



COST ACTION B28
“Array technologies for BSL3 and BSL4 pathogens”

**5th Management Committee and
WG1, WG2, WG3, WG4 and WG5 meetings**

December 10 – 12, 2007
Vienna, Austria

Venue:

Hotel Ibis Wien Messe
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Local Organiser:

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Monday, 10.12.2007.

- 12:00 – registration
- 13:00 – 14:00 lunch buffet*
- 14:30 – 15:00 COST B28 welcome & opening Patrick Butaye
- 15:00 – 16:00 invited speaker – Dr. Sachse: DNA microarray assays for detection and genotyping of Chlamydiae
- 16:00 – 17:00 invited speaker – Prof. Hashsham: Parallel detection and quantification of pathogens using DNA biochips, on-chip PCR and hand-held devices
- 17:00 – 17:30 coffee*
- 17:30 – 18:30 invited speaker – Dr. Wu: Tools for understanding the pathogen in the post genomic era
- 18:30 – 19:30 invited speaker – Dr Wenzel and Dr. Hackl: Diversity Arrays Technology (DART) as a generic tool for microbial diagnostics
- 20:00 dinner*

Tuesday, 11.12.2007.

07:00 - 08:00 breakfast

08:00 – 13:00 WG1: Array technologies

- 08:00 – 08:10 introduction WG1 chair Jacques Schrenzel
- 08:10 – 08:30 Ebran Piskin: Self Assembling monolayers for array technologies
- 08:30 – 08:50 Jasper Kieboom: Brucella genotyping
- 08:50 – 09:10 Antoine Huyghe: Microarray concepts for reliable bacterial pathogen identification applied to biological threats diagnosis
- 09:10 – 09:30 Jean-Luc Gala: (to be announced)
- 09:30 – 09:50 Joachim Frey: Diagnosis of *Francisella tularensis* in organs of a naturally infected common squirrel monkey (*Saimiri sciureus*) and zoonotic transmission to a veterinarian.

10:00 - 10:45 coffee

- 10:45 – 11:05 Angela Sessitsch: (to be announced)
- 11:05 – 11:25 Cornelis Schoen: Quantitative multiplex detection of plant pathogens using a novel ligation probe-based system coupled with universal, high-throughput real-time PCR on OpenArrays™
- 11:25 – 11:45 Henrik Nordström: DNA microarray technique for detection and identification of viruses causing encephalitis and hemorrhagic fever
- 11:45 – 12:05 Karen Kempell: Advances toward development of microarrays for routine diagnostic use

12:05 – 12:25 Karl Walravens: The use of Multiple Locus VNTR Analysis (MLVA) for the typing of *Brucella*

12:25 – 12:45 Future activities and planning of actions of WG1

WG1 participants to reimburse:

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| 1 | Bodrossy Levente | Austria | Not to be reimbursed |
| 2 | Frangoulidis Dimitrios | Germany | To be reimbursed as MC member |
| 3 | Frey Joachim | Switzerland | To be reimbursed as MC member |
| 4 | Gala Jean-Luc | Belgium | To be reimbursed as MC member |
| 5 | Kempsell Karen | UK | To be reimbursed as WG1 member |
| 6 | Kieboom Jasper | The Netherlands | To be reimbursed as WG1 member |
| 7 | Kostic Tanja | Austria | Not to be reimbursed |
| 8 | Nordstrom Henrik | Sweden | To be reimbursed as WG1 member |
| 9 | Piskin Erhan | Turkey | To be reimbursed as MC member |
| 10 | Sanoussi Souzan | Austria | Not to be reimbursed |
| 11 | Schoen Cornelis | The Netherlands | To be reimbursed as WG1 member |
| 12 | Schrenzel Jacques | Switzerland | To be reimbursed as MC member |
| 13 | Sessitsch Angela | Austria | Not to be reimbursed |
| 14 | Walravens Karl | Belgium | To be reimbursed as WG1 member |
| 15 | Huyghe Antoine | Switzerland | To be reimbursed as WG1 member |
| 16 | Weilharter Alexandra | Austria | Not to be reimbursed |

13:00 - 14:00 lunch

14:00 – 16:00 WG2: Antigenicity

14:00 – 14:10 introduction WG2 chair Claude P. Muller

14:10 – 14:30 Claude P. Muller: Low genetic and antigenic diversity despite high prevalence and geographic spread of HBV

14:30 – 14:50 Claude P. Muller: AIV in Africa: the story goes on

14:50 – 15:10 Jutta Eichler: Synthetic Mimicry of the CD4-Binding Site of HIV-1 gp120 for the Design of Immunogens

15:10 – 15:30 Claude P. Muller: (to be announced)

15:30 – 15:50 Future activities and planning of actions of WG2

WG2 participants to reimburse:

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| 3 | Irengé Leonid | Belgium | To be reimbursed as WG2 member |
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| 6 | Ihnatko Robert | Slovakia | To be reimbursed as WG2 member |
| 7 | Betinova Eva | Slovakia | To be reimbursed as WG2 member |
| 8 | Eichler Jutta | Germany | To be reimbursed as WG2 member |
| 9 | Glisic Sanja | Serbia | To be reimbursed as WG2 member |

16:00 - 16:45 coffee

16:45 – 19:00 WG3: Proteomics and Glycomics

- 16:45 – 16:55 introduction WG3 chair replacement
- 16:55 – 17:15 Willy Zorzi & Benaissa ElMoualij: Immuno PCR: a new tool for the ultra-sensitive detection of biomolecules & applications in the STREP Neuroscreen Project
- 17:15 – 17:35 Rudolf Toman: Recent advances in the structural and functional elucidations of the outer membrane components of *Coxiella burnetii*
- 17:35 – 17:55 Adela Straskova: Hypotetical lipoprotein FTT 1103 - a new potential virulence factor of *Francisella tularensis*
- 17:55 – 18:15 Anetta Hartlova: Proteomic analysis of lipid rafts of mouse monocyto-macrophage cell line J774 during *Francisella tularensis* LVS infection
- 18:15 – 18:35 Veljko Veljkovic: Identification of EMILINs as novel human proteins that bind anthrax protective antigen
- 18:35 – 18:55 Future activities and planning of actions within WG3

WG3 participants to reimburse:

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| 9 | Veljkovic Nevena | Serbia | To be reimbursed as WG3 member |

20:00 conference dinner

Wednesday, 12.12.2007.

07:00 - 08:00 breakfast

08:00 – 10:00 WG4: Genomics

- 08:00 – 08:10 introduction WG4 chair Stefan Panaiotov
- 08:10 – 08:30 Ingmar Janse: Multiplex detection of Biothreat agents
- 08:30 – 08:50 Pierre Wattiau: Exposure of workers to B anthracis spores in a Belgian wool cleaning manufacture
- 08:50 – 09:10 Sally Cutler: (to be announced)
- 09:10 – 09:30 Jens Jacob: (to be announced)
- 09:30 – 09:50 Future activities and planning of actions of WG4

WG4 participants to reimburse:

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| 8 | Hufert Frank | Germany | To be reimbursed as WG4 member |

10:00 - 10:45 coffee

10:45 – 12:05 WG5: Microbiology

10:45 – 10:55 introduction WG5 chair Mandy Elschner

10:55 – 11:15 Paula Pilo: Characterisation of Swiss Isolates of Francisella tularensis and Bacillus anthracis

11:15 – 11:35 Manfred Weidmann: Development of a microarray for European arboviruses

11:35 – 11:55 David Albert: Development of a real-time PCR targeting IS711 gene for rapid detection of Brucella in samples

11:55 – 12:15 Maria Nica: (to be announced)

12:15 – 12:35 Nora Mandani: (to be announced)

12:35 - 12:55 Future activities and planning of actions of WG5

WG5 participants to reimburse:

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| 6 | Nica Maria | Romania | To be reimbursed as MC member |
| 7 | Pilo Paula | Switzerland | To be reimbursed as WG5 member |
| 8 | Weidmann Manfred | Germany | To be reimbursed as MC member |
| 9 | Nadya Brankova | Bulgaria | To be reimbursed as WG5 member |

13:00 - 14:00 lunch

14:00 5th Management Committee meeting

**5th Management Committee Meeting
14:00-17:00**

1. **Welcome**
Patrick Butaye, the Chair of COST ACTION B28 welcomed the participants to the Fifth Management Committee Meeting.
2. **Adoption of the Agenda - P. Butaye**
3. **Adoption of the Minutes of the 4th MC – Plovdiv- P. Butaye**
4. **Report of the Chair of the Working Group 1 - J. Schrenzel**
5. **Report of the Chair of the Working Group 2 - C. Muller**
6. **Report of the Chair of the Working Group 3 - J. Stulik**
7. **Report of the Chair of the Working Group 4 - S. Panaiotov**
8. **Report of the Chair of the Working Group 5 - M.Elschner**
9. **Annual report - P. Butaye**
10. **Operation of COST B28 web site <http://www.cost-b28.be/> – P. Butaye**
11. **STSMs: current status. R. Toman**
12. **Discussion on the place and programme of next meetings**

COST ACTION B28
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Registered participants for the meeting
December 10 – 12, 2007
Vienna, Austria

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INVITED SPEAKER

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INVITED SPEAKER

Total list of participants:

| No | Name | Country | Status |
|----|------------------------|-----------------|--------------------------------|
| 1 | Wenzel Peter | Australia | INVITED SPEAKER |
| 2 | Bodrossy Levente | Austria | Not to be reimbursed |
| 3 | Hackl Evelyn | Austria | Not to be reimbursed |
| 4 | Kostic Tanja | Austria | Not to be reimbursed |
| 5 | Sanoussi Souzan | Austria | Not to be reimbursed |
| 6 | Sessitsch Angela | Austria | Not to be reimbursed |
| 7 | Bearzatto Bertrand | Belgium | To be reimbursed as WG2 member |
| 8 | Butaye Patrick | Belgium | To be reimbursed as MC chair |
| 9 | ElMoualij Benaissa | Belgium | To be reimbursed as WG3 member |
| 10 | Gala Jean-Luc | Belgium | To be reimbursed as MC member |
| 11 | Ireng Leonid | Belgium | To be reimbursed as WG2 member |
| 12 | Walravens Karl | Belgium | To be reimbursed as WG1 member |
| 13 | Wattiau Pierre | Belgium | To be reimbursed as WG4 member |
| 14 | Zorzi Willy | Belgium | To be reimbursed as WG3 member |
| 15 | Levterova Victoria | Bulgaria | To be reimbursed as WG3 member |
| 16 | Nadya Brankova | Bulgaria | To be reimbursed as WG5 member |
| 17 | Panaitov Stefan | Bulgaria | To be reimbursed as MC member |
| 18 | Todor Kantardjiev | Bulgaria | To be reimbursed as WG4 member |
| 19 | Hartlova Anetta | Czech Republic | To be reimbursed as WG3 member |
| 20 | Straskova Adela | Czech Republic | To be reimbursed as WG3 member |
| 21 | Nicolaisen Mogens | Denmark | To be reimbursed as MC member |
| 22 | Albert David | France | To be reimbursed as WG5 member |
| 23 | Madani Nora | France | To be reimbursed as WG5 member |
| 24 | Renesto Patricia | France | To be reimbursed as MC member |
| 25 | Eichler Jutta | Germany | To be reimbursed as WG2 member |
| 26 | Elschner Mandy | Germany | To be reimbursed as MC member |
| 27 | Frangoulidis Dimitrios | Germany | To be reimbursed as MC member |
| 28 | Hufert Frank | Germany | To be reimbursed as WG4 member |
| 29 | Jacob Jens | Germany | To be reimbursed as WG4 member |
| 30 | Sachse Konrad | Germany | INVITED SPEAKER |
| 31 | Weidmann Manfred | Germany | To be reimbursed as MC member |
| 32 | Muller Claude P. | Luxembourg | To be reimbursed as MC member |
| 33 | Brum Laura | Portugal | To be reimbursed as MC member |
| 34 | Apostol Iuliana | Romania | To be reimbursed as MC member |
| 35 | Nica Maria | Romania | To be reimbursed as MC member |
| 36 | Glisic Sanja | Serbia | To be reimbursed as WG2 member |
| 37 | Veljkovic Nevena | Serbia | To be reimbursed as WG3 member |
| 38 | Veljkovic Veljko | Serbia | To be reimbursed as MC member |
| 39 | Betinova Eva | Slovakia | To be reimbursed as WG2 member |
| 40 | Ihnatko Robert | Slovakia | To be reimbursed as WG2 member |
| 41 | Palkovicova Katarina | Slovakia | To be reimbursed as WG3 member |
| 42 | Toman Rudolf | Slovakia | To be reimbursed as MC member |
| 43 | Escudero Raquel | Spain | To be reimbursed as MC member |
| 44 | Aldernborn Anders | Sweden | To be reimbursed as MC member |
| 45 | Nordstrom Henrik | Sweden | To be reimbursed as WG1 member |
| 46 | Sjöstedt Anders | Sweden | To be reimbursed as MC member |
| 47 | Francois Patrice | Switzerland | To be reimbursed as WG2 member |
| 48 | Frey Joachim | Switzerland | To be reimbursed as MC member |
| 49 | Huyghe Antoine | Switzerland | To be reimbursed as WG1 member |
| 50 | Pilo Paula | Switzerland | To be reimbursed as WG5 member |
| 51 | Schrenzel Jacques | Switzerland | To be reimbursed as MC member |
| 52 | Janse Ingmar | The Netherlands | To be reimbursed as WG4 member |

| | | | |
|----|-----------------|-----------------|--------------------------------|
| 53 | Kerstjens Huib | The Netherlands | To be reimbursed as MC member |
| 54 | Kieboom Jasper | The Netherlands | To be reimbursed as WG1 member |
| 55 | Schoen Cornelis | The Netherlands | To be reimbursed as WG1 member |
| 56 | Piskin Erhan | Turkey | To be reimbursed as MC member |
| 57 | Cutler Sally | UK | To be reimbursed as WG4 member |
| 58 | Kempsell Karen | UK | To be reimbursed as WG1 member |
| 59 | Wu Guanghui | UK | INVITED SPEAKER |
| 60 | Hashsham Syed | USA | INVITED SPEAKER |

Total number of participants (including invited speakers): 60
Participants to be reimbursed (including invited speakers): 55



COST ACTION B28
“Array technologies for BSL3 and BSL4 pathogens”

**5th Management Committee and
WG1, WG2, WG3, WG4 and WG5 meetings**

December 10 – 12, 2007
Vienna, Austria

Invited speaker’s program

DNA microarray assays for detection and genotyping of chlamydiae

Konrad Sachse¹, Helmut Hotzel¹, Peter Slickers², Ralf Ehricht²

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The diagnostic test of the future will be expected to provide complex information on the causative agent of an infection beyond the mere identification of the species. As soon as the diagnostician is confronted with this demand for "multi-dimensional" evidence on the pathogenic microorganism the limitations of PCR-based tests become obvious. Detection of subtle differences among strains, such as single-nucleotide polymorphisms and other intra-species variations, is problematic with standard amplification assays, and even in multiplex PCR only a few target regions can be examined in parallel.

DNA microarray technology opens up new possibilities that may be particularly beneficial for laboratory diagnosis of infectious diseases. This highly parallel approach allows any sample DNA to be simultaneously examined using a large number of probes, which may be derived from a polymorphic gene segment and/or from different genomic regions. In essence, this amounts to sequencing the respective genomic site. Thus, DNA microarray-based tests can attain far higher specificity than PCR.

Although microarrays have become a widely accepted tool for mRNA expression monitoring in gene transcription analysis, their use in rapid diagnosis of bacterial and viral pathogens is only emerging. Recent applications include identification and genotyping of mycobacteria, staphylococci, *Escherichia coli*, *Listeria* spp., *Clostridium perfringens*, *Vibrio* spp., as well as neuroinvasive viruses.

In a recent study, we developed a microarray assay for detection and differentiation of *Chlamydia* spp. and *Chlamydophila* spp. [1]. We used the commercially available ArrayTube™ system (Clondia Chip Technologies, Jena, Germany), which represents a less expensive system for processing low- and high-density DNA arrays. It involves spotted or *in situ* synthesized DNA chips of 3x3 mm size, which are assembled to form the bottom of 1.5-ml plastic micro-reaction tubes. In contrast to other microarray equipment, hybridization and signal processing can be conducted in an easy and rapid fashion on standard laboratory equipment without additional devices, such as hybridisation chambers. Hybridisation signals are amplified by an enzyme-catalysed precipitation reaction. A CCD camera integrated in the ATR-01 reader is used to monitor DNA duplex formation by kinetic measurement of the precipitation reaction at each spot via specific changes in red light transmission.

Hybridisation probes were designed on the basis of a multiple sequence alignment that included 16S rRNA genes, 16S-23S intergenic spacer regions and 23S rRNA genes from 44 chlamydial strains. To identify genomic segments of high discriminatory power, we developed a sequence analysis algorithm designated the "most variable window approach". Using this procedure, species-specific nucleotide polymorphisms in a region of generally high sequence similarity in the 23S rRNA gene were identified. The selected 26-nt probe sequences were used on two different series of customized microarrays, i.e. combinatorial high-density *in situ* synthesized arrays and low-density spotted arrays. Target DNA was prepared by consensus PCR using

one biotinylated primer. Unique species-specific hybridization patterns were obtained for all nine species of the family *Chlamydiaceae* on both microarray types.

After proving the suitability of the present assay for unambiguous species identification of chlamydial cell cultures we used it for direct detection of chlamydiae from clinical tissue [2]. As many clinical samples contain only low numbers of bacteria, sensitivity becomes the crucial parameter in this application. We were able to show that the sensitivity of the microarray assay was equivalent to that of real-time PCR [3], thus rendering it suitable for use in the diagnostic lab.

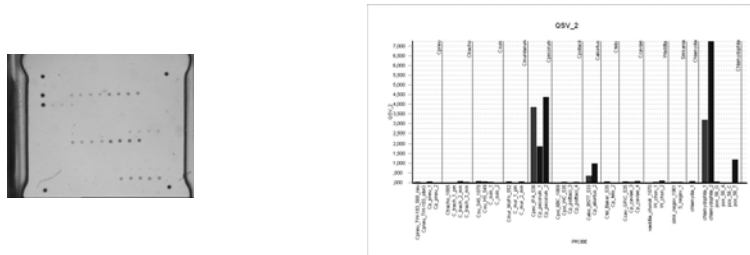


Fig. 1 Identification of *Chlamydomphila pecorum* from an ocular swab of an infected calf.

Meanwhile, the above microarray for species differentiation has been complemented with a new array for genotyping of *Chlamydomphila psittaci*, the causative agent of psittacosis in birds and humans.

[1] Sachse, K, Hotzel, H, Slickers, P, Ellinger, T, Ehricht, R: DNA microarray-based detection and identification of *Chlamydia* and *Chlamydomphila* spp. Mol Cell Probes 2005;19:41-50.

[2] Borel, N, Kempf, E, Hotzel, H, Schubert, E, Torgerson, P, Slickers, P, Ehricht, R, Tasara, T, Pospischil, A, and Sachse, K: Direct identification of chlamydiae from clinical samples using a DNA microarray assay - a validation study. Mol. Cell. Probes (2007), doi:10.1016/j.mcp.2007.06.003

[3] Ehricht, R, Slickers, P, Goellner, S, Hotzel, H, Sachse, K: (2006) Optimized DNA microarray assay allows detection and genotyping of single PCR-amplifiable target copies. Mol. Cell. Probes 2006;20:60-63.

Curriculum vitae Dr. Konrad Sachse

Name Konrad K.W. Sachse
Address Vor dem Obertore 18, 07751 Jena, Germany
Born 25 December 1949, Schleuskau, Germany
Nationality German
Marital status Married, 2 children

Education

1968-1973 Voronezh State University/Russia, Study of chemistry, specialization on polymer chemistry, Diploma with distinction
1982 Doctor's degree (Dr. rer. nat.) at University of Leipzig/Germany, Thesis "Liquid Chromatographic Analysis of Substituent Distribution in Cellulose Ethers", ("magna cum laude")
1987 Sabbatical year in the Molecular Biology Lab of Prof. H. Malke, Central Institute for Microbiology and Experimental Therapy, Jena

Professional Experience

1973-1977 Research chemist at an industrial polymer and rubber company (Chemische Werke Buna, Schkopau)
1977-1983 Head of a research group on liquid chromatography at the same company
1984-1990 Head of a research group on biochemistry, later molecular biology of pasteurellae and mycoplasmas, at the Research Institute for Bacterial Animal Diseases, Jena
1990-1991 Head of the Dept. of Molecular Biology at the same institute
01/1992-10/2002 Head of Dept. of Genetic Engineering at the Federal Institute for Health Protection of Consumers and Veterinary Medicine (BgVV), Division 4, Jena
11/2002-04/2006 Head of the Institute of Bacterial Infections and Zoonoses at the Friedrich-Loeffler-Institut (Federal Research Institute for Animal Health), Jena
since 05/2006 Deputy Head of the Institute of Molecular Pathogenesis at the Friedrich-Loeffler-Institut (Federal Research Institute for Animal Health), Jena

Professional Appointments

1995 - 2000 Member of the Management Committee of the European COST Action 826 (Ruminant Mycoplasmoses), Coordinator of Working Group 2
1995 - 2000 Member of the Working Team on Bovine Mycoplasmas of the International Research Programme on Comparative Mycoplasmaology (IRPCM)
since 1996 Member of the Subcommittee on the Taxonomy of Mollicutes of the International Committee of Systematic Bacteriology
since 2000 Leader of the Working Team on Ruminant Mycoplasmas of the International Research Programme on Comparative Mycoplasmaology (IRPCM)
2000-2001 Member of the Working Group on Psittacosis for the European Commission
2002-2007 Chairman of COST Action 855 "Animal Chlamydioses and the Zoonotic Implications" (18 member countries)
2003 Evaluator of the EU FP6 (6th Framework Programme of the European Union)
2003 Invited reviewer of research projects of DEFRA's Statutory and Exotic Disease Programme (Ministry of Agriculture of the UK)
since 2004 Member of the Editorial Advisory Board of Veterinary Research Communications

since 2005 Member of the Editorial Advisory Board of Veterinary Microbiology
since 2006 Member of the Editorial Advisory Board of the Veterinary Journal
since 2007 Candidate for Editorial Advisory Board Member of Molecular and Cellular Probes

Major Research Grants

- 1991-1995 German Research Foundation (DFG): "Development of rapid diagnostic methods for the detection of animal mycoplasmas"
- 1993-1995 German Research Foundation (DFG): "Cloning and expression of the P26 gene from *Mycoplasma bovis*"
- 1996-1998 German-Israeli Foundation for Scientific Research and Development (GIF): "The role of variable surface lipoproteins and their genes in mycoplasma-host interactions"
- 1996-1999 EU Project: SMT4-CT96-2072 "Development of methods to identify foods produced by means of genetic engineering"
- 1999-2000 The Ministry of Agriculture, Fisheries and Food of the UK: Research Project ZG0203 "Studies on mycoplasmas responsible for reproductive disorders in small ruminants"
- 2005-2007 German Research Foundation (DFG): "Investigation of transcriptional response by *Chlamydophila psittaci* and *Chlamydophila abortus* in the state of induced persistence"
- 2007-2010 Federal Ministry of Education and Research (BMBF): Coordinator of the national research network "Zoonotic Chlamydiae - Models of Chronic and Persistent Infections in Humans and Animals"

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- SACHSE, K.** (1993): Risk assessment of biological research and applications involving microorganisms to be used in food. Proceedings of the International Symposium on Potential and limitations of biotechnology in livestock production in developing countries (Eds. H.-J. Schwartz, H. Franzen). Humboldt University Berlin, pp.156-160.

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Parallel Detection and Quantification of Pathogens using DNA Biochips, On-Chip PCR, and Hand-Held Devices

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With more than 1400 different pathogenic microorganisms having dynamic genomes and extensive allelic variability in their signature genes, it is critical to develop high throughput tools that are able to screen for many pathogens in parallel. There are many desired attributes for such tools including sensitivity, specificity, speed, cost, robustness, sample processing, portability, and the ability to quantify the pathogens. Optimizing all attributes in a single assay is difficult but it is possible to optimize a limited number of parameters for a set of pathogens relevant to a given niche. Using a comprehensive virulence and marker (VMG) gene database encompassing 90 bacterial pathogens and 500 virulence and marker genes, we have developed four different types of parallel detection tools. These are: i) a microfluidic biochip using in situ synthesized 18-mer and 50-mer probes focusing on the specificity, ii) an on-chip PCR array focusing on quantification and enhancing the throughput with respect to the number of samples as well as target pathogens, iii) a high density 50-mer glass slide array using gold and silver nanoparticles focusing on cost, and iv) a microPCR based hand-held device focusing on portability, speed, and sensitivity. On the microfluidic biochip a highly specific assay for 20 waterborne pathogens with little or no false positives was developed. This was accomplished using three to six VMGs per organism and 5 to 20 probes per gene to make the call. To achieve a sensitivity of 0.01 to 0.001%, a multiplex PCR amplification step (combining up to 25 amplicons per reaction mixture) was necessary prior to fluorescent labeling and hybridization. Hybridization signals were highly specific as observed by non-equilibrium dissociation curves obtained for both 18- and 50-mer probes. For the on-chip PCR assay focusing on quantification, approximately 215 genes from 30 pathogens were used to examine the amplification and quantitative capacity of multiple primer sets in 33 nano-liter reaction holes for 12 to 36 samples in parallel. Quantification, sensitivity, and specificity were highly dependent on the genome size and GC content of the targeted organisms and primer characteristics. An empirical equation was developed to predict the copy number of detected amplicons without the use of standard curves. This equation is expected to play a key role in developing parallel screening tools without extensive validation. The third approach focused on reducing the cost of parallel screening by using high density in situ synthesized arrays, labeling with gold and silver nano-particles and scanning using a flatbed scanner (replacing fluorescent dyes and laser scanners). Using this approach, the total cost for equipment and analysis was reduced by 10- to 100-fold without compromising the power of parallel detection and

specificity. Automation of gold and silver labeling protocols may further reduce the cost of such assays. The fourth approach focused on developing a rapid microPCR chip for selected pathogens to be used with portable and point-of-use devices. The PCR chip consisted of microfluidic channels in doped silicon allowing rapid temperature control with minimal power, transparent base and cover for easy visualization with chemiluminescent light source and CCD imaging, and lyophilized PCR reagents. Using these components it is possible to develop less than 2-minute PCR based assays for multiple pathogens. The information gathered during the development of these tools suggests that parallel screening of pathogens using genetic markers has many advantages and should be considered as a method for routine monitoring both under laboratory and field conditions.

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1. EDUCATION

| | | | |
|------|-------|---------------------------------------|--|
| 1996 | Ph.D. | Environmental Engineering and Science | University of Illinois at Urbana-Champaign |
| 1986 | M.S. | Environmental Science and Engineering | Indian Institute of Technology, Bombay |
| 1984 | B.S. | Civil Engineering | Aligarh Muslim University, Aligarh, India |

2. PROFESSIONAL EXPERIENCE

| | | |
|--------------|-----------------------------------|--|
| 2005-present | Edwin Willits Associate Professor | Michigan State University |
| 1999-2005 | Assistant Professor | Michigan State University |
| 1998-99 | Post-doctoral Res. Associate | Stanford University |
| 1996-98 | Visiting Research Associate | Michigan State University |
| 1991-96 | Graduate Assistant | University of Illinois at Urbana-Champaign |
| 1990-91 | Graduate Assistant | University of Texas at Arlington |
| 1986-90 | Lecturer | Aligarh Muslim University, India |
| 1985-86 | Project Assistant | Indian Institute of Technology, Bombay |

3. RESEARCH INTERESTS

My current research interests include development of in situ synthesized and glass slide based biochips for water safety, microbial ecology, and biotechnology and mathematical modeling of molecular data. I am interested in developing and using genomic and molecular tools to solve environmental engineering problems. Currently two post-doctoral (shared), seven doctoral (out of which two are shared), three masters, and one undergraduate student are part of my research group. In addition, two visiting scholars and one research technician are also part of the group. Details of their individual research interest can be seen at the lab webpage listed above.

4. TEACHING INTERESTS

I teach Biological Processes in Environmental Engineering, Environmental Microbiology, and Feasibility Studies in Environmental Engineering to graduate and undergraduate students. I am also developing learner's modules as a tool to teach molecular biology to environmental engineers (named MOBEE, short for Molecular Biology Explorer for Engineers). The modules will be equally useful to anyone interested in learning the basics of molecular biology with a focus on detection and waterborne pathogens. It is still under development but its general approach can be seen at http://www.egr.msu.edu/~hashsham/mobee_dev.

5. PUBLICATIONS

Summary: A total of 28 peer-reviewed journal articles, 6 peer-reviewed book chapters, 9 peer-reviewed conference proceedings, 44 peer-reviewed abstracts, 8 non-reviewed (but invited) conference proceedings, and 17 submitted conference proceedings were published. In addition, 21 sponsored or invited platform

presentations were also made. The rate of peer-reviewed publications (including the journals and book chapters) is **4 per year**.

5.1 Reviewed archival journal publications

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3. Pozhitkov AE, Stedtfeld RD, Hashsham SA, Noble PA. Revision of the nonequilibrium thermal dissociation and stringent washing approaches for identification of mixed nucleic acid targets by microarrays. *Nucleic Acids Res.* 2007;35(9):e70. **2007**.
4. Stedtfeld, R.D., Jean-Marie Rouillard, Erdogan Gulari, James M. Tiedje, and Syed A. Hashsham. 2006. Influence of dangling end and surface proximal tail of targets on probe- target duplex formation in 16S rRNA gene-based diagnostic arrays. *Applied and Environmental Microbiology*. 73(2): 380-389. **2007**.
5. Maqbool, S.B., A. Ahmad, M.B. Sicklen, and S.A. Hashsham. Real time PCR assay for polyphosphate kinase genes in activated sludge. 2007. *Research Journal of Environmental Sciences* 1 (5): 229-236, **2007**
5. Parnell J.J., Park, J., Deneff, V., Tsoi, T., Hashsham, S., Quensen, J., and J.M. Tiedje. Coping with PCB toxicity: Physiological and genome-wide responses of *Burkholderia xenovorans* LB400 to PCB (polychlorinated biphenyl)-mediated stress. *Applied and Environmental Microbiology*. doi:10.1128/AEM.01129-06. **2006**.
6. Alok Dhawan, Julian S. Taurozzi, Alok K. Pandey, Wenqian Shan, Sarah M. Miller, Syed A. Hashsham, and Volodymyr V. Tarabara. Stable colloidal dispersions of C₆₀ fullerenes in water: Evidence for genotoxicity. *Environmental Science and Technology*. (40) 7394-7401. **2006**.
7. Stedtfeld, R.D. Duran M., Alm, E.W., and Hashsham, S.A. Detection and occurrence of indicator organisms and pathogens. *Water Environment Research*. 78(10) 1054-1077: **2006**.
8. Wick, Lukas M^{1,3}, Jean-Marie Rouillard, Thomas S. Whittam, Erdogan Gulari, James M. Tiedje, and Syed A. Hashsham. On-chip non-equilibrium dissociation curves and dissociation rate constants as methods to assess specificity of oligonucleotide probes. *Nucleic Acids Research*. 34(3):e26; doi:10.1093/nar/gnj024. **2006**.
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10. Duran M., Alm, E.W., Stedtfeld, R.D.¹, Haznedaroglu, B., and Hashsham, S.A.³. Detection and occurrence of indicator organisms and pathogens. *Water Environment Research*. 77: 659-717: **2005**.
11. Hashsham, S.A., Wick, L.M.¹, Rouillard¹, J-M., Gulari, E., and J.M. Tiedje, Potential of DNA microarrays for developing parallel detection tools (PDTs) for microorganisms relevant to biodefense and related research needs. *Biosensors and Bioelectronics*. Volume 20, Issue 4, Pages 668-683; **2004**.
12. Hashsham, S.A., Alm, E.W., Stedtfeld, R.D., Traver, R.G., and M. Duran. Detection and occurrence of indicator organisms and pathogens. *Water Environment Research*. Vol. 75, No. 6. **2004**.
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18. Musarrat J. and S.A. Hashsham. Customized cDNA microarray for expression profiling of environmentally important genes of *Pseudomonas stutzeri* strain KC. *Teratogenesis Carcinogenesis and Mutagenesis*. 283-294. Suppl. **2003**.
19. Deneff, V.J.^{1,3}, J. Park¹, J.L.M. Rodrigues¹, T.V. Tsoi, S.A. Hashsham and J.M. Tiedje. Validation of a more sensitive method for using spotted oligonucleotide DNA microarrays for functional genomics studies on bacterial communities. *Environmental Microbiology*. 5 (10), 933-943. **2003**.
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22. Dollhopf, S.L.^{1,3}, S.A. Hashsham, and J.M. Tiedje. Interpreting 16S rDNA T-RFLP Data: Application of self-organizing maps and principal component analysis to describe community dynamics and convergence. *Microbial Ecology*. 42 (4): 495-505. **2001**.
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25. Hashsham, S.A. and D.L. Freedman². Enhanced biotransformation of carbon tetrachloride by *Acetobacterium woodii* upon addition of hydroxocobalamin and fructose. *Applied and Environmental Microbiology*. 65(10): 4537-4542. **1999**.
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28. Qasim, S.R. , S.A. Hashsham, and G.M. Middleton. Study aimed at odor reduction in water from reservoir. *Water/Engineering and Management*. April 1992, p. 58-62. **1992**.

5.2 Book chapters and reports

1. Hashsham, S.A., Culture Techniques, In *Methods in Microbiology*, Editors, Academic Press, Inc. New York. (In Press). **2007**.
2. Hashsham, S.A. and Samuel Baushke. Energetics, Stoichiometry, and Kinetics of Microbial Growth, In *Methods in Microbiology*, Academic Press, Inc. New York. (In Press). **2007**.
3. Hashsham, S.A., S. Callister, and M. Tijdens. Oligonucleotide probe design for mixed microbial community microarrays and other applications and important considerations for data analysis. *Molecular Microbial Ecology Manual*. 2nd Edition, Kluwer Academic Publishers, The Netherlands. 1.7.8:1-26, **2004**.

4. Dollhopf, S., Ayala-del-Rio H.L., Hashsham, S.A. and J.M. Tiedje. Multivariate statistical methods and artificial neural network for analysis of microbial community molecular fingerprints. *Molecular Microbial Ecology Manual*. 2nd Edition, Kluwer Academic Publishers, The Netherlands. 1.7.6:1-40, 2004.
5. Tiedje, J., A. Fernandez, S. Hashsham, S. Dollhopf, F. Dazzo, R. Hickey, and C. Criddle. Stability, persistence and resilience in anaerobic reactors; a community unveiled. In *Advances in Water and Wastewater Treatment Technologies: Molecular Technology, Nutrient Removal, Sludge production and Environmental Health*. Ed. T. Matsuo, K. Hanaki, S. Takizawa, and H. Satoh. Elsevier, Amsterdam. pp. 13-20. 2001.
6. Hashsham, S.A., T.L. Marsh, S. Dollhopf, A.S. Fernandez, F.B. Dazzo, R.F. Hickey, C. Criddle, and J.M. Tiedje. Relating function and community structure of complex microbial systems using neural networks. In *Advances in Water and Wastewater Treatment Technologies: Molecular Technology, Nutrient Removal, Sludge Production and Environmental Health*. Ed. T. Matsuo, K. Hanaki, S. Takizawa, and H. Satoh. Elsevier, Amsterdam. pp. 67-77. 2001.

5.3 Reviewed conference proceedings

5.3.1 Full publications- reviewed

1. Jenkins, T.M., T.M. Scott, J.R. Cole, S.A. Hashsham, and J.B. Rose. Assessment of virulence factor activity relationships (VFARs) for waterborne diseases. In *Proceedings of the International Water Association (IWA) on Health Related Waterborne Microbiology*, Cape Town, South Africa, Sep 14-19th, 2003.
2. Musarrat, J., C.S. Criddle, and S.A. Hashsham. 2001. Development of DNA microarray technology for environmental applications. *Proceedings of the Water Environment Federation Technology Conference*, Oct 14-17, Atlanta, Georgia. CD-ROM.
3. Tiedje, J., A. Fernandez, S. Hashsham, S. Dollhopf, F. Dazzo, R. Hickey, and C. Criddle. 2001. Stability, persistence and resilience in anaerobic reactors; a community unveiled. *Proceedings of the COE Symposium on Establishment and Evaluation of Advanced Water Treatment Technology Systems Using Functions of Complex Microbial Community*. March 6-8, 2000. pp. 13-17. The University of Tokyo, Japan.
4. Hashsham, S., T. Marsh, S. Dollhopf, A. Fernandez, F. Dazzo, R. Hickey, C. Criddle and J. Tiedje. 2000. Relating function and community structure of complex microbial systems using neural networks. *Proceedings of the COE Symposium on Establishment and Evaluation of Advanced Water Treatment Technology Systems Using Functions of Complex Microbial Community*. March 6-8, 2000. pp. 70-79. The University of Tokyo, Japan.
5. Freedman, D.L., and S.A. Hashsham. 1997. Enhanced biotransformation of carbon tetrachloride by an anaerobic enrichment culture. Proceedings of the Fourth International Symposium, *In Situ and On-Site Bioremediation*, sponsored by Battelle, New Orleans, Louisiana, April 28-May 1.
6. Freedman, D.L., M. Lasecki, S.A. Hashsham, and R. Scholze. 1995. Accelerated biotransformation of carbon tetrachloride by sulfate-reducing enrichment cultures. Proceedings of the Third International Symposium, *In Situ and On-Site Bioreclamation*, sponsored by Battelle, San Diego, CA, April 24-27.
7. Hashsham, S.A. and D.L. Freedman. 1994. Enhanced biotransformation of carbon tetrachloride with added cyanocobalamin. Proceedings of the 4th Annual WERC Technology Development Conference, sponsored by U.S. Department of Energy, Las Cruces, NM, April 13-15.
8. Freedman, D.L., S.A. Hashsham, and R. Scholze. 1993. Enhanced biotransformation of carbon tetrachloride under methanogenic conditions, Proceedings of the Second International Symposium, *In Situ and On-Site Bioreclamation*, sponsored by Battelle, San Diego, CA, April 5-8.
9. Gupta, S.K., S.A. Hashsham, and S.G. Joshi. 1987. Biological treatment of nitrogenous wastewater- a nitrification study. Proceedings of the International Congress, *Management of Hazardous and Toxic Waste in the Process Industries*, Vienna, March 13-17.

5.3.2 Abstracts- reviewed

1. Alok Dhawan, Julian S. Taurozzi, Alok K. Pandey, Wenqian Shan, Sarah M. Miller, Syed A. Hashsham, and Volodymyr V. Tarabara. 2006. Stable Colloidal Dispersions of C60 Fullerenes in Water: Evidence for Genotoxicity. The 2006 AIChE Annual Meeting, November 12-17, 2006, San Francisco.
2. R. D. Stedtfeld, S. W. Baushke, S. Miller, J. M. Tiedje, S. A. Hashsham. Microchamber Biochip for Quantitative Detection of Human Pathogens in Environmental Samples. *106th General Meeting of the American Society for Microbiology*, Orlando, Florida, May 21-25, 2006.
3. Dieter M Turlousse, Sarah M Miller, Robert D Stedtfeld, Amanda B Herzog, Samuel W Baushke, Lukas M Wick, Jean Marie Rouillard, Erdogan Gulari, James M Tiedje, and Syed A Hashsham: Validation of an *in situ* synthesized oligonucleotide biochip for parallel detection of 12 waterborne pathogens. *106th General Meeting of the American Society for Microbiology*, Orlando, Florida, May 21-25, 2006.
4. Ruifang Xu, Robert D. Stedtfeld, Erdogan Gulari, James M. Tiedje, and Syed A. Hashsham. Influence of Target Preparation on Microarray Based Community Analysis of Indicator Organisms. *106th General Meeting of the American Society for Microbiology*, Orlando, Florida, May 21-25, 2006.
5. Sarah M. Miller, Alok Dhawan, Robert D. Stedtfeld, Dieter M. Turlousse, James M. Tiedje, and Syed A. Hashsham. Real-Time Hybridization Behavior in a Flow-Through DNA Biochip as a Function of Temperature, Flow Rate, and Time. *106th General Meeting of the American Society for Microbiology*, Orlando, Florida, May 21-25, 2006.
6. Vidya Srinivasan, Alok Dhawan, Robert D Stedtfeld, Sarah M Miller, Trinh Pham, Jean Marie Rouillard, James M Tiedje, Erdogan Gulari, and Syed A Hashsham. Detection of Pathogens in Environmental Samples with Gold Nanoparticles and Silver Enhancement on Microarray Using a Flatbed Scanner. *106th General Meeting of the American Society for Microbiology*, Orlando, Florida, May 21-25, 2006.
7. Yu Yang, Robert D. Stedtfeld, Jean Marie Rouillard, Erdogan Gulari, James M. Tiedje, Syed A. Hashsham. Synthetic virulence and marker gene targets as surrogates for validation of pathogen biochips. *106th General Meeting of the American Society for Microbiology*, Orlando, Florida, May 21-25, 2006.
8. Ahsan Munir, Sarah M Miller, Alok Dhawan, Robert D Stedtfeld, Jean Marie Rouillard, Erdogan Gulari, James M Tiedje, and Syed A Hashsham. Hybridization Behavior of Vancomycin Resistance Genes in a Flow-Through DNA Biochip using a Computational Approach. *106th General Meeting of the American Society for Microbiology*, Orlando, Florida, May 21-25, 2006.
9. S.A. Hashsham, L. M. Wick, R. Stedtfeld, D. Turlousse, S. Baushke, J-M. Rouillard, J. Klappenbach, J. R. Cole, E. Gulari, J. M. Tiedje. DNA Biochips for Diagnostics, Source Tracking, and Microbial Community Analysis: Challenges and Selected Solutions, *105th General Meeting of the American Society for Microbiology*, Atlanta, Georgia, June 5-9, 2005.
10. S. Baushke, S. Kravchenko, L. Wick, R. Stedtfeld, D. Turlousse, A. Herzog, T. Huynh, J-M. Rouillard, E. Gulari, J.M. Tiedje, and S.A. Hashsham. Statistical Analysis of DNA Microarray Data for Parallel Microbial Detection and Mixed Community Fingerprinting, *105th ASM General Meeting*, Atlanta, Georgia, June 5-9, 2005.
11. Herzog, A, R. Stedtfeld, T. Huynh, S. Baushke, J. Weiss, J-M. Rouillard, E. Gulari, J. Tiedje, and S.A. Hashsham. Challenges in Developing Community Fingerprinting Oligonucleotide Microarrays using the 16S rRNA Gene, *105th General Meeting of the American Society for Microbiology*, Atlanta, Georgia, June 5-9, 2005.
12. Elizabeth Kruszewski, Y. Matsumura, V. Deneff, L. Wick, K. Yadav, R. Xu, S. Baushke, R. Stedtfeld, J. Tiedje and S. Hashsham. Detection of Vancomycin Resistant *Enterococcus faecalis* (N00-410) and *Enterococcus faecium* (N97-330) Using Microarrays, *105th General Meeting of the American Society for Microbiology*, Atlanta, Georgia, June 5-9, 2005.
13. R. D. Stedtfeld, L. M. Wick, S. W. Baushke, J.M. Rouillard, X. Yongmei, J. Cole, J. Klappenbach, E. Gulari, J. M. Tiedje, S. A. Hashsham. Validation Strategy for a DNA Biochip: Comparison of Synthetic Targets and Extracted DNA Fragments for 16S rDNA Probes, *105th General Meeting of the American Society for Microbiology*, Atlanta, Georgia, June 5-9, 2005.

14. Ruifang Xu, R. Stedtfeld, J-M. Rouillard, E. Gulari, J. Tiedje, and S.A. Hashsham. Evaluation of Labeling Protocols for Enhanced Sensitivity of In Situ Synthesized Biochips, *105th General Meeting of the American Society for Microbiology*, Atlanta, Georgia, June 5-9, 2005.
15. Tourlousse, D., Herzog, A., Baushke, S., Tiedje, J.M., Hashsham, S.A. Development and validation of a DNA chip for the detection of virulence genes of human pathogens. *105th General Meeting of the American Society for Microbiology*, Atlanta, Georgia, June 5-9, 2005.
16. Robert Stedtfeld, Sam Baushke, Ruifang Xu, Tam Hyunh, Amanda Herzog, Jean-Marie Rouillard, Erdogan Gulari, James Tiedje, and Syed Hashsham. Phylogenetic Microarrays as Microbial Community Analysis Tools. 8th Symposium on Bacterial Genetics and Ecology; BAGECO 8 - Lyon (France) - June 26-29, 2005.
17. Wick, L. M., T. S. Whittam, E. Gulari, J. M. Tiedje, and S. A. Hashsham: 2005. On-chip non-equilibrium dissociation curve and dissociation rate constant as methods to assess specificity of oligonucleotide probes. 8th Symposium on Bacterial Genetics and Ecology; BAGECO 8 - Lyon (France) - June 26-29, 2005.
18. Hashsham, S.A. and J.M. Tiedje. DNA biochips for diagnostics, source tracking, and microbial community analyses: issues and selected solutions. *10th International Symposium on Microbial Ecology, Microbial Planet: Subsurface to Space*, Cancun, Mexico, August 22nd -27th, 2004.
19. Deneff, V.J., Park, J., Wick, L., Vervaeren, H., Sys, K., Matsumura, Y., Goris, J., Tsoi, T., Verstraete, W., J.M. Tiedje and S. A. Hashsham. Use of highly flexible, in situ synthesized microarrays for differential gene expression, comparative genomics and environmental microbial detection. *Genomes 2004: International Conference on the Analysis of Microbial and Other Genomes*, Cambridge, United Kingdom, April 14-17, 2004.
20. Weiss, J., Wick, L., Yang, Y., Hashsham, S., Whittam, T., J.M. Tiedje, DNA microarrays as parallel microbial detection tools for water biosafety. *ASM Biodefense Research Meeting*, Baltimore, Maryland, March 7-10, 2004.
21. Weiss, J., Deneff, V., Klappenbach, J., Cole, J., Gulari, E., Rouillard, J.-M., Whittam, T., Rose, J., Hashsham, S., and J.M. Tiedje. Microarrays for the parallel detection and identification of microbes indicative of fecal matter contamination in water. *10th International Symposium on Microbial Ecology, Microbial Planet: Subsurface to Space*, Cancun, Mexico. August 22nd -27th, 2004.
22. Tourlousse, D., Weiss, J., Stedtfeld, R., J.M. Tiedje and S. Hashsham. Development of a DNA chip targeting virulence genes of human pathogens. *10th International Symposium on Microbial Ecology, Microbial Planet: Subsurface to Space*, Cancun, Mexico. August 22nd -27th, 2004.
23. Stedtfeld, R.D., Weiss, J., Tourlousse, D., J.M. Tiedje, and S.A. Hashsham. Innovative validation strategy for optimizing DNA biochips: comparison of synthetic targets and real DNA fragments. *10th International Symposium on Microbial Ecology, Microbial Planet: Subsurface to Space*, Cancun, Mexico. August 22nd - 27th, 2004.
24. Wick, L.M., Whittam, T.S., Gulari, E., J.M. Tiedje and S.A. Hashsham. Development of in situ synthesized oligonucleotide microarrays (XeoChips) as parallel detection tools for water biosafety. *10th International Symposium on Microbial Ecology, Microbial Planet: Subsurface to Space*, Cancun, Mexico. August 22nd - 27th, 2004.
25. Deneff, V.J., Patrauchan, M.A., Florizone, C.M., Park, J., Tsoi, T.V. , Hashsham, S.A., Verstraete, W., Eltis, L.D., J. M. Tiedje. A transcriptomic, proteomic and genetic investigation of cellular processes involved in biphenyl and benzoate metabolism by *Burkholderia xenovorans* LB400. *10th International Symposium on Microbial Ecology, Microbial Planet: Subsurface to Space*, Cancun, Mexico. August 22nd - 27th, 2004.
26. Hashsham, S., J-M. Rouillard, X. Gao, S. Callister, J. Cole, V. Deneff, T. Tsoi, J. Wibbenmeyer, E. Gulari, and J. Tiedje. Highly parallel microbial detection using in situ synthesized flexible biochips. 103rd General Meeting of the American Society for Microbiology to be held in Washington D.C., May 18-22, 2003.
27. Deneff, V.J., T.V. Tsoi, J. Park, J.-M. Rouillard, H. Zhang, X. Gao, J. Wibbenmeyer, S.A. Hashsham, J.M. Tiedje. Outlining genome-wide carbon source specific metabolic networks in *Burkholderia* sp. nov. LB400. 103rd General Meeting of the American Society for Microbiology, Washington D.C., May 18-22, 2003.

28. Jenkins, TM, SA Hashsham, JR Cole, and JB Rose. Virulence-factor activity relationships (VFARs) database for waterborne pathogens. ASM the 103rd General Meeting of the American Society for Microbiology to be held in Washington D.C., May 18-22, 2003.
29. Callister, S. and S.A. Hashsham. 2002. Quantifying the relationship between genomic response and perturbation by modulus of stability. *Proceedings of the 102nd General Meeting of the American Society for Microbiology*. Salt Lake City, Utah, May 19-21.
30. Musarrat, J., J. Larabee, C. Criddle, and S.A. Hashsham, Monitoring the abundance of mRNA transcripts associated with carbon tetrachloride dechlorination in *Pseudomonas stutzeri* strain KC under different environmental conditions using DNA microarray. *Proceedings of the 101st General Meeting of the American Society for Microbiology*. Orlando, Florida, May 20-24, 2001.
31. Deneff, V.J., C.A. Kachel, J.L.M. Rodrigues, S.A. Hashsham, T.V. Tsoi, and J.M. Tiedje. DNA microarray approach to monitor engineered mixed PCB-degrading communities. *Proceedings of the 101st General Meeting of the American Society for Microbiology*. Orlando, Florida, May 20-24, 2001.
32. Callister, S.J., H.L. Ayala, and S.A. Hashsham. Quantification of *Pseudomonas cepacia* G4 in a dense mixed microbial community using a dual laser microarray scanner. *Proceedings of the 101st General Meeting of the American Society for Microbiology*. Orlando, Florida, May 20-24, 2001.
33. Dollhopf, S.L., S.A. Hashsham, and J.M. Tiedje. Interspecies interactions affecting spirochete species in methanogenic environments. *Proceedings of the 101st General Meeting of the American Society for Microbiology*. Orlando, Florida, May 20-24, 2001.
34. Hashsham, S.A., J. Musarrat, M. Tijdens, V. Deneff, T. Tsoi, C.S. Criddle, and J.M. Tiedje. Development of DNA microarray technology for mixed microbial communities. *Proceedings of the 9th International Society for Microbial Ecology*, Amsterdam, Netherlands, August 27-September 1, 2001.
35. Dollhopf, S.L., S.A. Hashsham, and J.M. Tiedje. Application of neural networks in microbial community analysis: Detecting patterns and making predictions. *Proceedings of the 9th International Society for Microbial Ecology*, Amsterdam, Netherlands, August 27-September 1, 2001.
36. Hashsham, S.A., S. Dollhopf, F.B. Dazzo, and J.M. Tiedje, Molecular data and mathematical modeling in biological wastewater treatment. *Proceedings of the NSF Sponsored U.S.-Egypt Workshop in Microbial Ecology*, National Research Center, Cairo, Egypt. May 6-11, 2001.
37. Dollhopf, S., S.A. Hashsham, F. Dazzo, C. Criddle, R. Hickey, and J. Tiedje. Community succession in methanogenic digesters to spirochete dominated communities *Proceedings of the 99th General Meeting of the American Society for Microbiology*. Chicago, Illinois, May 27-June 02, 1999.
38. Hashsham, S., A. Fernandez, S. Dollhopf, F. Dazzo, R. Hickey, J. Tiedje, and C. Criddle. Modeling microbial ecosystems: implications of molecular techniques. *Proceedings of the 99th General Meeting of the American Society for Microbiology*. Chicago, Illinois, May 27-June 02, 1999.
39. Hashsham S., T. Marsh, A. Fernandez, L. Raskin, S. Seston, F. Dazzo, R. Hickey, C. Criddle, and J. Tiedje. Microbial ecology of anaerobic digesters: stability and persistence during shock loads. *Proceedings of the 8th International Symposium on Microbial Ecology*. Halifax, Canada, August 9-14, 1998.
40. Fernandez A., S. Huang¹, S. Hashsham, R. Hickey, C. Criddle, and J. Tiedje. Dynamics of bacterial populations and functional stability in perturbed anaerobic reactors. *Proceedings of the 8th International Symposium on Microbial Ecology*. Halifax, Canada, August 9-14, 1998.
41. Dollhopf S.L., S. Hashsham, F. Dazzo, C. Criddle, R. Hickey, and J. Tiedje. Novel community structure in a model methanogenic system: implications for carbon and energy flow. *Proceedings of the 8th International Symposium on Microbial Ecology*. Halifax, Canada, August 9-14, 1998.

42. Hashsham S., T. Marsh, S. Seston, F. Dazzo, R. Hickey, C. Criddle, and J. Tiedje. Perturbed anaerobic microbial communities: structure and stability. *Proceedings of the 98th General Meeting of the American Society for Microbiology*. Atlanta, Georgia, May 14-19, 1998.
43. Fernandez A.T., J. Xing, S. Hashsham, C. Criddle, and R. Hickey. Diversity among glucose fermenters in methanogenic reactors. *Proceedings of the 97th General Meeting of the American Society for Microbiology*. Miami, Florida, May 19-23, 1997.
44. Hashsham, S.A., and D.L. Freedman. Enhanced biotransformation of carbon tetrachloride by *Acetobacterium woodii* using hydroxocobalamin. *Proceedings of the 96th General Meeting of the American Society for Microbiology*, New Orleans, LA, May 19-23, 1996.

5.4 Non-reviewed conference proceedings

5.4.1 Invited papers/presentations

1. Hashsham, S., X. Zhao, R. Musleh, S. Maher, and T. Voice. "Bioreactor landfill research and demonstration project at the Northern Oaks Landfill, MI" In National Solid Wastes Management Association's WasteTech 2003 Landfill Conference, New Orleans, Louisiana, Feb 16-18, 2003.
2. "GeneScreen: A high-density biochip for phylogenetic and functional analysis of microbial communities" Environmental Genomics Workshop, Idaho Falls, Idaho. Sponsored by the Environmental Biotechnology Institute at the University of Idaho and the Biotechnology Department at the Idaho National Engineering and Environmental Laboratory, September 19-20, 2002.
3. Callister, S.J. and S.A. Hashsham, Modulus of stability and genome expression data. 2002. Poster to be presented at the "*Environmental Genomics Workshop*", sponsors: University of Idaho and Idaho Engineering and Environmental Laboratory's Biotechnology Department. Idaho Falls, Idaho, September 19-20, 2002.
4. Molecular Data and Mathematical Modeling in Biological Wastewater Treatment. Invited lecture at the NSF Sponsored U.S.-Egypt Workshop in Microbial Ecology, National Research Center, Cairo, Egypt, May 6-11, 2001.
5. Development of DNA microarray technology for environmental applications. Invited lecture presented to the Biotechnology Group, Idaho National Engineering and Environmental Laboratory, May, 2001.
6. Microarray technology for mixed microbial communities. Invited Seminar presented at the Department of Civil and Environmental Engineering, University of Michigan, Ann Arbor, MI. August 2001.
7. DNA chip technology and environmental applications. Invited lecture presented to the Biotechnology Group, Idaho National Engineering and Environmental Laboratory, April, 2000.
8. Relating function and community structure of complex microbial systems using neural networks. COE Symposium on Establishment and Evaluation of Advanced Water Treatment Technology Systems Using Functions of Complex Microbial Community The University of Tokyo, Japan. March 6-8, 2000.

5.4.2 Submitted papers/presentations

1. Hashsham, S. (presented for J. Rose). A bioinformatics database to assess risk from waterborne pathogens. 104th General Meeting of the Society for Microbiology, New Orleans, May 22-27th, 2004.
2. S. Dobbelaere, N. Boon, H. Vervaeren, J. Matthys, K. Sys V. Deneef, S. Hashsham and W. Verstraete. 2004. Development and validation of a microarray for the identification of microorganisms responsible for bulking and foaming in WWTPs. European Symposium on Environmental Biotechnology, 25-28 April 2004, Oostende, Belgium.
3. Issues Related to the development of a virulence factor activity relationships (VFARs) biochip for waterborne pathogens. American Water Works Association's 2004 Annual Conference and Exposition, Orlando, Florida. June 13-17th, 2004.

4. Voice, TC, Hashsham, S., Khire, M., Maher, S., Musleh, R., and X. Zhao, Full-scale evaluation of bioreactor landfill technology. 8th Conference on Environmental Science and Technology (CEST), Lemnos Island, Greece, September 8th - 10th, 2003.
5. Zhao, X., Maher, S., Khire, M., Musleh, R., Voice, T., and Hashsham, S., "Bioreactor Landfill Research and Demonstration Project at the Northern Oaks Landfill, MI," *Proceedings of the Waste Tech Landfill Conference 2003*, New Orleans, Louisiana, Feb. 16-18.
6. D. M. Kirk, WG Bickert, S. Hashsham, S. Davies. Methane generation potential of processed liquid dairy manure. 2003. American Society of Agricultural Engineers Annual International Meeting Las Vegas, NV.– July 27-30, 2003.
7. Zhao, X., TC Voice, M. Khire, P Heerwani, R. Musleh, S. Maher, and S. Hashsham. Full-scale evaluation of bioreactor landfill technology. 8th Annual Landfill Management Symposium. Atlantic City, New Jersey, June 16-20th, 2003.
8. Park, Joonhong, Tamara Tsoi, Syed A. Hashsham, Jim Cole, Stephen Callister, and James M. Tiedje. 2002. Design of specific nucleotide probes for genomic microarrays that explore gene expression patterns in a polychlorobiphenyl-degrading bacterium. "Bioremediation and biodegradation: current advances in reducing toxicity, exposure and environmental consequences" June 9-12. Asilomar, CA. (poster presentation).
9. Callister, Stephen, Joonhong Park, Tamara Tsoi, James Cole, James Tiedje, and Syed Hashsham. 2002. Examining DNA-DNA hybridization between chlorinated compounds oxidizing species using a combination PCR/oligo microarray. "Bioremediation and biodegradation: current advances in reducing toxicity, exposure and environmental consequences" June 9-12. Asilomar, CA.
10. Deneff, Vincent^{1,3}, Bergholz, P.¹, Joonhong Park¹, Syed Hashsham, Tamara Tsoi, James Tiedje. 2002. Assessing the sensitivity of oligonucleotide microarrays when studying bioaugmented bacterial communities in the environment. "Bioremediation and biodegradation: current advances in reducing toxicity, exposure and environmental consequences" June 9-12. Asilomar, CA.
11. Hashsham, Syed³: Development of DNA microarray technology for mixed microbial communities. Platform presentation at the 9th International Society for Microbial Ecology, Amsterdam, Netherlands, August 27-September 1, 2001.
12. Dollhopf, S.L., S.A. Hashsham, A. Fernandez, F.B. Dazzo, and J.M. Tiedje. 2000. Microbial community structure as a function of hydraulic retention time length and number in lab-scale methanogenic chemostats. *COE Symposium on the Evaluation of Advanced Waster Water Treatment Technology Systems using Functions of Complex Microbial Communities*. Tokyo, Japan.
13. Dollhopf, S.L., S.A. Hashsham, A. Fernandez, F.B. Dazzo, and J.M. Tiedje. 2000. Spirochete populations in methanogenic chemostats: Population dynamics and ecophysiology VAAM Microbiology 2000 Meeting. Munich, Germany.
14. Seston, S., Hashsham, S., Dazzo, F., Criddle, C., Hickey, R, and Tiedje, J. 1998. Comparison of spirochete populations in three anaerobic reactors using ARDRA and cultivation techniques. Midwest Molecular Microbial Ecology Meeting 1998. Michigan State University, East Lansing, MI.
15. Seston, S. Fernandez, A., Hashsham, S., Criddle, C., Hickey, R., and Tiedje, J. 1997. Community change during stable reactor function: A phylogenetic analysis. Midwest Molecular Microbial Ecology Meeting 1997. University of Wisconsin-Milwaukee, Milwaukee, WI.
16. Hashsham, S. and D. L. Freedman . 1994. Cobalamin-enhanced biotransformation of carbon tetrachloride. Las Cruces, New Mexico. The 4th Annual WERC Technology Development Conference, 13-15 April 1994, Las Cruces, NM.
17. Hashsham, S. and D. L. Freedman. 1992. Enhanced biotransformation of carbon tetrachloride under methanogenic conditions," The University of Notre Dame Hazardous Waste Conference, Notre Dame, IN, (August 31-September 4).

5.5 Sponsored or invited lectures

1. Detection and genotyping of 20 waterborne pathogens in parallel using a microfluidic DNA biochip. Department of Agricultural & Biosystems Engineering, University of Arizona, Tucson, AZ. September 18, 2006.

2. Microfluidics Based Assays for Parallel Detection and Quantification of Infectious Agents. Lab-in-a-Box and Other Cutting-Edge Technology in Microbial Detection, 106th General Meeting of the American Society for Microbiology, Orlando, Florida, May 23, 2006.
3. Screening for All Pathogens in Parallel: Is It Possible? Nucleic Acid-Based Technologies, Arlington, VA, June 27, 2006.
4. Past, Present, and Future of Environmental Biotechnology. A one-day workshop sponsored by National University of Science and Technology (NUST) and held at Pakistan Navy College of Engineering, Karachi, December 31st, 2005. The Power and Beauty of Environmental Genomics. National Academy of Sciences, Central drug Research Institute, Lucknow, India, December 14th, 2005.
6. Microbial Community Analysis using Microarrays: Potential and Challenges. Symposium--Molecular Based Approaches to Soil Microbiology. The ASA-CSSA-SSSA International Annual Meetings (November 6-10, 2005), Salt Lake City, Utah. 2005.
7. Next Generation Real Time Polymerase Chain Reaction (RT-PCR). SERDP/ESTCP Workshop on Molecular Biology Tools. August 9-10th, Charlottesville, Virginia. 2005.
8. In situ Synthesized Biochips for Parallel Microbial detection and Community Fingerprinting: Realized Potential and Remaining Challenges. 4th Montreal Microarray Symposium, BRI, Montreal, Canada, March 17-18, 2005.
9. Environmental Genomics: Challenges and Opportunities, Invited presentation to the students and faculty of the Center for Environmental Science and Engineering, Indian Institute of Technology, Mumbai, December 7th, 2004.
10. Bioinformatics Tools and Techniques for Various Microarray Platforms and Applications Presented at the Workshop on Genetic Approaches to DNA Damage. Indian Toxicology Research Center, Lucknow, U.P., India. December 11th, 2004.
11. Bioinformatics: Challenges and Business Opportunities; Presentation at the Bioinformatics Conference at Biotechnology Park, December 7-13th, 2004.
12. Emerging Areas of Environmental Biotechnology and Nanotechnology, Presentation to the faculty and students of Biotechnology, Computer Science, Environmental Biotechnology, and Pharmacy, Integral University, Lucknow, December 29th, 2004.
13. Full scale bioreactor landfill design, installation, and performance, WasteTech Landfill Technology Conference, May 23-25, 2004. Dallas, TX.
14. Bioreactor landfill research and demonstration project at the northern Oaks Landfill, MI. Proceedings of the National Solid Waste Management Association, WasteTech 2002 Technical Landfill Conference. New Orleans, Louisiana, Feb 16-18, 2003. This is accompanied by publication of the manuscript in the corresponding proceedings.
15. GeneScreen: A high density biochip for phylogenetic and functional analysis of microbial communities" Environmental Genomics Workshop, September 19-20, 2002. Idaho Falls, Idaho. Sponsored by the Environmental Biotechnology Institute at the University of Idaho and the Biotechnology Department at the Idaho National Engineering and Environmental Laboratory.
16. Molecular data and mathematical modeling in biological wastewater treatment. Invited lecture at the NSF Sponsored U.S.-Egypt Workshop in Microbial Ecology, May 6-11, 2001. National Research Center, Cairo, Egypt.*
17. Development of DNA Microarray technology for mixed microbial communities. 9th International Society for Microbial Ecology, Amsterdam, Netherlands, August 27-September 1, 2001.*
18. Development of DNA microarray technology for environmental applications. Idaho National Engineering and Environmental Laboratory, May, 2001.
19. Microarray technology for mixed microbial communities. Department of Civil and Environmental Engineering, University of Michigan, Ann Arbor, MI. August 2001.
20. DNA chip technology and environmental applications. Invited lecture presented to the Biotechnology Group, Idaho National Engineering and Environmental Laboratory, April, 2000.

21. Relating function and community structure of complex microbial systems using neural networks. COE Symposium on Establishment and Evaluation of Advanced Water Treatment Technology Systems Using Functions of Complex Microbial Community March 6-8, 2000. The University of Tokyo, Japan. *

*Abstracts of these presentations were also published in the corresponding proceedings.

6. SUMMARY OF RESEARCH-RELATED ACTIVITIES

A summary of research proposals as per the Contracts and Grants information is given below This indicates a success rate of approximately **60%** by dollar amount and **53%** by the number of proposals awarded. The sections that follow describe the title of the successful proposals and the corresponding granting agency.

| Total Award Amount | Total Requested Amount | Award Count | Proposal Count |
|--------------------|------------------------|-------------|----------------|
| \$9,535,826 | \$15,812,045 | 26 | 49 |

6.1 ACTIVE RESEARCH GRANTS

| Lead Investigator or Co-Principal Investigator | | | | |
|--|---|---|-------------|----------------|
| <i>PIs</i> | <i>Title</i> | <i>Granting Agency</i> | <i>Date</i> | <i>Funding</i> |
| Hashsham and Tiedje | A PCR-chip for simultaneous detection of 50 threat agents relevant to air and water safety | Michigan Economic Development Corporation | 2006-2011 | \$966,558 |
| Tiedje (Project Leader), Hashsham, Cole, Quensen, and Cole | <i>Environmental, Microbial, and Mammalian Biomolecular Responses to Ahr Ligands: Director: Dr. Norb Kaminski</i> | NIEHS-SBRP | 2006-2011 | \$17M |
| JB Rose (25%), Hashsham (25%), C. Bolin (25%), and E. Todd (25%) | Center for Advancing Microbial Risk Assessment (CAMRA) (It is a 10 M Grant to MSU and partner institutions) | Environmental Protection Agency and Department of Homeland Security | 2005-2010 | \$1,970,833 |
| Hashsham (PI), Tiedje, and Tarabara | On-Chip PCR, Nanoparticles, and Virulence/Marker Genes For Simultaneous Detection Of 20 Waterborne Pathogens | Environmental Protection Agency | 2006-2008 | \$600,000 |
| Hashsham (34%), Cole, Klappenbach, Gulari, Tiedje | Flexible Biochip for Highly Parallel Microbial Detection | National Center for Research Resources, National Institutes of Health | 2003-2006 | \$1,370,788 |
| Tiedje (34%), Hashsham (33%), | GeneScreen: A low-cost and high-density DNA biochip for detecting | Michigan economic | 2003-2007 | \$1,151,562 |

| | | | | | |
|------------------------------------|--|-----------------------------------|-----------|-----------|--|
| Cole, Klappenbach, Gulari | up to 30,000 microorganisms (pre-proposal) | Development Corporation | | | |
| Rose Hashsham Gulari (34%), (33%), | Development of a virulence factor biochip and its validation for microbial risk assessment in drinking water | Environmental Protection Agency | 2004-2007 | \$600,000 | |
| Hashsham (100%) | Development of microarrays for evaluating phylogenetic and functional diversity of the microbial world | National Science Foundation (NSF) | 2002-2007 | \$25,000 | |

Center Participation as Co-Principal Investigator:

| <i>PIs</i> | <i>Title</i> | <i>Granting Agency</i> | <i>Date</i> | <i>Funding</i> |
|---|--|--|-------------|----------------|
| Tiedje, Whittam, Lenski, Hashsham, and Gulari (Microbial detection group) | Center for Water Security | MSU Foundation Strategic Partnership Grant | 2002-2007 | \$900,000 |
| Rose, Voice, Boyd, and Hashsham | REF Center on Water Quality and Health | MSU | 2004-2009 | \$750,000 |
| Tiedje, Schmidt, Hashsham, Lenski, Voice, | Center for Microbial Ecology | MSU Vice President for Research | 2002-2006 | \$500,000 |

6.2 COMPLETED RESEARCH GRANTS

| <i>PIs</i> | <i>Title</i> | <i>Granting Agency</i> | <i>Date</i> | <i>Funding</i> |
|---|---|--|-------------|----------------|
| Tiedje, Boyd, Quensen, Tamara, Maltseva, Hashsham (5%), Kukor | Health Hazards from Groundwater Contamination | National Institute of Env Health Sc. (NIEHS) | 2002-2005 | \$244,241 |
| Hashsham (100%), Tiedje | Real time scanning and hybridization capabilities at MSU for quantitative and parallel detection of microorganisms on DNA chips | Department of Defense (DOD) | 2004-2005 | \$150,495 |
| Tiedje, Park, Ostrom, Hashsham (10%) | Probing microbial communities by stable isotope and RNA/DNA analyses | Environmental Protection Agency | 2003-2005 | \$397,454 |
| Hashsham (100%) | Gift to be used for research at the bioreactor cell at northern oaks landfill | Daimler Chrysler Corp | 2004-2005 | \$50,000 |
| Hashsham (100%) | Emerging microbial indicator technologies | Environmental Protection Agency | 2003-2005 | \$20,496 |
| Zhao, Hashsham (15%), Khire, Voice | Development of aeration strategy for elevating temperature in bioreactor landfill | Waste Management, Inc. | 2004-2005 | \$127,263 |
| Khire, Hashsham | Evaluation of landfill gas | National | 2003-2004 | \$44,652 |

| | | | | | |
|--|--|---|-----------|-----------|--|
| (15%), Voice | emissions from an instrumented bioreactor landfill cell | Science Foundation | | | |
| Khire, Hashsham (15%), Voice | Evaluation of landfill gas emissions from an instrumented bioreactor landfill cell | National Science Foundation | 2003-2005 | \$43,772 | |
| Tiedje, Hashsham (32%) | Characterization and application of reductive dehalogenase genes in enhancement and monitoring of biodegradation of chlorinated pollutants | Environmental Protection Agency (EPA) | 2002-2004 | \$249,842 | |
| Hashsham (34%), Zhao, Khire, and Voice | Landfill Bioreactors: Field Scale Evaluation | Department of Energy (DOE) & Env Res & Ed Fdn | 2001-2004 | \$207,000 | |
| Hashsham (34%), Cole, and Rose | Bioinformatics Pilot Program for the Assessment of Virulence-Factor and Activity relationships (VFARs) for Waterborne Disease Microorganisms | Environmental Protection Agency | 2002-2004 | \$95,786 | |
| Hashsham (100%), Bickert | Pilot scale evaluation of sustainable manure management by struvite recovery | National Center for Manure Management | 2002-2004 | \$35,000 | |
| Khire, Hashsham (15%), Voice | Evaluation of landfill gas emissions from an instrumented bioreactor landfill cell | National Science Foundation | 2003-2004 | \$24,950 | |
| Hashsham (100%) | Development of Experimental and Computational Tools in Environmental Genomics | Intramural Research Grant Proposal (MSU) | 2001-2003 | \$49,923 | |
| Dybas, Wiggert, Hashsham, Hyndman | Tiedje, Voice, (8%), Evaluation of Remediation in Schoolcraft Plumes G and F Contaminated with Chlorinated Solvents and Metals | Michigan Department of Environmental Quality (DEQ) | 2000-2002 | \$58,581 | |
| Hashsham (100%) with C. Criddle | Proof of Gene Expression during Bioremediation | EPA HSRC WR | 1999-2002 | \$84,388 | |
| Hashsham (100%) with W. Bickert | Phosphorous Removal from Dairy Manure | Michigan Animal Industry Coalition | 2001-2002 | \$30,000 | |
| Hashsham (100%) | State of Science: Landfill Bioreactors | EPA HSRC CR | 2001-2002 | \$25,000 | |
| Hashsham (100%) | Assessing Market Potential for GeneScreen: A Low Cost and High Density DNA Biochip for Parallel Detection of up to 30,000 Microorganisms | MSU Case Center's Management of Technology and Innovation program | 2002-2002 | \$5,000 | |

7. SUMMARY OF TEACHING-RELATED ACTIVITIES

My teaching experience at MSU is focused on three biological courses: ENE804: Biological Processes in Environmental Engineering; ENE 806: Feasibility studies in Environmental Engineering, and CE487: Environmental Microbiology. Occasionally, I have taught other courses as well.

| Number | Title | Semester/Y | Size |
|----------------------------|---|------------|-------|
| ENE804: 3 Credits (Grad) | Biological Processes in Environmental Engineering | Fall 99 | 8 |
| | | Fall 00 | 9 |
| | | Fall 01 | 10 |
| | | Fall 02 | 10 |
| | | Fall 03 | 10 |
| | | Fall 05 | 9 |
| | | Fall 06 | 4 |
| CE487: 3 Credits (UG/Grad) | Microbiology for Environmental Health Engineering | Spring 00 | 14 |
| | | Spring 01 | 17 |
| | | Spring 02 | 20 |
| | | Spring 03 | 22 |
| | | Spring 04 | 17 |
| | | Spring 05 | 12 |
| ENE806: 3 Credits (Grad) | Feasibility studies in Environmental Engineering | Spring 01 | 8 |
| | | Spring 02 | 6 |
| | | Spring 03 | 9 |
| | | Spring 04 | 9 |
| | | Spring 05 | 7 |
| | | Spring 06 | 6 |
| CE 280: 3 Credits (UG) | Introduction to Environmental Engineering | Summer 03 | 40 |
| ENE800: 1 Credit (Grad) | Environmental Engineering Seminar | Spring 01 | 3/20 |
| | | Spring 05 | ~2/18 |

7.1 COURSE DESCRIPTION:

ENE 804: Biological Processes in Environmental Engineering: This is a design course for environmental engineers focusing on the biological principles. Earlier this course used the 3rd edition Metcalf and Eddy: Wastewater Treatment Plant Design. I have changed it to a more suitable textbook by Rittmann and McCarty, 2001, Environmental Biotechnology: Principles and Applications.

CE 487: Microbiology for Environmental Health Engineering: This course is the only course that teaches microbiological principles to civil and environmental engineers. I use Brock Biology of Microorganisms by Madigan, Martinko, and Parker as the textbook but heavily supplement with handouts on quantitative aspects. This is the course in which I am incorporating learner's modules (e.g., MOBEE) as a teaching tool.

ENE 806: Treatability Studies in Environmental Engineering: This course is designed to provide and overarching experience in literature review, experimental design, laboratory scale reactor set up and operation, data collection and analysis (using gas chromatography, high performance liquid chromatography, atomic absorption spectrophotometer, ion chromatography, PCR, plate count, and other routine laboratory instruments), report writing, and presentation. It also requires group meetings, team effort, task scheduling and problem solving. Overall it provides a short exposure to many aspects of research and team approach within one semester. It does not have a formal textbook.

CE 280: Introduction to Environmental Engineering: This is the basic environmental engineering course taken by many students on campus. It is considered as a service course and is taken by approximately 140 students in each of the Spring and Fall semester. During summer it is taken by ~40 students.

ENE 800: Environmental Engineering Seminar: A seminar in which 6-8 speakers over a given semester (external and internal) are invited to share their research. Attendance by graduate students and faculty is expected.

8. GRADUATE STUDENT ADVISING

During the period August 1999-November 2006, I have graduated two doctoral and four MS students. During the same period, I have also hosted a total of eleven visiting scholars and six post-doctoral associates. I am currently advising seven doctoral candidates, two of them are shared with other colleagues. I am currently serving as a committee member of three doctoral and MS students. In the past, I have served as committee member for 12 MS thesis/projects and 12 doctoral students. The following table provides further details.

8.1 AS ADVISOR

| Name | Start date | Research Topic |
|---------------------------|-------------|---|
| Doctoral | | |
| <i>Completed:</i> | | |
| Musleh, Reem | 2001-2005 | Bioreactor landfill field scale design considerations |
| Callister, Stephen | 1999-2003 | Resistance and resilience of gene expression in biological systems |
| <i>Continuing:</i> | | |
| Robert Stedtfeld | 2004-contd. | High throughput validation strategies using synthetic targets |
| Dieter Tourlousse | 2004-contd. | Parallel microbial detection biochips using functional genes |
| Farhan Ahmad | 2006-contd. | On-chip PCR for pathogens and indicator organisms |
| Samuel Baushke | 2005-contd. | Design and validation of on-chip PCR |
| Muneer Ahsan | 2005-contd. | Modeling of hybridization in microfluidic biochips |
| James Wallace | 2003-contd. | Anaerobic membrane reactor (Dual degree with W. Bickert) |
| Yu Yang | 2004-contd. | Nanomaterials and biosensors (with Jim Tiedje) |
| Gregoire Seyrig | 2007-contd. | On-chip PCR assay for pathogens |
| <i>Changed Dept/Univ:</i> | | |
| Ruifeng Xu | 2004-2006 | Validation strategies to improve detection limit |
| Kamlesh Yadav | 2004-2005 | Innovative and cost effective array-based validation and detection |
| Maria Rodriguez | 2000-2002 | Change of research focus, program and advisor |
| Post-doctoral | | |
| Alok Pandey | 2006-contd. | CAMRA: Quantum dots as surrogates and their genotoxicity |
| Lukas Wick | 2003-2005 | Development of strain fingerprinting biochips (Lukas Wick is shared with James Tiedje and Thomas Whittam) |
| Park, Joonhong | 2001-2005 | NIH PCB project (shared with Prof. James Tiedje and Dr. Tamara Tsoi) |
| Sun, Baolin | 2000-2004 | DEQ Plume G Schoolcraft project (shared with Prof. James Tiedje) |
| Maqbool, Shahina | 2001-2002 | Development of DNA Microarrays |
| Musarrat, Javed | 2000-2001 | Proof of gene expression during bioremediation |

| Visiting Scholars | | |
|---------------------------|-------------|---|
| Imran Hashmi | 2006-contd. | Microbial community structure analysis of anaerobic reactors |
| Angela Wang | 2006-contd. | Microbial Fuel Cell community structure (shared with J. Tiedje) |
| Abu-Bakr Gomaa | 2007-contd. | Microbial community analysis of rhizosphere roots |
| Alok Dhawan | 2005-2006 | On-chip melting curves for antibiotic resistance gene chips |
| Yu Xin | 2005-2006 | Genotyping biochips for <i>Cryptosporidium</i> and <i>Giardia</i> |
| Ian Marshall | 2004-2004 | Sample processing for PDT arrays (shared with J. Tiedje) |
| Tam N.T. Huynh | 2004-2004 | Fingerprinting of bioreactor landfill microbial communities |
| Matsumura, Yoshinobu | 2002-2003 | Antibiotic Resistance Gene chip (shared with Prof. James Tiedje) |
| Han Vervaeren | 2003-2004 | PDT arrays for wastewater treatment communities |
| Sys Kurt | 2003-2004 | PDT arrays for wastewater treatment communities |
| Tijdens, Marjoline | 2000-2001 | Source tracking using <i>E. coli</i> toxin gene microarrays |
| Denef, Vincent | 2000-2001 | Development of DNA Microarrays |
| Research Assistant | | |
| Tiffany Stedtfeld | 2006-contd. | PCR chips |
| Sarah Miller | 2005-contd. | Biochips validation for parallel microbial detection |
| Jeff Weiss | 2004-2005 | Biochips for indicator microorganisms (Res. Tech.) |
| Masters | | |
| Amanda Herzog | 2006-contd. | Detection limit and microbial risk assessment (CAMRA) |
| Vidya Srinivasan | 2005-contd. | Use of Gold and silver Nanoparticles and Flatbed Scanner PDTs |
| Bryant Miller | 2004-contd. | Project topic not yet decided |
| Arora, Salil | 2001-contd. | Exploration of unique genes in whole genome sequences |
| Yang, Yu | 2003-2004 | Sample concentration issues for DNA biochips (with J. Tiedje) |
| Siple, Scott | 2002-2006 | Project option, works with Dr. Terry Marsh for research advising |
| Heerwani, Priya | 2001-2004 | Dynamics of CH ₄ , N ₂ , CO ₂ , and O ₂ in a bioreactor landfills |
| Maher, Seth | 2002-2003 | Bioreactor landfills: field scale design and operation: DOE Project |
| Undergraduates | | |
| Herzog, Amanda | 2004-2006 | DNA biochip fingerprinting of activated sludge foam communities |
| Baushke, Sam | 2004-2005 | Automation of data analysis for parallel detection biochips |
| Aldrich, Keith | 2004-2004 | Establishment of a nuclear pore filter train for sample concentration |
| Kruszewski, Elizabeth | 2004-2005 | Validation of antibiotic resistance genes chip |
| Katherine M. Rubrich | 2004-2004 | Works on an hourly basis on waterborne pathogen chips |
| High School | | |

| | | |
|-------------|------|---|
| Hon Chu | 2006 | Hand-held PCR chip |
| Asra Shaik | 2006 | Visualization of amplicons in a PCR-chip |
| Sarah Asrar | 2006 | Dispensing and amplification on a microfluidic chip |

8.2 AS COMMITTEE MEMBER

| Name | Date | Dept. | Primary advisor | Thesis/Dissertation Area |
|--------------------|-------------|---------|-----------------|--|
| Doctoral | | | | |
| Brian Campbell | 2006-contd. | CSS/CME | James Tiedje | Antibiotic resistance gene tracking |
| Erick Cardenas | 2005-contd. | CSS/CME | James Tiedje | Metal reduction at DOE sites |
| James Wallace | 2004-contd. | AgE/CEE | William Bickert | Fixed film digesters |
| Fujimoto Masanori | 2004-contd. | CEE | Thomas Voice | Water safety in the developing nations |
| Dana Kirk | 2002-contd. | AgE | William Bickert | Anaerobic digestion of manure |
| Jacob Parnell | 2002-2006 | CME | James Tiedje | LB400 expression microarrays |
| Elica Monique Moss | 2002-2005 | CME | James Tiedje | Aerobic TCE community structure |
| Claribel Kruzgarc | 2001-2005 | CME | James Tiedje | <i>Shewanella</i> microarray studies |
| K. Konstantinidis | 2001-2005 | CME | James Tiedje | LB400 genome informatics |
| Xiayun Qiu | 2002-2004 | CME | James Tiedje | Effect of UV radiation on <i>Shewanella</i> genomics |
| Tim Mayotte | 2000-2004 | CEE | Mike Dybas | TCE remediation- column studies |
| Hayk-Yung Kim | 2000-2004 | CEE | Mike Dybas | TCE transformation studies |
| Mohammad Sajjad | 2003-2005 | CEE | Thomas Voice | Bioavailability of PAHs in soil |
| Jaime Graulau | 2000-2002 | CEE | David Wiggert | Modeling of Plume G (changed to Phanikumar) |
| Jerry Wu | 4/30/01 | CEE | Susan Masten | Ozonation of lake water |
| Kyung-Hyuk Lee | 4/30/01 | CEE | Susan Masten | Ozonation of lake water |
| Masters | | | | |
| Fan Wang | 2005-2006 | Micro | Terry Marsh | Chromium uptake by <i>Ralstonia</i> |
| Shashi Bhatta | 5/2/00 | CEE | Susan Masten | MTBE degradation |
| Dongwan Kim | 12/7/00 | CEE | Thomas Voice | Bioreactor landfills |
| Asad Qazi | 5/10/00 | CEE | Susan Masten | Manure management |
| Georgina Gavilan | 6/30/00 | CEE | David Wiggert | TCE remediation |
| Mohsin Mir | 4/27/01 | CEE | Voice/Hashsham | Anaerobic digestion |
| Adnan Javed | 4/27/01 | CEE | Voice/Hashsham | Anaerobic digestion |
| Ya-Yun Wu | 12/14/01 | CEE | Susan Masten | Ozonation of manure |
| Park Daewook | 8/31/02 | CEE | Thomas Voice | Arsenic removal from drinking |

| | | | | |
|------------------------|------|-----|----------------------|---|
| Mike Ayallo | 2002 | CME | James Tiedje | water TCE remediation- RT PCR |
| Dana Kirk | 2001 | AgE | William Bickert | Phosphorus removal from manure |
| Kevin Arthur Kowalk | 2002 | AgE | William Northcott | Wetland remediation of manure |
| Aruna Yellai | 2004 | CEE | Milind Khire | Field scale evaluation of landfill gas emissions |

9. SUMMARY OF ACTIVITIES AS A REVIEWER

| Organization/Journal | Period or year | Number manuscripts/proposals/books reviewed | of |
|--|----------------|---|----|
| Manuscripts: | | | |
| Environmental Science and Technology | 2003-2006 | 5 | |
| Applied and Environmental Microbiology | 2003-2006 | 12 | |
| Journal of Bacteriology | 2004 | 2 | |
| Critical Reviews in Microbiology | 2004 | 1 | |
| Journal of Environmental Engineering | 2003-2006 | 4 | |
| Journal of Microbiological Methods | 2006 | 1 | |
| Water Research | 2003-2006 | 5 | |
| Chemosphere | 2002 | 1 | |
| Biotechnology and Bioengineering | 2002-2006 | 2 | |
| Environmental Engineering | 2003-2004 | 2 | |
| Microbial Ecology | 2002-2006 | 3 | |
| | Total | 37 | |
| Books: | | | |
| McGraw Hill Book Company, Metcalf & Eddy 4 th Ed, Wastewater Treatment | 2000 | 1 | |
| Proposals: | | | |
| Graham Environ. Sustainability Institute | 2006 | 3 | |
| Singapore National Science Foundation | 2005 | 1 | |
| National Science Foundation | 2002-2006 | 20 | |
| National Sea Grant College Program | 2001 | 2 | |
| Unites State Department of Agriculture | 2004-2006 | 4 | |
| A proposal for National Center for Genomics, United Kingdom | 2000 | 1 | |
| | Total | 31 | |
| MS Theses | | | |
| As member, AEESP review committee | 2002 | 10 | |
| As member, AEESP review committee | 2003 | 11 | |
| As chair, AEESP review committee | 2004 | 12 | |

10. HONORS AND AWARDS

1. Edwin Willits Associate Professor Award, One of four chaired Associate Professors in the College of Engineering, 2005-2007
2. Session Chair, International Water Association's 2nd Leading Edge Conference on Sustainability, Nov 8-10, 2004, Sydney, Australia.
3. Distinguished Service Award for Outstanding Service as Chair of the Masters Thesis Award Subcommittee, Association of Environmental Engineering and Science Professors, 2004
4. Lilly Teaching Fellowship Award, Michigan State University, Office of the Vice President, 2003-2004
5. Racheff Travel Award, University of Illinois at Urbana-Champaign, 1995
5. Member, Tau Beta Pi, University of Texas at Arlington, 1991-contd.
6. Ranked 2nd in M.Tech., Indian Institute of Technology, Bombay. 1986
7. GATE Fellowship, Indian Institute of Technology, Bombay, 1984
8. Member, Vice Chancellor's Student Council for Academic Excellence, AMU, Aligarh, 1984
9. Ranked 2nd, Class of 1984.
10. Merit Scholarships during the last three years of B.Sc. Engineering. 1981-1984

11. CURRENT COLLABORATIONS

1. James Tiedje, University Distinguished Professor and Director, The Center for Microbial Ecology, Michigan State University, MI.
2. Joan Rose, Homer Nowlin Chair, Fisheries and Wildlife, Michigan State University, MI.
3. Erdogan Gulari, Department of Chemical Engineering, University of Michigan.
4. James Cole, Center for Microbial Ecology, Michigan State University
5. Tamara Tsoi, Center for Microbial Ecology, Michigan State University
6. Thomas Voice, Department of Civil and Environmental Engineering, Michigan State University.
7. Norb Kaminski, National Center for Food Safety and Toxicology, Michigan State University

12. COMMITTEE SERVICES

Department Committees:

| | |
|-------------|---|
| 2006-contd. | Faculty search committee (Biosystems Engineering) |
| 2005-contd. | Department's ENE Space Committee |
| 2005-contd. | Department's Advisory Committee |
| 2006. | Faculty search committee (Crops and Soil Sciences) |
| 2004-2005 | Faculty search committees (CEE) |
| 2002-2004 | Department's Advisory Committee |
| 2002 | Faculty search committees (Pathogen position, CEE) |
| 2001 | Graduate Studies Committee and ENE program Graduate Coordinator |
| 2001 | Faculty search committees (Fluids position in CEE) |
| 2000 | Faculty search committees (Chemical Engineering) |
| 2000 | Faculty search committees (Geo-environmental position in CEE) |

University Committee:

| | |
|-------------|---|
| 2007-contd. | ESPP Recruiting Fellowship Committee |
| 2006-cond. | Environmental Science and Policy Science Advisory Committee |
| 2004 | Invited Faculty Participant of Lilly Fellowship Selection Committee |
| 2001 | Genomic Technology User Committee |

National Committees:

| | |
|-------------|---|
| 2004-contd. | Chair, American Society for Testing Materials (ASTM) Committee on Microbial Corrosion of Concrete, C13.03 |
| 2004 | Review Committee: VFAR Workshop Document organized by EPA, Nov 27-29, 2004 |

| | |
|-----------|--|
| 2004 | Chair, Montgomery-Watson-Herza MS Thesis Review Committee, AEESP |
| 2003-2004 | Member, American Society for Testing Materials (ASTM) Committee on Microbial Corrosion of Concrete, C13.03 |
| 2002-2003 | Member, Montgomery-Watson-Herza MS Thesis Review Committee, AEESP |

13. PROFESSIONAL AFFILIATIONS

| | |
|-------------|---|
| 1994-Contd. | Association of Environmental Engineering and Science Professors (AEESP) |
| 1995-Contd. | American Society for Microbiology (ASM) |
| 1999-2005 | International Society for Microbial Ecology (ISME) |
| 2004-2005 | International Water Association (IWA) |
| 2002-2004 | Water Environment Federation (WEF) |
| 1999-2001 | American Society of Civil Engineers (ASCE) |

(Last Revised: January 21, 2007)

Tools for understanding the pathogen in the post genomic era

Guanghai Wu and Muna Anjum

Department of Food and Environmental Safety, Veterinary Laboratories Agency-Weybridge, New Haw, Addlestone, Surrey KT15 3NB, U.K.

The DNA microarray chip offers a new way for biologists to understand the complexities of a pathogen. In our laboratory we have been using microarrays to detect the presence and absence of genes from field and clinical isolates of *Escherichia coli* and *Salmonella*, of both animal and human origins, in comparison to sequenced strains. Information regarding the relationship between different isolates and pathogenetic potential of these isolates have been obtained to understand the evolution of these organisms and develop a better detection method for these pathogens (1, 2, 4, 8)). The microarray platform including array printing, slide processing and procedures for hybridization and data analyzing, using statistical methods, have been well established in our laboratory (7). We have also developed miniturised microarrays using an eppendorf-based system (ArrayTubes) for use in detection of virulence in *E. coli* for pathotyping (3) or antimicrobial resistant genes in Gram negative bacteria, especially *E. coli* and *Salmonella* (6), and in serotyping of somatic *E. coli* antigens (5). Due to the simplicity and rapidness of the ArrayTube systems, they have the potential for development as high-throughput systems in the future and for automation. We are also collaborating and providing technical training to scientists from a number of institutes and universities in the UK and continental Europe in the various array technologies currently being used at the VLA. The virulence and antimicrobial arrays have been commercialized (www.identibac.com.) and are being well received by our users.

References:

1. **Aktan, I., B. Carter, H. Wilking, R. La Ragione, L. Wieler, M. J. Woodward, and M. F. Anjum.** 2007. The influence of geography, host animal and stx gene on the virulence characteristics of *Escherichia coli* O26 strains. *JMedMicro* **in press**.
2. **Anjum, M. F., S. Lucchini, A. Thompson, J. C. Hinton, and M. J. Woodward.** 2003. Comparative genomic indexing reveals the phylogenomics of *Escherichia coli* pathogens. *Infect Immun* **71**:4674-83.
3. **Anjum, M. F., M. Mafura, P. Slickers, K. Ballmer, P. Kuhnert, M. J. Woodward, and R. Ehricht.** 2007. Analysis of virulence diversity in clinical isolates of *Escherichia coli* using miniaturised DNA-microarrays. *Appl Environ Microbiol* **73**:5692-7.
4. **Anjum, M. F., C. Marooney, M. Fookes, S. Baker, G. Dougan, A. Ivens, and M. J. Woodward.** 2005. Identification of core and variable components of the *Salmonella enterica* subspecies I genome by microarray. *Infect Immun* **73**:7894-905.

5. **Anjum, M. F., J. D. Tucker, K. A. Sprigings, M. J. Woodward, and R. Ehricht.** 2006. Use of miniaturized protein arrays for Escherichia coli O serotyping. *Clin Vaccine Immunol* **13**:561-7.
6. **Batchelor, M., K. L. Hopkins, E. Liebana, P. Slickers, Ehricht, R.,, M. Mafura, and F. Aarestrup, Mevius, D., Clifton-Hadley, F. A., Woodward, M. J., Davies, R. H.,Threlfall, E. J., Anjum, M. F.** 2007. Development of a microarray based assay for the rapid identification of antimicrobial resistance genes from Gram-negative bacteria. in preparation.
7. **Carter, B., G. Wu, M. J. Woodward, and M. F. Anjum.** 2007. A process for analysis of microarray comparative genomic hybridisation studies. *BMC Genomics* **submitted**.
8. **Wu, G., B. Carter, M. Mafura, E. Leibana, M. J. Woodward, and M. F. Anjum.** 2007. Genetic Diversity among Escherichia coli O157:H7 Isolates and identification of genes linked to human infections. *Infect Immun* **submitted**.

Curriculum vitae Dr. Guanghui Wu

PROFESSIONAL EXPERIENCE

Molecular Microbiologist, Veterinary Laboratories Agency (2004-present)

Studies pathogenic mechanism of enteric bacteria. Study the global responses of pathogenic *E. coli* in tissue cultures and animal models.

Postdoctoral Research Associate, University of Sheffield (1996-2004)

Responsible for several research projects, writing grant proposals, supervision and training of postdoctoral/postgraduate research assistants and the technician, presenting results within the group, as well as at international conferences.

Postdoctoral Research Associate, King's College London, University of London (1992-1996)

Successfully completed all research projects. Performed scientific research independently and involved in the supervision of Ph.D students and project students, initiated and set up the recording systems for the culture and plasmid collections in the laboratory.

Research Assistant, Institute of Microbiology, Academy of Sciences of China, Beijing (1986-1988)

Screened thousands of bacteria for those with high enzymatic activities, optimised fermentation conditions, participated in trials in a pilot plant and collaboration with industry.

EDUCATION

Ph.D in Microbiology, King's College London, University of London, 1992.

M.Sc in Microbiology, Academy of Sciences of China, Institute of Microbiology, Beijing, 1986.

B.Sc. in Microbiology, Sichuan University, China, 1983.

RESEARCH GRANTS

- | | |
|-----------|--|
| 1999-2001 | BBSRC research grant on Respiration and oxygen tolerance in <i>Campylobacter coli</i> and <i>C. jejuni</i> : implication for food safety and colonisation together with Professor R. K. Poole and Dr. S. Park. |
| 2003-2006 | BBSRC research grant on Mechanisms underlying survival of <i>Campylobacter jejuni</i> during oxidative and nitrosative stresses together with Professor R. K. Poole and Dr. S. Park. |

PUBLICATIONS

1. **Wu, G.**, Williams, H. D., Zamanian, M., Gibson, F. and Poole, R. K. (1992). Isolation and characterisation of *Escherichia coli* mutants affected in aerobic respiration: the cloning and nucleotide sequence of *ubiG*. Identification of an *S*-adenosylmethionine-binding motif in protein, RNA, and small-molecule methyltransferases. *Journal of General Microbiology* **138**, 2101-2112.
2. **Wu, G.**, Williams, H. D., Gibson, F. and Poole, R.K. (1993). Mutants of *Escherichia coli* affected in respiration: the cloning and nucleotide sequence of *ubiA*, encoding the membrane-bound *p*-hydroxybenzoate:octaprenyltransferase. *Journal of General Microbiology* **139**, 1795-1805.
3. Poole, R.K., Hatch, L., Cleeter, M. W. J., Gibson, F., Cox, G. B. and **Wu, G.** (1993). Cytochrome *bd* biosynthesis in *Escherichia coli*: the sequences of the *cydC* and *cydD* genes suggest that they encode the compounds of an ABC membrane transporter. *Molecular Microbiology* **10**, 421-430.
4. Poole, R. K., Gibson, F. and **Wu, G.** (1994). The *cydD* gene product, component of a heterodimeric ABC transporter, is required for assembly of periplasmic cytochrome *c* and cytochrome *bd* in *Escherichia coli*. *FEMS Microbiology Letters* **117**, 217-224.
5. Vargas, C., **Wu, G.**, Davies, A. and Downie, J. A. (1994). Identification of a gene encoding a thioredoxin-like product necessary for cytochrome *c* biosynthesis and symbiotic nitrogen fixation in *Rhizobium leguminosarum*. *Journal of Bacteriology* **176**, 4117-4123.
6. Poole, R. K., D'mello, R., Hill, S., Ioannidis, N., Leung, D. and **Wu, G.** (1994). The oxygen reactivity of bacterial respiratory haemoproteins: oxidases and globins. *Biochimica et Biophysica Acta* **1187**, 226-231.

7. Vargas, C., **Wu, G.**, Delgado, M.-J., Poole, R. K. and Downie, J. A. (1996). Identification of symbiosis-specific *c*-type cytochromes and a putative oxidase in bacteroids of *Rhizobium leguminosarum* biovar *viciae*. *Microbiology* **142**, 41-46.
8. Delgado, M.-J., Yeoman, K. H., **Wu, G.**, Vargas, C., Davies, A. E., Poole, R. K., Johnston, A. W. B., and Downie, J. A. (1995). Characterization of the *cycHJKL* genes involved in cytochrome *c* biogenesis and symbiotic nitrogen fixation in *Rhizobium leguminosarum*. *Journal of Bacteriology* **177**, 4927-4934.
9. **Wu, G.**, Vargas, C., Downie, J. A. and Poole, R. K. (1996). The cytochrome *bc₁* complex but not CymM is necessary for symbiotic nitrogen fixation by *Rhizobium leguminosarum*. *Microbiology* **142**, 3381-3388.
10. **Wu, G.**, Hill, S. Kelly, M., Sawers, G. and Poole, R. K. (1997). The *cydR* gene product, required for regulation of cytochrome *bd* expression in obligate aerobe *Azotobacter vinelandii*, is an Fnr-like Protein. *Microbiology*, **143**, 2197-2207.
11. **Wu, G.**, Cruz-Ramos, H., Hill, S., Green, J., Sawers, G. and Poole, R. K. (2000) Regulation of cytochrome *bd* expression in the obligate aerobe *Azotobacter vinelandii* by CydR (Fnr). Sensitivity to oxygen, reactive oxygen species, and nitric oxide. *J Biol. Chem.* **18**, 4679-4686.
12. Beard S.J, Hashim R, **Wu G**, Binet M.R, Hughes M.N, Poole R.K. (2000). Evidence for the transport of zinc (II) ions *via* the Pit inorganic phosphate transport system in *Escherichia coli*. *FEMS Microbiol. Lett.* **184**, :231-235.
13. Edwards, S. E., Loder, C. S., **Wu, G.**, Corker, H., Bainbridge, B. W., Hill, S. and Poole, R. K. (2000). Mutation of cytochrome *bd* quinol oxidase results in reduced stationary phase survival, iron deprivation, metal toxicity and oxidative stress in *Azotobacter vinelandii*. *FEMS Microbiol. Lett.* **185**, 71-77.
14. **Wu, G.** Moir, A., Hill, S. Sawers, G. and Poole, R. K. (2001). Biosynthesis of poly- β hydroxybutyrate acids (PHB) is controlled by CydR (FNR) in the obligate aerobe *Azotobacter vinelandii*. *FEMS Microbiol Lett.* **194**, 215-220.
15. Cruz-Ramos, H., **Wu G**, Poole, R. K. (2002). NO sensing by FNR: regulation of the *Escherichia coli* NO-detoxifying flavohaemoglobin, Hmp. *The EMBO Journal* **21**: 3235-3244.
16. Pittman, M. S., Corker, H., **Wu, G.**, Binet, M. B., Moir, A. J. G. and Poole, R. K. (2002) Cysteine is Exported from the *Escherichia coli* cytoplasm by *cydDC*, an ATP-binding cassette-type transporter required for cytochrome assembly. *Journal of Biological Chemistry* **277**: 49841-49849.
17. **Wu, G.**, Wainwright, L. M. and Poole, R. K. (2003) Microbial Globins. *Advances in Microbial Physiology* **47**: 255-310.
18. **Wu, G.**, Wainwright, L. M. Membrillo-Hernandez, J. and Poole, R. K. (2004) Bacterial haemoglobins: old protein with 'new' function? Roles in respiratory and nitric oxide metabolism. *Respiration in Archaea and Bacteria*. (ed. Davide Zannoni).
19. Cruz-Ramos, H., Cook, G. M. **Wu G**, Cleeter, M. Poole, R. K. (2004) Membrane topology and mutational analysis of *Escherichia coli* *CydDC*, an ABC-type cysteine exporter required for cytochrome assembly. *Microbiology* **150**: 3415-342720.
20. Elvers, K., **Wu, G.**, Poole, R. K. Park, S., (2004) Role of an Inducible Single-Domain Hemoglobin in Mediating Resistance to Nitric Oxide and Nitrosative Stress in *Campylobacter jejuni* and *Campylobacter coli*. *J. bacteriol.* **186**: 5332-5341.
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22. Barynin, V., **Wu. G.**, Poole, R. K. and Rice, D (2003) Crystal structure of the single domain hemoglobin from *Campylobacter jejuni* at 1.2 Å resolution. In preparation.
23. **Wu, G.** Cooker, H., Orii, Y., Poole, R. K. (2004) *Escherichia coli* Hmp, an "oxygen-binding flavohaemoprotein", produces superoxide anion and self-destructs. *Archives of Microbiology* **182**: 193-203.
24. **Wu, G.**, Yeh, S. Poole, R. K. (2006). *Escherichia coli hmp* mutant is more resistant to organic peroxides than its wild-type parent. In preparation.
26. **Wu, G.** et al Purification and spectroscopic characterisation of a monomeric 'myoglobin-like' haemoglobin, Cgb, from the microaerophilic foodborne pathogen, *Campylobacter jejuni*.
27. Lu, C.; Mukai, M., Lin, Y., **Wu, G.** Poole, R. K. and Yeh, S. R (2007). Structure and functional properties of a single-domain hemoglobin from the food-borne pathogen *Campylobacter jejuni*. *JBC in the press*.
28. Pickford, J. L., Wainwright, L., Wu, G. and Poole, R. K. Expression and Purification of Both NO-Inducible Globins from the Foodborne Bacterial Pathogen *Campylobacter jejuni*: Cgb and Ctb. *Methods in Enzymology in the press*.
29. Wu, G., Mafura, M., Liebana, E., Woodward, M. J. and Anjum, M. F. (2007) Genetic Diversity among *Escherichia coli* O157:H7 Isolates and identification of genes linked to human infections. Submitted.
30. Carter, B, Wu, G., Woodward M. J. and Anjum, M. F. (2007). A process for analysis of microarray comparative genomics hybridisation studies. Submitted.

Diversity Arrays Technology (DArT) as a generic tool for microbial diagnostics

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Diversity Arrays Technology (DArT) is a sequence-independent microarray platform that measures the abundance of DNA fragments in 'representations' prepared from (meta)genomic DNA samples. A representation is a well-defined subset of DNA fragments, generated by restriction-enzyme digestion, adapter ligation and amplification of adapter-ligated fragments (or any other robust and reproducible 'complexity reduction' method). The abundance of fragments in representations is a reflection of single-nucleotide polymorphisms (SNP) at restriction-enzyme sites, insertion-deletion (InDel) polymorphisms between restriction-enzyme sites, or the relative abundance of different species in the case of metagenomic samples.

DArT is established without using any DNA sequence information, yet it produces sequence-ready diagnostic clones. This feature makes DArT particularly attractive for applications that would otherwise require extensive sequencing to capture the genetic diversity of a phylogenetic group. In addition, diagnostic clones are identified and assayed using exactly the same procedure, which obviates the need for a separate assay-development step. To this date, DArT has been applied to more than two dozens of plant species (from mosses to higher plants), several fungal plant pathogens and a few animal species.

We first outline the technology principles, review the most common areas of application in plant science and highlight potential opportunities in the area of microbial diagnostics. We then present the results of a first evaluation of DArT for the purpose of *Salmonella* phage typing. In this project, DArT was adapted to the subtyping of *Salmonella enterica* ssp. *enterica* Enteritidis and Typhimurium serovars as a means to overcome drawbacks associated with traditional, phenotypic phage typing. Phage typing of *S. enterica* is required to precisely identify strains, e.g. in order to trace back sources of infection. We successfully applied DArT-based molecular markers to discriminate among various *S. enterica* phage types by hybridizing representations prepared from individual *S. enterica* strains onto microarrays. By further refining the complexity reduction method used for *Salmonella*, or by using multiple complexity reduction methods, DArT offers a vast potential for identifying significant numbers of phage type-specific markers. The encouraging results of this proof-of-concept study suggest that DArT is a useful, yet underexploited tool for microbial diagnostics.

Curriculum vitae Dr. Peter Wenzl

Personal

Name: Peter Wenzl
Born: 1967 in Vienna, Austria
Citizenship: Austria
Marital status: Married, one child
Languages: English (fluent), Spanish (fluent), German (mother tongue)
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University education

2000 **PhD in Plant Physiology**, University of Vienna, Austria
1993 **MSc in Biophysics**, Swiss Federal Institute of Technology (ETH), Zürich, Switzerland, and University of Vienna, Austria
1992 **BSc in Genetics & Biochemistry**, University of Vienna, Austria

Employment history

2004 to date **Principal Scientist**, Diversity Arrays Technology P/L, Canberra, Australia
2002-04 **Senior Scientist**, Diversity Arrays Technology P/L and Center for the Application of Molecular Biology to International Agriculture (CAMBIA), both in Canberra, Australia
2002 **Consultant**, Biotechnology Research Unit, International Center for Tropical Agriculture (CIAT), Cali, Colombia
2001 **Staff Scientist**, CAMBIA, Canberra, Australia
1998-2000 **Postdoctoral Fellow/Experimental Scientist**, CAMBIA, Canberra, Australia
1997 **Visiting Fellow**, Bioactive Natural Products Laboratory, Michigan State University, USA
1994-98 **Visiting Scientist**, Biotechnology Research Unit, CIAT, Cali, Colombia
1990-93 **Research Assistant**, Biophysical Chemistry Laboratories, University of Vienna, Austria, and ETH, Zürich, Switzerland
1991 **Freelance Researcher**, Institute for Interdisciplinary Research, Vienna, Austria

Awards, grants and fellowships

2003 **Outstanding Research Publication Award** of CIAT in 2003
1999 Nominated by CIAT for the **Promising Young Scientist Award** of the CGIAR
1997 **Travel Fellowship** of the Austrian Ministry of Science and Education
1996-98 **Fellowship** of Colombian Ministry of Agriculture and Rural Development
1993 Secured **US\$ 120,000 Research Grant** of Austrian Academy of Sciences for my PhD project
1985-2000 Completed PhD, MSc, BSc degrees and high school-leaving examination **with distinction**

Teaching and student supervision

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| 2003 to date | Co-supervised and trained a large number of visiting scientists and students from Australia and overseas at Diversity Arrays Technology P/L, Canberra, Australia |
| 1997-2004 | Principal supervisor of three BSc theses , CIAT, Cali, Colombia |
| 1995-2004 | Principal supervisor of two MSC theses , CIAT, Cali, Colombia |
| 1994-2000 | Supervised and trained six visiting students and scientists from North Korea, Germany, Colombia, USA and South Africa at CAMBIA, Canberra, Australia and CIAT, Cali, Colombia |

Publications in refereed journals

1. Bedo J, **Wenzl P**, Kowalczyk A, Kilian A. Precision-mapping and statistical validation of quantitative trait loci by machine learning. Submitted
2. Mace ES, Xia L, Jordan DR, Halloran K, Parh DK, **Wenzl P**, Huttner E, Kilian K. DArT markers: diversity analyses and mapping in *Sorghum bicolor*. Submitted
3. **Wenzl P**, Raman H, Wang J, Zhou M, Huttner E, Kilian A (2007) A DArT platform for quantitative bulked segregant analysis. *BMC Genomics* **8**, 196
4. Akbari M, **Wenzl P**, Caig V, Carling J, Xia L, Yang S, Uszynski G, Mohler V, Lehmensiek A, Kuchel H, Hayden MJ, Howes N, Sharp P, Rathmell B, Huttner E, Kilian A (2006) Diversity Arrays Technology (DArT) for high-throughput profiling of the hexaploid wheat genome. *Theoretical and Applied Genetics* **113**, 1409-1420
5. **Wenzl P**, Li H, Carling J, Zhou M, Raman H, Paul E, Hearnden P, Maier C, Xia L, Caig V, Ovesná J, Cakir M, Poulsen D, Wang J, Raman R, Smith KP, Muehlbauer GJ, Chalmers KJ, Kleinhofs A, Huttner E, Kilian A (2006) A high-density consensus map of barley linking DArT markers to SSR, RFLP and STS loci and phenotypic traits. *BMC Genomics* **7**, 206
6. Yang S, Pang W, Ash G, Harper J, Carling J, **Wenzl P**, Huttner E, Kilian A (2006) Low level of genetic diversity in cultivated pigeonpea compared to its wild relatives is revealed by Diversity Arrays Technology (DArT). *Theoretical and Applied Genetics* **113**, 585-595
7. Sessitsch A, Hackl E, **Wenzl P**, Kilian A, Kostic T, Stralis-Pavese N, Tankouo Sandjong B, Bodrossy L (2006) Diagnostic microbial microarrays in soil ecology. *New Phytologist* **171**, 719-736
8. **Wenzl P**, Arango A, Chaves AL, Buitrago ME, Patiño GM, Miles J, Rao IM (2006) A greenhouse method to screen brachiariagrass genotypes for aluminum resistance and root vigor. *Crop Science* **46**, 968-973
9. Xia L, Peng K, Yang S, **Wenzl P**, de Vicente C, Fregene M, Kilian A (2005) DArT for high-throughput genotyping of cassava (*Manihot esculenta*) and its wild relatives. *Theoretical and Applied Genetics* **274**, 30-39
10. Wagatsuma T, Khan MSH, Rao IM, **Wenzl P**, Tawaraya K, Yamamoto T, Kawamural T, Hosogoe K, Ishikawa S (2005) Methylene blue stainability of root-tip protoplasts as an indicator of aluminum tolerance in a wide range of plant species, cultivars and lines. *Soil Science and Plant Nutrition* **51**, 991-998
11. **Wenzl P**, Wong L, Kwang-won K, Jefferson RA (2005) A functional screen identifies lateral transfer of β -glucuronidase (*gus*) from bacteria to fungi. *Molecular Biology and Evolution* **22**, 308-316
12. Ishitani M, Rao IM, **Wenzl P**, Beebe S, Tohme J (2004) Integration of genomics approach with traditional breeding towards improving abiotic stress adaptation: Drought and aluminum toxicity as case studies. *Field Crops Research* **90**, 35-45
13. **Wenzl P**, Carling J, Kudrna D, Jaccoud D, Huttner E, Kleinhofs A, Kilian A (2004) Diversity arrays technology (DArT) for whole-genome profiling of barley. *Proceedings of the National Academy of Sciences* **101**, 9915-9920
14. **Wenzl P**, Mancilla LI, Mayer JE, Albert R, Rao IM (2003) Simulating infertile acid soils with nutrient solutions: The effects on *Brachiaria* species. *Soil Science Society of America Journal*, **67**, 1457-1469
15. **Wenzl P**, Patiño GM, Mayer JE, Rao IM (2002) Aluminium stress stimulates the accumulation of organic acids in root apices of *Brachiaria* species. *Journal of Plant Nutrition and Soil Science* **165**, 582-588

16. **Wenzl P**, Mayer JE, Rao IM (2002) Al stress inhibits accumulation of P in root apices of Al-sensitive but not Al-resistant *Brachiaria* cultivar. *Journal of Plant Nutrition* **25**, 1821–1828
17. **Wenzl P**, Patiño GM, Chaves AL, Mayer JE, Rao IM (2001) The high level of aluminium resistance in signalgrass is not associated with known mechanisms of external aluminium detoxification in root apices. *Plant Physiology* **125**, 1473–1484
18. **Wenzl P**, Chaves AL, Mayer JE, Rao IM, Nair MG (2000) Roots of nutrient-deprived *Brachiaria* species accumulate 1,3-di-*O*-*trans*-feruloylquinic acid. *Phytochemistry* **55**, 389–395
19. **Wenzl P**, Mancilla LI, Rao IM, Mayer JE (2000) Isolation of rare cDNAs by "asymmetric self-hybridization". *Analytical Biochemistry* **286**, 303–305
20. Prozesky VM, Pineda CA, Mesjasz-Przybylowicz J, Przybylowicz WJ, Churms CL, Springhorn KA, Moretto P, Michelet C, Chikte U, **Wenzl P** (2000) The biological research programme of the nuclear microprobe at the National Accelerator Centre, Faure. *Nuclear Instruments and Methods in Physics Research B* **161**, 852–859
21. Pineda CA, **Wenzl P**, Mayer JE, Mesjasz-Przybylowicz J, Przybylowicz WJ, Prozesky VM (1997) Study of the nutrient distribution in root tips of the tropical forage *Brachiaria*. *Nuclear Instruments and Methods in Physics Research B* **130**, 362–367
22. **Wenzl P**, Fringeli M, Goette J, Fringeli UP (1994) Supported phospholipid bilayers prepared by the "LB/vesicle method": a Fourier transform infrared attenuated total reflection spectroscopic study on structure and stability. *Langmuir (Journal of the American Chemical Society)* **10**, 4253–4264

Patent

1. **Wenzl P** (2004) Fungal β -glucuronidase genes and gene products. *US patent application* No. 10/757,093

Curriculum vitae Dr. Evelyn Hackl

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Date of birth: 14 September 1970, Vienna
Nationality: Austria

Education

- 1996-2001 University of Vienna, Austria.
Institute of Ecology and Conservation Biology, Chemical Physiology of Plants;
Ph.D. Thesis advisors: Prof. Marianne Popp and Dr. Sophie Zechmeister-Boltenstern (Austrian Federal Office and Research Centre for Forests). Ph.D. Title: „Microbial nutrient turnover in soils under natural forest stands“.
- 1989-1996 University of Vienna, Austria.
M.Sc. in Biology with distinction.
Diploma thesis at the Institute for Soil Management (now Austrian Agency for Health and Food Safety) and at the Austrian Federal Office and Research Centre for Forests. Thesis advisors: Prof. Ellen Kandeler and Dr. Sophie Zechmeister-Boltenstern. Thesis title: „Nitrogen dynamics in different types of pasture in the Austrian Alps“.
- 1989 A-level exams with distinction.

Professional Experience

- 2006-present **Microbiologist** at the Austrian Research Centers GmbH, Dept. of Bioresources.
Diversity Arrays Technology (DArT) application for microarray-based molecular typing of food-borne pathogenic bacteria; research on bacterial diversity in agricultural and forest soils by using various molecular cultivation-dependent and –independent techniques based on phylogenetic and functional marker genes; molecular analysis of bacteria involved in nitrogen turnover by diversity and expression analysis of functional genes and correlation with chemical analyses, metagenomics for the identification of novel genes.
- 2006 **Research scholar** at the Department of Plant Pathology of the University of Wisconsin-Madison, Madison, U.S.A. for studying high-throughput screening technologies for microbial metagenomics.
- 2002–2006 **Post doctoral** position at the ARC Seibersdorf research GmbH, Dept. of Bioresources / Microbiology.
Bacterial diversity studies in forest soils by using various molecular cultivation-dependent and –independent techniques; molecular analysis of bacteria involved in nitrogen turnover by diversity and expression analysis of functional genes and correlation with chemical analyses, involvement in microarray analyses; research on microbial metagenomics.
- 2004 Research on **Diversity Arrays Technology (DArT)** for microbial ecology application at Diversity Arrays Technology Pty Ltd., Canberra, Australia
- 2001-2002 **Post doctoral** position at the University of Applied Life Sciences (BOKU) Vienna, Institute of Agricultural, Environmental and Energy Engineering.

Research on biogas production from energy plants. Experiments to optimize co-fermentation of manure with energy plants (grasses). Development of a lab-scale fermentor system for the study of biogas production from energy plants.
Lecturer at the University of Vienna "Bodenbiologische Übungen, Teil I".

- 2000-2001 Research on methane, nitrous oxide and ammonia emissions from management of liquid and solid manures at the University of Applied Life Sciences (BOKU) Vienna, Institute of Agricultural, Environmental and Energy Engineering.
- 1996-2001 **Doctoral** position at the Austrian Federal Office and Research Centre for Forests (BFW). Research on microbial nitrogen turnover, microbial biomass dynamics, biogenic trace gas fluxes from soil, and PLFA based microbial community analysis.
Lecturer at the University of Vienna "Bodenbiologische Übungen, Teil I".
- 1995 Research within the soil monitoring programme „Soil monitoring in Salzburg, Austria“ at the Institute for Soil Management (now Austrian Agency for Health and Food Safety).

Awards

- 2006 Research scholarship within the **Call all-round excellence** of the **brainpower initiative (bmvit)** for a six months research visit at the University of Wisconsin-Madison (Department of Plant Pathology) for collaboration and research in the field of metagenomics with an emphasis on high-throughput screening technologies.
- 2004 **OECD-research fellowship** within the co-operative research programme "Biological Resource Management for Sustainable Agricultural Systems" to undertake research on Soil Microbial Diversity Arrays for the analysis of soil quality and plant-microbe interactions at the Diversity Arrays Technology Pty Ltd. in Canberra, ACT 2601, Australia.
- 2001 **Bodenkulturpreis 2001** of the Wiener Wirtschaftskammer.
- 1996 **DOC-fellowship** of the Austrian Academy of Sciences.

Foreign Languages

English (fluent), French (good working knowledge)

Selected publications

- Hackl E.**, Bachmann G., Zechmeister-Boltenstern S. (2000) Soil microbial biomass and rhizosphere effects in natural forest stands. *Phyton* Vol. 40, 83-90.
- Hackl E.**, Zechmeister-Boltenstern S., Kandeler E. (2000) Nitrogen dynamics in different types of pasture in the Austrian Alps. *Biol. Fertil. Soils* 32, 321-327.
- Zechmeister-Boltenstern S., **Hackl E.**, Bachmann G., Donat C., Pfeffer M. (2000) Microbial nutrient turnover in forests with natural tree species composition. *Proceedings of the International Conference on Forest Ecosystem Restoration*, 10.-12. April 2000, Universität für Bodenkultur, Vienna, 296-297.
- Hackl E.**, Bachmann G., Zechmeister-Boltenstern S. (2004). Microbial nitrogen turnover in soils under different types of natural forest. *Forest Ecol. Manag.* 188, 101-112.
- Hackl E.**, Zechmeister-Boltenstern S., Bodrossy L., Sessitsch A. (2004). Comparison of diversities and compositions of bacterial populations inhabiting natural forest soils. *Appl. Environ. Microbiol.* 70, 5057-5065
- Hackl E.**, Pfeffer M., Donat C., Bachmann G., Zechmeister-Boltenstern S. (2005) Composition of the microbial communities in the mineral soil under different types of natural forest. *Soil Biol. Biochem* 37, 661-671.
- Zechmeister-Boltenstern S., **Hackl E.**, Bachmann G., Pfeffer M., Englisch M. (2005) Nutrient turnover, greenhouse gas exchange and biodiversity in natural forests of Central Europe. In: Binkley, D., and O. Menyailo (eds). 2005. *Tree Species Effects on Soils: Implications for Global Change*. NATO Science Series 55, Kluwer Academic Publishers, Dordrecht, 31-49.
- Sessitsch A., **Hackl E.**, Wenzl P., Kilian A., Kostic T., Stralis-Pavese N., Tankouo Sandjong B., and Bodrossy L. (2006). Diagnostic microbial microarrays in soil ecology. *New Phytologist*, 171: 719-736.