared the growth rate and maximum biomass of evolved, ancestral, and mixed history communities. All *D. vulgaris* and most *M. maripaludis* improved in growth rate and maximum biomass relative to the ancestor. These evolutionary changes affected the functioning of the communities. Evolved communities grew on average 70% faster than ancestral communities, and improvements in growth rate were higher when communities evolved in a chemically uniform environment. Communities also evolved greater efficiency of conversion of lactate into biomass, and the magnitude of improvement was higher in the heterogeneous evolution environment. Overall, evolved communities reached an OD_{600} that was 30% higher than the ancestors growing on the same quantity of resource. Together, these results show that the characteristics of a community can change dramatically in a relatively short amount of time as one or more of its component populations adapt to new environmental conditions.

P19. PROFILING THE MICROBIAL COMMUNITY RESPONSE TO HYDROCARBON CHALLENGES

<u>Sinéad M. Ní Chadhain</u> and Gerben J. Zylstra

Biotechnology Center for Agriculture and the Environment, Rutgers University, New Brunswick, NJ 08901

Bacteria play a critical role in the remediation of contaminants such as alkanes and polycyclic aromatic hydrocarbons (PAHs) in the environment. While pure cultures have been isolated and characterized for their ability to grow on alkanes and PAHs, many of the organisms involved in these biodegradation pathways are difficult to culture and study under laboratory conditions. We have undertaken a culture independent approach to the study of the metabolic potential and diversity of bacteria in a variety of contaminated environments. We have designed PCR primers to the metal-binding motifs found in key enzymes in a variety of hydrocarbon degradation pathways. To date we have utilized primers which amplify the Rieske center of the PAH dioxygenase gene, the enzyme catalyzing the initial step in aerobic PAH degradation; the diiron histidine motif of alkane hydroxylase, the enzyme catalyzing the initial step in aerobic alkane degradation; and the heme binding motif of cytochrome P450 alkane hydroxylase, which also catalyzes the initial step in aerobic alkane degradation. These primers were used in conjunction with primers to the 16S rRNA gene to track shifting microbial gene profiles in soil following exposure to different hydrocarbons. We hypothesized that different suites of genes would dominate after enrichment on different hydrocarbons and that degradation gene profiles are indicative of microbial biodegradative activity. 16S rRNA and functional gene fragments were amplified from DNA extracted from each enrichment culture and an unamended treatment. The PCR products were cloned and sequenced. Molecular monitoring of the enrichment cultures before and after PAH degradation using 16S rRNA DGGE and gene libraries suggests that specific phylotypes of bacteria were associated with the degradation of each hydrocarbon. The PCR products were cloned and sequenced. Sequencing of the cloned functional gene fragments showed that different suites of degradation genes were present in soil microbe populations under each enrichment condition. Indeed distinct gene

populations were detected in response to the different enrichment conditions. While some sequences aligned with previously described genes for alkane and PAH oxygenases, many novel groups were identified. Our PCR products thus represent a convenient and rapid biomarker for monitoring the microbial response to contaminant exposure.

P20. INVESTIGATION OF ANAEROBIC DEGRADATION OF MONOCHLOROBENZENE USING STABLE ISOTOPE TRACERS

Paula M. Martinez[2], Anja-Bettina Dohrmann[3], Christoph C. Tebbe[3], Stefanie Weber[1], Nicole Stelzer[1], Hans-Hermann Richnow[1], and Ivonne Nijenhuis[1]

[1]Department of Isotope Biogeochemistry, Contact: ivonne.nijenhuis@ufz.de; [2]Bioremediation, Helmholtz Centre for Environmental Research - UFZ, Permoserstrasse 15, 04318 Leipzig, Germany; [3]Institut für Biodiversität, Johann Heinrich von Thünen-Institut (vTI), Bundesforschungsinstitut für Ländliche Räume, Wald und Fischerei, Bundesallee 50, 38116 Braunschweig, Germany.

Monochlorobenzene (MCB) is a groundwater contaminant almost ubiquitously found throughout the Bitterfeld/Wolfen region in Germany. MCB degradation has been well studied under aerobic conditions but under anaerobic conditions, as it commonly exist in most aquifers, complete degradation of MCB was, until recently, not described. Our previous study investigating the stable isotope composition of MCB in a contaminated aquifer in Bitterfeld suggested anaerobic degradation of MCB (1). To further investigate the degradation of MCB under anaerobic conditions, laboratory microcosms were applied using a ^{13}C-labelled tracer. In the laboratory tracer experiment, the fate of carbon from ^{13}C-labeled MCB was followed over time. In several anaerobic microcosms, prepared with groundwater from the contaminant plume, the isotope composition of potential products such as CO_2 and methane was monitored. While the methane isotope signature remained stable over the monitored period, CO_2 became highly enriched in ^{13}C indicating a mineralization of the added MCB. Furthermore, the ^{13}C-label could be retrieved in the total lipid fatty acid fraction demonstrating the incorporation of MCB derived carbon into biomass (2). The microbial community from two sets of microcosms prepared with groundwater of two different wells in the contamination plume was investigated using single-strand conformation polymorphism (SSCP) and sequencing of PCR amplified 16S rRNA genes from directly extracted DNA. Individual microcosms showed a certain variability in community structure among each other, as revealed by the SSCP patterns. Most of the dominant bacteria in both sets of cultures were closely related or identical in their 16S rRNA gene sequence to yet uncultured bacteria previously reported in environmental samples. Additionally, the microcosms were screened for presence of potential metabolites of MCB degradation and a DNA-SIP approach was used to assess the involved microbial population. Further research should provide more insights into the identity and physiology of the MCB degrading bacterial communities.

References:

1. Kaschl, A., Vogt, C., Uhlig, S., Nijenhuis, I., Weiss, H., Kästner, M., Richnow, H.H., 2005. Isotopic fractionation indicates anaerobic monochlorobenzene biodegradation. Environ Toxicol Chem 24, 1315-1324.
2. Nijenhuis, I., Stelzer, N., Kastner, M., Richnow, H.H., 2007. Sensitive detection of anaerobic monochlorobenzene degradation using stable isotope tracers. Environ Sci Technol 41, 3836-3842.

P21. CHANGES IN SOIL *Acidobacteria* COMMUNITIES AFTER 2,4,6-TRINITROTOLUENE CONTAMINATION

Isabelle George[1], Mark R. Liles[2§], Manuela Hartmann[3], Wolfgang Ludwig[3], Robert M. Goodman[2§§] and Spiros N. Agathos[1]

[1]Unité de Génie Biologique, Université Catholique de Louvain, Place Croix du Sud 2/19, 1348 Louvain-la-Neuve, Belgium; [2]Dept. Plant Pathology, Univ. Wisconsin-Madison, 1630 Linden Drive, Russell Laboratories, Madison, WI 53706, USA; [3]Lehrstuhl für Mikrobiologie, Technische Univ. München, Am Hochanger 4, 853530 Freising, Germany;
[§] Present address: Dept. Biological Sciences, Auburn University, Rouse Life Sciences Bldg, 120 W. Samford Avenue, Auburn, AL 36849, USA; [§§] Present address: Dean's Office, Cook College and New Jersey Agricultural Experiment Station, Rutgers - The State University of New Jersey, 88 Lipman Drive, New Brunswick, NJ 08901-8525, USA

Despite their widespread occurrence in soils, the ecology of *Acidobacteria* and their response to environmental perturbations due human activities remain very poorly documented. This study was aimed at assessing the impact of one xenobiotic compound, 2,4,6-trinitrotoluene (TNT), on the diversity and abundance of *Acidobacteria* in soils. The analysis of *Acidobacteria* communities at two sites with long-term and short-term contamination revealed that TNT has a drastic impact on the relative abundance of *Acidobacteria* in soil bacterial communities. The disapperance of most *Acidobacteria* from these soils was concomitant with a shift in *Acidobacteria* community composition and a loss of diversity, although the extent of diversity erosion depended on the sampling site.

P22. CHANGES IN DIVERSITY AND GENE EXPRESSION OF MARINE BACTERIAL COMMUNITIES UNDER EXPERIMENTAL HYDROCARBON POLLUTION

Mariana P. Lanfranconi, Vanesa Capó, Rafael Bosch and Balbina Nogales

Group of Microbiology, Dept. Biology, University of the Balearic Islands, Spain. Ctra Valldemossa km 7.5, E-07122. E-mail: bnogales@uib.es

Metagenomics has greatly advanced our understanding of the ecology and diversity of microbial communities in the ocean. However, many questions concerning regulation and dynamics of genes in the environment remain unanswered. Furthermore, the information regarding the physiological responses of marine microbial communities under stress conditions in the environment is scarce. To evaluate the impact of diesel pollution on bacterial communities we proposed to study changes in diversity and the expression of relevant genes by carrying out laboratory microcosms experiments, which were prepared with 45 L of unpolluted surface water from Western Mediterranean Sea. With this approach we expected to reduce the influence of uncontrollable environmental variables that we encountered in our previous studies *in situ*. In diesel-treated microcosms we observed an increase in the number of prokaryotic cells, a higher bacterial activity, and changes in the bacterial community structure. We also detected a negative effect of diesel on picocyanobacteria (*Synechococcus*) microscopic counts. A decrease in the expression of genes involved in oxygenic photosynthesis (*rbcL*IA and *psbA*) was also observed, as evidenced by lower

amplification signal in mRNA RT-PCR analysis when compared to control microcosms. However, regarding aerobic anoxygenic photosynthesis, no changes in *bchL*/*bchX* expression were observed between diesel-treated and control microcosms. Comparison of clone sequences from cDNA libraries with reference sequences in databases was performed to analyze diversity of gene transcripts. Our results will help to understand which physiological pathways are turned on (or off) under a stress condition like hydrocarbon pollution and which microorganisms are involved in different key processes.

LIST OF PARTICIPANTS

Spiros N. Agathos
Unit of Bioengineering (GEBI)
University of Louvain
Place Croix du Sud 2/19
B-1348 Louvain-la-Neuve
BELGIUM
Tel: +32 10473644
Fax: +32 10473062
E-mail: spiros.agathos@uclouvain.be

Ana Beloqui
Instituto de Catálisis y Petroleoquímica (CSIC)
C/ Marie Curie 2
Campus Cantoblanco
28049 Madrid
SPAIN
Tel: +34 915855479
E-mail: anabeloqui@icp.csic.es

Punyasloke Bhadury
Department of Geosciences
Princeton University
Guyot Hall
Princeton NJ 08544
USA
Tel: +1 6092586294
Fax: +1 6092580796
E-mail: pbhadury@princeton.edu

Sinéad M Ní Chadhain
Biotechnology Center for Agriculture and the Environment
Rutgers University
Foran Hall, 59 Dudley Road
New Brunswick, NJ 08901-8520
USA
Tel: +1 7329328165 ext. 321
Fax: +1 7329320312
E-mail: sinead@AESOP.rutgers.edu

Yin Chen
Department of Biological Sciences,
The University of Warwick,
Coventry, CV4 7AL
UNITED KINGDOM
Tel: +44 2476522557
cheny98@gmail.com

Ioannis Economidis
European Commission. Biotechnologies Unit
Directorate for Biotechnology, Agriculture and Food
Directorate-General for Research
European Commission.
Square de Meeûs 8,
B-1050 Brussels.
BELGIUM
Tel: +32 22951574
Fax: +32 22991860
E-mail: ioannis.economidis@ec.europa.eu

Víctor de Lorenzo
Centro Nacional de Biotecnologia, CSIC
Campus de Cantoblanco
28049 Madrid
SPAIN
Tel: +34 915854536
Fax: +34 915854506
E-mail: vdlorenzo@cnb.uam.es

Tamas Feher
Institute of Biochemistry,
Biological Research Center
of the Hungarian Academy of Sciences
P.O. Box 521
Szeged, H6701
HUNGARY
E-mail: fehert@brc.hu

James R. Frank
Bio-Molecular Science & Engineering
U.S. Office of Naval Research Global (ONRG)
Edison House at Blenheim Crescent Bldg 288, 86
Blenheim Crescent Ruislip
Mddx HA4 7HL
UNITED KINGDOM
Tel: +44 (0)18952356282
Email: jfrank@onrglobal.navy.mil

Caroline S. Harwood
Dept. Microbiology, Box 357242
University of Washington
1705 NE Pacific Street
Seattle, WA 98195-7242
USA
Tel: + 1 2062212848
Fax: +1 2062215041
E-mail: csh5@u.washington.edu

Kristina L. Hillesland
Dept. Civil and Environmental Engineering
University of Washington
Campus Box 355014
616 NE Northlake Place
Seattle WA 98109
USA
Tel: +1 2066853493
Fax: +1 2066165721
E-mail: kristina.stredwick@gmail.com

Wei E. Huang
Kroto Research Institute
The University of Sheffield
North Campus

Broad Lane
Sheffield S3 7HQ
UNITED KINGDOM
Tel.: +44 (0)1142225796
Fax: +44 (0)1142225701
w.huang@sheffield.ac.uk

Kimberly L. Keller
Biochemistry Department
University of Missouri- Columbia
117 Schweitzer Hall
Columbia, MO 65211
USA
Tel: +1 5738829771
Fax: +1 5738825635
kellerkl@missouri.edu

Maia Kivisaar
Institute of Molecular and Cell Biology
University of Tartu
Riia 23
Tartu 51010
ESTONIA
Tel: +372 7375036
Fax: +372 7420286
E-mail: maiak@ebc.ee

Mary S. Lipton
Environmental Molecular Sciences Lab.
Pacific Northwest National Laboratory
3335 Q. Avenue MSIN-K8-98
Richland, WA 99354
Tel: +1 5093716589
Fax: +1 5093716546
E-mail: mary.lipton@pnl.gov

Luis Lopes da Fonseca
Cell Physiology and NMR Group
Instituto de Tecnologia Química e Biológica
Rua da Quinta Grande 6, Apartado 127
2780-156 Oeiras
PORTUGAL
Tel: +351 214469829
Fax: +351 214428766
E-mail: llf@itqb.unl.pt

Marianna Lucio
Helmholtz Zentrum München
Institut für Ökologische Chemie
Ingolstädter Landstrasse 1
85764 Neuherberg
GERMANY
Tel.: +49 (0)8931873775
E-mail: marianna.lucio@helmholtz-muenchen.de

Andrey Mardanov
Centre "Bioengineering" RAS
Prosp. 60-let Oktiabria, bld. 7-1
Moscow, 117312
RUSSIA
mardanov@biengi.ac.ru

James "Jake" McKinlay
Dept. Microbiology, Box 357242
University of Washington
Health Sciences Bldg. Rm K-327
1705 NE Pacific Street
Seattle, WA 98195-7242
USA
Tel: +1 206 2212798
Fax: +1 206 5438297
E-mail: mckinla1@u.washington.edu

Barbara Methé
Institute for Genomic Research
J. Craig Venter Institute
9704 Medical Center Drive
Rockville, MD 20850
USA
Tel: +1 3017957515
Fax: +1 3018380208
E-mail: bmethe@jcvi.org

J. Colin Murrell
Department of Biological Sciences
University of Warwick
Coventry CV4 7AL,
UNITED KINGDOM
Tel: +44 (0) 2476523553
Fax: +44 (0) 2476523568
E mail: J.C.Murrell@Warwick.ac.uk

Ivonne Nijenhuis
Dept. Isotope Biogeochemistry
Helmholtz Centre for Environmental Research - UFZ
Permoserstrasse 15
D-04318 Leipzig
GERMANY
Tel: +49 (0) 3412351356
Fax: +49 (0) 3412352625
E-mail: ivonne.nijenhuis@ufz.de

Balbina Nogales
Dept. Biologia
Universitat de les Illes Balears
Crtra. Valldemossa km 7.5
07122 Palma de Mallorca
SPAIN
Tel: +34 971172068
Fax: +34 971173184
E-mail: bnogales@uib.es

Anna Palmisano
Biological and Environmental Research
Office of Science
Department of Energy
Tel: +1 3019033251

Fax: +1 3019035051
anna.palmisano@science.doe.gov

Balázs Papp
Evolutionary Systems Biology Group
Biological Research Center
Szeged,
HUNGARY
Tel: +36 62 599600 / 586
E-mail: pappb@brc.hu

Victoria Parisi
Department of Botany and Microbiology and
Institute for Energy and the Environment
University of Oklahoma
770 Van Vleet Oval, Rm 135
Norman OK 73019-6131
USA
Tel: +1 4053253771
Fax: +1 4053257619
E-mail: vparisi@ou.edu

Katharina Pflüger
Dept Biotecnología Microbiana
Centro Nacional de Biotecnología, CSIC
UAM Campus Cantoblanco
28049 Madrid
SPAIN
Tel: +34-91 5854573
Fax: +34-91 5854506
E-mail: kpfluger@cnb.uam.es

Marta Putrins
Institute of Molecular and Cell Biology
University of Tartu
Riia 23
Tartu 51010
ESTONIA
Tel: +372 7375015
Fax: +372 7420286
martake@ut.ee

Juan Luis Ramos
Estación Experimental del Zaidín, CSIC
C/ Prof. Albareda, 1
E-18008 Granada
SPAIN
Tel: +34 958181600 (ext. 289)
Fax: +34 958135740
E-mail: jlramos@eez.csic.es

Nikolai Ravin
Centre "Bioengineering" RAS
Prosp. 60-let Oktiabria, bld. 7-1
Moscow, 117312
RUSSIA
Tel: +7 4997833264
Fax: +7 4991350571
E-mail: nravin@biengi.ac.ru

Helena Santos
Cell Physiology and NMR Group
Instituto de Tecnologia Química e Biológica
Universidade Nova de Lisboa
Rua da Quinta Grande, 6, Apt. 127
2780-156 Oeiras,
PORTUGAL
Tel: +351 214469828
Fax: +351 214428766
E-mail: santos@itqb.unl.pt

Uwe Sauer
ETH Zurich
Institute of Molecular Systems Biology
ETH Hönggerberg HPT D 78
8093 Zurich
SWITZERLAND
Tel: +41 446333672
Fax: +41 446331051
E-mail: sauer@imsb.biol.ethz.ch

Philippe Schmitt-Kopplin
Institut für Ökologische Chemie
Helmholtz Zentrum München,
German Research Center for Environmental
Health
Ingolstaedter Landstrasse 1
85764 Neuherberg
GERMANY
Tel:+49 (0) 8931873246
Fax: +49 (0) 8931873358
E-mail: schmitt-kopplin@helmholtz-
muenchen.de

David A. Stahl
Dept. of Civil and Environmental Engineering
University of Washington
302 More, Box 352700
Seattle WA 98195-2700
USA
Tel: +1 2066853464
E-mail: dastahl@u.washington.edu
Currently at: Univ. of Vienna (Tel: +43
1427754207; Fax: +43 1427754389)

Joseph M. Suflita
Sarkeys Energy Center
University of Oklahoma
100 E Boyd, Rm 534
Norman, OK 73019-0628
USA
Tel: +1 4053255761
Fax: +1 4053257541
E-mail: jsuflita@ou.edu

Kenneth N. Timmis
Environmental Microbiology Laboratory
Helmholtz Centre for Infection Research

Inhoffenstrasse 7
D-38124 Braunschweig
GERMANY
Tel:+49 53161814000
Fax: +49 53161814199
E-mail: kti@helmholtz-hzi.de

Ron S. Tjeerdema
Dept. Environmental Toxicology
University of California- Davis
Davis, CA 95616-8588
USA
Tel: +1 5307545192
Fax: +1 5307523394
E-mail: rstjeerdema@ucdavis.edu

Joshua E. Turse
Environmental Molecular Sciences Lab.
Pacific Northwest National Laboratory
3335 Q. Avenue, MSIN-K8-98
Richland, WA 99354
USA
Tel: +1 509 3716224
Fax: +1 5093716546
E-mail: joshua.turse@pnl.gov

April Van Scoy
Department Environmental Toxicology
University of California- Davis
Davis, CA 95616-8588
USA
Tel: +1 5307522534
E-mail: avanscoy@ucdavis.edu

Patricia J. Waldron
Institute for Environmental Genomics
University of Oklahoma
101 David L., Boren Blvd.
Norman, OK 73072
USA
Tel: +1 4053253052
E-mail: pwaldron@ou.edu

Judy D. Wall
Biochemistry Dept
University of Missouri- Columbia
117 Schweitzer Hall
Columbia, MO 65211
USA
Tel: +1 5738828726
Fax: +1 5738825635
E-mail: wallj@missouri.edu

Yun Wang
Kroto Research Institute
The University of Sheffield
North Campus
Broad Lane
Sheffield S3 7HQ
UNITED KINGDOM
E-mail: cip07yw@sheffield.ac.uk

Bess B. Ward
Department of Geosciences
Princeton University
Guyot Hall
Princeton NJ 08544
USA
Tel: +1 6092585150
Fax: +1 6092580796
E-mail: bbw@princeton.edu

Lily Young
Biotechnology Center
Rutgers University
Foran Hall, 59 Dudley Rd.
New Brunswick, NJ 08901-8520
USA
Tel: +1 7329328165, ext. 312
Fax: +1 7329320312
E-mail: lyoung@AESOP.rutgers.edu

Nicola Zamboni
ETH Zurich
Institute of Molecular Systems Biology
ETH Hönggerberg HPT D 78
8093 Zürich,
SWITZERLAND
Tel: +41 44 633 3141
Fax: +41 44 633 1051
E-mail: zamboni@imsb.biol.ethz.ch

Jizhong Zhou
Stephenson Research and Technology Center
University of Oklahoma
101 Davil L., Boren Blvd.
Norman OK 73072
USA
Tel: +1 4053256073
Fax: +1 4053257552
E-mail: jzhou@ou.edu

EUROPE DIRECT is a service to help you find answers to your questions about the European Union

Freephone number (*):
00 800 6 7 8 9 10 11

(*) Certain mobile telephone operators do not allow access to 00 800 numbers or these calls may be billed.

LEGAL NOTICE
Neither the European Commission nor any person acting on behalf of the Commission is responsible for the use which might be made of the following information.
The views expressed in this publication are the sole responsibility of the author and do not necessarily reflect the views of the European Commission.
A great deal of additional information on the European Union is available on the Internet.
It can be accessed through the Europa server (http://europa.eu).
Cataloguing data can be found at the end of this publication.
Luxembourg: Office for Official Publications of the European Communities, 2008

ISSN 1831-2322
EUR Collection 1018-5593
ISBN 978-92-79-09796-6

© European Communities, 2008
Reproduction is authorised provided the source is acknowledged.
Printed in Belgium
PRINTED ON WHITE CHLORINE-FREE PAPER

KI-AN-08-001-EN-N

A "*Workshop on Metabolomics and Environmental Biotechnology*" was organised on 16-17 June 2008 in Mallorca (Spain) as an initiative of the Environmental Biotechnology Working Group of the EC-US Task Force on Biotechnology Research. The Workshop covered topics on metabolomics and functional analysis of microbial communities. Over the last few years a vast amount of information on the (meta)genomes of microorganisms has been generated. The potential exploitation of these discoveries in environmental biotechnology is enormous but requires profound knowledge of the functioning of microbial cells as complex networks of interacting metabolites, which is the aim of metabolomic studies. The potential outcome in relevant aspects for environmental biotechnology, such as the development of novel biocatalysts, novel biomarkers or more efficient and safer processes were also discussed in the workshop.

Publications Office
Publications.europa.eu

ISBN 978-92-79-09796-6