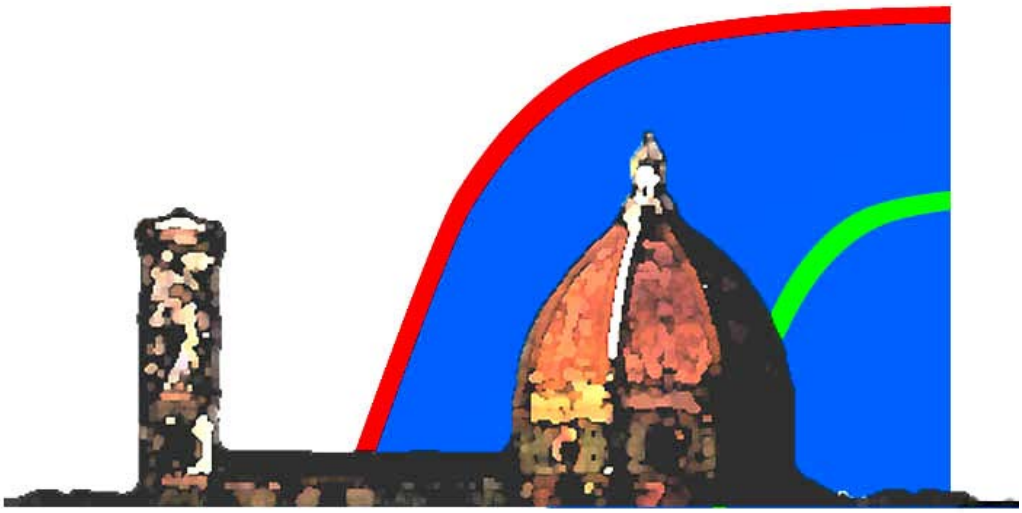
Florence Conference on Phenotype MicroArray Analysis of Microorganisms

The Environment, Agriculture, and Human Health



Firenze-Italy, March 19-21, 2008

Programme and Abstracts

Università degli Studi di Firenze

Florence Conference on Phenotype MicroArray Analysis of Microorganisms

The Environment, Agriculture, and Human Health

Firenze-Italy, March 19-21, 2008

Programme and Abstracts

Edited by: F. Decorosi, L. Giovannetti, E. Tatti, C. Viti

Contents

Organizing and Scientific Committee

Carlo Viti Department of Agricultural Biotechnology, University of Firenze, Italy

Luciana Giovannetti Department of Agricultural Biotechnology, University of Firenze, Italy

Alessandro Camussi, Department of Agricultural Biotechnology, Genexpress laboratory, University of Firenze, Italy

Barry Bochner, CEO & CSO Biolog, Usa.

Fabio Zenna AES - Chemunex , Italy

Scientific Secretariat

Enrico Tatti Department of Agricultural Biotechnology, University of Firenze, Italy

Francesca Decorosi Department of Agricultural Biotechnology, University of Firenze, Italy

Partners-Sponsors and Patronages

Programme

Wednesday, 19th

15.00 16.45	**Registration**	
16.45-17.00	The Authorities Carlo Viti and Luciana Giovannetti (Firenze, Italy)	Welcome to Florence University Opening of Florence Conference on Phenotype MicroArrays
	Opening lecture	
17.00-18.00	Tom Cebula (Laurel, MD, USA)	Phenotypic analysis of foodborne pathogens
18.00-19.00	**Welcome cocktail**	

Thursday, 20th
Morning

8.00-8.30 **Registration**

Section: *Principles, practice and data analysis*

Chairs: Camussi A. (Firenze, Italy), Canganella F. (Viterbo, Italy)

8.30-10.30 ***Lectures***

8.30	Barry Bochner (Hayward, CA, USA)	Phenotype MicroArray technology: principles and practice
9.00	Laurence Yang (Toronto, Canada)	Use of growth profiling data to improve constraint-based modelling of metabolism
9.30	Hirotada Mori (Nara, Japan)	Towards elucidation of physiological network in a cell using PM technology and genetic network analysis
10.00	Ian Paulsen (Sydney, Australia)	Unravelling membrane transport mechanisms in *Pseudomonas* sp. through phenomics

10.30-11.00 *Coffee break and poster viewing*

Section: *Phenotypic analysis of eukaryotes*

Chairs: Polsinelli M. (Firenze, Italy), Cavalieri D. (Firenze, Italy)

11.00-12.30 ***Lectures***

11.00	Enrico Casalone (Firenze, Italy)	Pleiotropic effects of mutations of the LEU4 gene in *Saccharomyces cerevisiae*
11.30	Irina Druzhinina (Vienna, Austria)	Phenotype MicroArrays as a tool to study physiology of industrially important fungi
12.00	Barry Bochner (Hayward, CA, USA)	PM assay of mammalian cells

12.30-12.45 ***Oral presentations selected by Organizing Committee***

Manal Abuoun (Surrey, United Kingdom)	Characterization of Porcine cell using Phenotype MicroArray

13.00-15.00 *Lunch and poster viewing*

Thursday, 20th
Afternoon

Section: *Phenotypic analysis of human and animal pathogens*

Chairs: Taccetti G. (Firenze, Italy), Fani R. (Firenze, Italy)

15.00-18.30 ***Lectures***

15.00	Anders Omsland (Hamilton, MT, USA)	Use of PM technology to characterize nutrient utilization and restriction in the obligate intracellular bacterial pathogen *Coxiella burnetii*
15.30	Muna Anjum (Surrey, United Kingdom)	Phenotypic analysis of *Salmonella*
16.00	Jean Guard-Bouldin (Athens, GA, USA)	Linking phenotype to genotype of epidemiologically prevalent *Salmonella* by comparative microarray analysis
16.30	Kunihiko Nishino (Osaka, Japan)	Phenotypic analysis of multidrug efflux pumps - not just for multidrug resistance

17.00-17.30 *Coffee break and poster viewing*

17.30	Carol Iversen (Dublin, Ireland)	Phenotypic analysis of *Enterobacter sakazakii* (*Cronobacter* spp.)
18.00	Alex Boehm (Basel, Switzerland)	Antimicrobials inducing *E. coli* biofilm formation, identified by Phenotype MicroArrays

18.30-19.00 ***Oral presentations selected by Organizing Committee***

Atin R. Datta (Laurel, MD, USA)	Comparative Phenotypic Microarray analysis of *Listeria monocytogenes* strains involved in invasive and gastroenteritis listeriosis outbreaks
Tyrrell Conway (Norman, OK, USA)	Carbon and energy metabolism of *Escherichia coli* in the intestine

19.15 ***Departure for the historic centre of the town***

20.15 Dinner

Friday, 21st
Morning

Section: *Phenotypic analysis of environmental microorganisms*

Chairs: Daffonchio D. (Milano, Italy), Bazzicalupo M. (Firenze, Italy)

8.30-10.30 ***Lectures***

8.30	Stefano Fedi (Bologna, Italy)	Physiological characterization of *Pseudomonas pseudoalcaligenes* KF707: biofilm development and adaptation to environmental stress
9.00	Enrico Tatti (Firenze, Italy)	Phenotypic analysis of Cr(VI)-sensitive mutants of *Pseudomonas corrugata* strain 28
9.30	Emanuele Biondi (Firenze, Italy)	Integrating Biolog phenomic analysis with genomic approaches to explore the diversity of natural *Sinorhizobium meliloti* strains
10.00	Terry C. Hazen (Berkeley, CA, USA)	Phenotype MicroArray analysis of anaerobes

10.30-11.00 *Coffee break and poster viewing*

11.00-12.15 ***Oral presentations selected by Organizing Committee***

Patrick Venail (Montpellier, France)	Functional diversity and productivity peak at intermediate dispersal rate in evolving bacterial metacommunities
Alexander Shearer (Menlo Park, CA, USA)	Metabolic network inference using Pathway Tools and MetaCyc
Bhagwati Upadhyay (Addlestone, United Kingdom)	Adapting Biolog Phenotype MicroArray technology to reveal the metabolomics of *Mycobacterium tubercolosis* and *Mycobacterium bovis*, slow-growing pathogens with d=0.03 to 0.05/h
Rachael L. Edwards (Ann Arbor, MI, USA)	Novel cues of *L. pneumophila* differentiation uncovered by Phenotype MicroArrays
Francesca Fabretti (Milano, Italy)	H-NS, a master controller of warm adaptation in *Escherichia coli*: a trascriptomic analysis

12.15-13.00 Daffonchio D., Fani R., Camussi A., Bazzicalupo M., Cavalieri D. — Round table and closing of the symposium

Friday, 21st
Afternoon

15.00-17.30 PM demonstration (Genexpress laboratory)

Poster contributions

ABSTRACTS

PART I

LECTURES AND ORAL PRESENTATIONS

PHENOTYPIC ANALYSIS OF FOODBORNE PATHOGENS

A. MUKHERJEE, J.E. LECLERC, M.K. MAMMEL, T.A. CEBULA

Office of Applied Research and Safety Assessment (HFS-25), US FDA. Laurel, MD, USA 20708.

Approximately 76 million cases of foodborne disease occur each year in the United States. While the large majority of foodborne disease cases are mild and transient, some are much more serious. The United States Center for Disease Control and Prevention morbidity and mortality estimates assign approximately 325,000 hospitalizations and 5,000 deaths each year to foodborne disease. The need for disease surveillance, alert monitoring of disease outbreaks, and prompt identification of the microbe involved are thus important elements in ensuring a safe food supply. The "-omics" era of research has brought new technologies to bear on identifying the causative agent in a foodborne outbreak. Genomics, transcriptomics, proteomics, and metabolomics, new words entered into our lexicon, are, or will be, exploited for identification purposes. So too will be the use of Phenotyping MicroArrays, a technology that allows over 1,000 cellular traits to be measured simultaneously. The use of the Omnilog PM system to profile *Salmonella enterica* and *Escherichia coli* strains will be the major focus of this discussion. We demonstrate how exploiting novel phenotypes within Salmonella and *E. coli* O157:H7 has led to specific assay development that enhances strain attribution.

PHENOTYPE MICROARRAY TECHNOLOGY: PRINCIPLES AND PRACTICE

B.R. BOCHNER

CEO & CSO, Biolog, Inc., Hayward, CA, USA.

Phenotypes provide a very useful way to describe biological differences between cells. They reflect both genetic and epigenetic differences among cells. Phenotype MicroArray (PM) technology was developed to provide scientists with a simple and efficient way to test nearly 2,000 phenotypes of a microbial cell and gain a comprehensive overview of pathway functions in a single experiment. The phenotypic assays are designed from a physiological perspective to survey, *in vivo*, the function of diverse pathways including both metabolic and regulatory pathways. Included in the phenotypes are basic cellular nutritional pathways for C, N, P, and S metabolism (800 tests), pH growth range and regulation of pH control (100 tests), sensitivity to NaCl and various other ions (100 tests), and sensitivity to 240 chemical agents that disrupt various biological pathways (1,000 tests). There are now more than 80 scientific publications and presentations that have used PM technology in basic cellular research, pathogenesis and epidemiology, gene function and genome annotation, drug discovery, bioprocess development, and cell identification and taxonomy. In this presentation I will describe details of how PM technology works and show examples of how it can be used in both basic and applied research.

USE OF GROWTH PROFILING DATA TO IMPROVE CONSTRAINT-BASED MODELLING OF METABOLISM

L. YANG, R. MAHADEVAN

University of Toronto, 200 College Street, Toronto, ON M5S 3E5, Canada.

Experimental technologies such as Phenotype Micro-arrays have provided unprecedented physiological data under different growth environments and genetic backgrounds. Similar advances in computational modeling techniques have allowed the development of genome-scale metabolic models of several organisms. In this talk, we will show two examples of how large-scale growth profiling data can be used to inform genome-scale models.

In the first example, we illustrate the use of PM data to obtain a highly accurate and expanded metabolic model of *Bacillus subtilis*. In this report, the computed growth rates under aerobic conditions were compared with high-throughput phenotypic screen data and the initial *in silico* model could predict the outcomes qualitatively in 142 of 270 cases considered. Detailed analysis of the incorrect predictions resulted in the addition of 84 reactions to the initial reconstruction and 214 of 270 cases were correctly computed. The resulting *in silico* computations of the growth phenotypes of knock-out strains were found to be consistent with experimental observations in 725 of 772 cases evaluated. Finally, the integrated analysis of the large-scale substrate utilization and gene essentiality data with the genome-scale metabolic model revealed the requirement of 89 specific enzymes (transport, 63; intracellular reactions, 26) that were not in the genome annotation. Subsequent sequence analysis resulted in the identification of genes that could be putatively assigned to 13 intracellular enzymes.

In the second example, fitness profiling data from growth competition experiments between strains of different genetic backgrounds were used to refine the metabolic model of *Escherichia coli*. Constraint-based models of metabolism seldom incorporate capacity constraints on intracellular fluxes due to lack of kinetic data. Here, the optimal capacity constraint identification (OCCI) algorithm was developed to identify capacity constraints necessary for accurately predicting experimental fitness profiles. In a case study, fitness profiles from 14 different genetic backgrounds in the same growth environment were used to identify capacity constraints in central metabolism of *E. coli*. This algorithm can be readily extended to handle Phenotype Micro-array data from multiple growth environments.

TOWARDS ELUCIDATION OF PHYSIOLOGICAL NETWORK IN A CELL USING PM TECHNOLOGY AND GENETIC NETWORK ANALYSIS IN *E. COLI* K-12

H. MORI

Graduate School of Biological Sciences, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara 630-0101, Japan - Advanced Institute of Biological Sciences, Kieo University, Tsuruoka, Yamagata, 997-0017, e-mail: hmori@gtc.naist.jp.

To elucidate metabolic network of *E. coli*, comprehensive phenotype analysis by PM technology is now underway. Single gene deletion mutants of 107 genes related to glycolysis, TCA cycle and Pentose Phosphate pathway have been subjected to Biolog analysis. We first developed the transformation algorithm from the time series raw data from OminiLog System to multi-dimensional simplified vector data. 1) Smoothing by calculation of moving average, 2) set a certain threshold of respiratory activity, 3) calculation the maximum speed of the respiration and 4) statistical comparison between the data from the wild type cell. Finally we classified data in every condition into three categories, +1, 0 and -1. If the deletion affects significant activation of respiration than the wild type cell, the value of 1 is set. Similarly, 0 for no difference and -1 for repression. Based on these spectrum data, correlation and clustering analyses were performed.
We are now expanding the number of target gene deletion mutants selected randomly. I will show the present situation of our approach using PM technology and also show the database of our data.
On the other hand, the growth profiles of single gene deletion mutant in LB and MOPs+glucose medium revealed that most of the singe gene deletion did not affect their growth both in LB and MOPs medium. This is robustness and cell might have alternative or bypass pathway(s) of the deleted gene. To elucidate the molecular mechanism of such robustness, synthetic lethal/sickness analysis is one of the powerful methods. To perform this, we prepared another set of single deletion mutant library and develop the efficient method to combine single deletion to double knockout strain by conjugation.
I would like to summarize our recent approaches to solve the cellular network and discuss about the future perspectives.

UNRAVELLING MEMBRANE TRANSPORT MECHANISMS IN *PSEUDOMONAS* SP. THROUGH PHENOMICS

I.T. PAULSEN

Macquarie University, Sydney, Australia.

The deluge of data generated by genome sequencing has led to an increasing reliance on bioinformatic predictions, since the traditional experimental approach of characterizing gene function one at a time cannot possibly keep pace with the sequence-based discovery of novel genes. We have utilized Biolog phenotype MicroArrays to identify phenotypes of gene knockout mutants in the opportunistic pathogen and versatile soil bacterium *Pseudomonas aeruginosa* in a relatively high throughput fashion. Seventy eight *P. aeruginosa* mutants defective in predicted sugar and amino acid membrane transporter genes were screened and clear phenotypes were identified for twenty seven of these. In all cases these phenotypes were confirmed by independent growth assays on minimal media. Using qRT-PCR, we demonstrate that the expression levels of eleven of these transporter genes were induced from 4- to 90-fold by their substrates identified via phenotype analysis. Overall, the experimental data showed the bioinformatic predictions to be largely correct in 22 out of 27 cases, and led to the identification of novel transporter genes and a potentially new histamine catabolic pathway. Thus rapid phenotype identification assays are an invaluable tool for confirming and extending bioinformatic predictions.

PLEIOTROPIC EFFECTS OF MUTATIONS OF THE LEU4 GENE IN *SACCHAROMYCES CEREVISIAE*

E. CASALONE[1], C. NATALI[1], M. ROSSI[1], E. TATTI[2], C. VITI[2], L. GIOVANNETTI[2]

[1]Departement of Evolutionary Biology, University of Florence, Italy
[2]Departement of Biotecnologie Agrarie, Sez. Microbiologia, University of Florence, Italy

In yeast, the gene regulation of the *LEU4* gene, encoding the first specific enzyme of the leucine biosynthetic pathway, α-isopropylmalate synthase (α-IPMS), is very complex. In addition, α-IPMS activity is finely regulated by two small molecules, leucine and CoA. This picture, with the unusual cell-partitioning of the leucine pathway enzymes, makes leucine metabolism very interesting by the point of view of its connection to other metabolisms and cell regulation mechanisms. To investigate these possible links, we have characterized *S. cerevisiae* strains with different *LEU4* alleles, encoding α-IPMSs no more responsive to leucine feedback inhibition and CoA reversible inactivation.
Characterization results strongly suggest an involvement of leucine metabolism in cell morphogenesis and energy metabolism.
Preliminary Phenotype Microarray results envisage new possible metabolic connections of the leucine biosynthetic pathway.

Acknowledgements. *We are grateful to the Genexpress laboratory (Departement of Biotecnologie Agrarie, University of Florence, Italy).*

PHENOTYPE MICROARRAYS AS A TOOL TO STUDY PHYSIOLOGY OF INDUSTRIALLY IMPORTANT FUNGI

I.S. DRUZHININA

Research Area Gene Technology and Applied Biochemistry, Institute of Chemical Engineering, Vienna University of Technology, Getreidemarkt 9-1665, A-1060 Vienna, Austria. Phone:+43 1 58801 17202, Fax:+43 1 58801 17299, e-mail: druzhini@mail.zserv.tuwien.ac.at.

Conidial fungi or molds and mildews formally called as Fungi imperfecti are important as human and animal pathogens, organisms causing dangerous epyphytotia, effective plant protectors and microorganisms used in biotechnology for the production of antibiotics, industrially important enzymes, chemicals and foods. The importance of this group of fungi has led to an increasing number of species for which genomic sequences are now available. To exploit this information, functional genomics methods are applied to study the impact of altered genes on the phenotype of the organism. These are mainly technologies that provide a cell-wide perspective such as genomics, transcriptomics and proteomics. It allows for global analysis of the information flow from DNA to RNA to protein, but fails to describe the resulting general phenotype. More recently, Phenotype MicroArrays (PM) have been introduced as a tool to characterize the metabolism of a fungal strain or a mutant.

We have adapted carbon source utilization PMs for the analysis of such industrially important fungi as *Trichoderma* and *Fusarium*. In this review, we will emphasize experimental prerequisites for the use of PMs with fungi and report on selected examples of their use to illustrate the versatility of the tool for their use in diverse investigations such as characterization of DNA-transformants, screening for enzyme inducers, identifying traits useful in screening of industrial fungi, as well as strain degeneration.

PM ASSAY OF MAMMALIAN CELLS

B.R. BOCHNER

CEO & CSO, Biolog, Inc., Hayward, CA, USA.

Phenotype MicroArray™ (PM) technology has now been extended to work for diverse mammalian cells, in addition to bacterial and fungal cells. Just as various microbial cells differ in their phenotypic properties, mammalian cells from different animals and from different organs and tissues also differ in their phenotypes. Mammalian cells can be assayed without modification, including primary cells as well as established cell lines. Nearly 1400 PM assays are now available for mammalian cells. Panels PM-M1 to M4 can be used to culture cells with nearly 700 different carbon and nitrogen sources. Panels PM-M5 to M8 can be used to assay the effects of ions, hormones, and other metabolic effectors. Panels PM-M11 to M14 can be used to determine the sensitivity of cells to 92 diverse cytotoxic agents. Information from these assays can enlighten the user on medium composition effects on (a) growth, (b) cell productivity, (c) cell viability, (d) cell stability/instability (e) cell differentiation. Major applications of the technology are in cell culture and bioprocess improvement, cell fingerprinting and authentication, basic nutrition and toxicology research, and drug development around metabolic disorders such as diabetes, obesity, and cancer. Very recently, laboratories have started to experiment with looking at how they can use PM technology to address issues of how infectious agents interact with animal cells that they infect.

CHARACTERISATION OF A PORCINE CELL LINE BY PHENOTYPE MICROARRAY

M. ABUOUN, J.W. COLLINS, M.J. WOODWARD, R.M. LA RAGIONE

Veterinary Laboratories Agency, Addlestone, United Kingdom.

Salmonella is a major cause of human and porcine enteric disease, with most reported cases attributed to *Salmonella enterica* serovar Typhimurium. In pigs, *S.* Typhimurium is implicated in enterocolitis in young piglets leading to diarrhoea, loss of appetite and lethargy. The pig gastrointestinal tract (GIT) represents an interface between the metabolism of the animal, microbiota and its external environment, and is a main site of metabolism and absorption of nutrients from ingested foods.
A further understanding how *Salmonella* subverts the host metabolism during infection will allow the development of effective intervention strategies. To investigate the effect of bacteria on the porcine host, an *in vitro* model was developed utilising the porcine jejunal cell line, IPEC-J2. The Biolog mammalian phenotype microarray was employed to characterise the basal nutrient requirements of IPEC-J2's. In addition, chemosensitivities of IPEC-J2 to ion stress, cytokines, growth hormones and other metabolic effectors were also determined. Ultimately, this model will be used to determine the effects of bacterial infection on the porcine metabolome.

USE OF PM TECHNOLOGY TO CHARACTERIZE NUTRIENT UTILIZATION AND RESTRICTION IN THE OBLIGATE INTRACELLULAR BACTERIAL PATHOGEN *COXIELLA BURNETII*

A. OMSLAND, D.C. COCKRELL, R.A. HEINZEN

Coxiella Pathogenesis Section, Laboratory of Intracellular Parasites, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rocky Mountain Laboratories, Hamilton, Montana, USA.

Replication of *Coxiella burnetii*, the agent of human Q fever, is strictly limited to colonization of a viable eukaryotic host cell. Following infection, the pathogen replicates exclusively in an acidified (pH 4.5-5) phagolysosome-like parasitophorous vacuole (PV). Axenic (host cell free) buffers have been described that activate *C. burnetii* metabolism *in vitro*, but metabolism is short-lived with bacterial protein synthesis halting after a few hours. Therefore, to improve conditions for *C. burnetii* axenic metabolic activity, we characterized the bacterium's responses to a variety of nutritional and biophysical conditions. Initially, several biological buffers (pH 4.5) were screened for *C. burnetii* metabolic permissiveness. ^{35}S Cys/Met incorporation showed that citrate and phosphate buffers support optimal metabolic activity. Citrate buffer was chosen as a medium buffering solution due to its high buffering capacity at pH 4.5-5. A nutrient medium termed Complex *Coxiella* Medium (CCM) was developed that allowed prolonged (> 24 h) *C. burnetii de novo* protein and ATP synthesis. The expression patterns of terminal respiratory chain oxidases suggested *C. burnetii* is a microaerophile. Consistent with this hypothesis, extended *C. burnetii* metabolic activity in CCM occurs only under microaerobic conditions, and Phenotype Microarray (PM) substrate utilization is severely restricted under aerobic (20% atmospheric oxygen) conditions while being maximal under microaerobic (2.5% atmospheric oxygen) conditions. Even under microaerobic conditions, *C. burnetii* utilized a limited number of substrates in PM analyses with only 17 of 94 substrates (PM1) efficiently oxidized. Moreover, several PM substrates inhibited *C. burnetii* metabolic activity. Overall, our data suggest that, like other intracellular bacteria that reside in vacuolar compartments, *C. burnetii* is adapted for low metabolic activity as a consequence of nutrient limitation. Moreover, the bacterium appears to prefer a low oxygen environment.

USE OF OMNILOG PHENOTYPE MICROARRAYS TO REVEAL DIFFERENCES BETWEEN *SALMONELLA ENTERICA* SUBSPECIES I STRAINS.

M. ANJUM

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

Infection with nontyphoid *Salmonella* spp. is one of the leading causes of diarrhoea in developed countries. Epidemiological studies have shown that relatively few *Salmonella* serovars/strains are responsible for the bulk of nontyphoidal *Salmonella* infections in humans. In fact serovars Enteritidis and Typhimurium are the current most prevalent causes of human inflammatory gastroenteritis, often referred to as food poisoning. In this study we constructed an *in silico* genome-scale metabolic network for the sequenced *S.* Typhimuiurm LT2 strain based on the model constructed for the laboratory *Escherichia coli* strain MG1655 (*i*AF1260), using the core genome shared between these closely related organisms. Data from Biolog PM panels were used to validate the model, and determine differences between these closely related species. Furthermore, phenotypic differences in other *Salmonella enterica* sequenced strains *S.* Typhimurium SL1344 (NCTC 13347), DT104 (NCTC 13348) and *S.* Enteritidis PT4 (NCTC 13349) were determined using Omnilog Phenotype microarrays with view to understanding these differences at the genomic level. The phenotypic and genotypic profiles of *S.* Entritidis phage types that have been found to be prevalent in human infection were compared to the sequenced strain and other phage types only involved sporadically in human infections. Similarly, differences in phenotypic and genotypic profiles of multi-resistant Typhimurium were also characterized. Our data shows that the Biolog PM panels are an useful mode for identifying differences between pathogenic strains, the challenge will be to correlate these differences at the genetic level to gain greater insight into the behaviour of pathogenic organisms.

LINKING PHENOTYPE TO GENOTYPE OF EPIDEMIOLOGICALLY PREVALENT *SALMONELLA* BY COMPARATIVE MICROARRAY ANALYSIS

J. GUARD-BOULDIN

U.S. Department of Agriculture Agricultural Research Service Athens, GA, 30605 USA

BACKGROUND: *Salmonella enterica* subsp. I includes over 1400 serovars, but only about 20 of these pose a threat to the public health on a routine basis. In spite of years of mutational analysis, it has been difficult to identify genetic markers associated with pandemic potential. **METHODOLOGY:** We combined whole genome sequencing with Phenotype Microarray (PM™) to analyze which genes and biochemical pathways of *Salmonella enterica* serovar Enteritidis (*S.* Enteritidis) are linked to epidemiological trends. *S.* Enteritidis was used as the index organism, because it has a more clonal population structure than many other Salmonellae and because it is the world's leading cause of food-borne salmonellosis. **RESULTS:** A panel of 12 ORF-disrupting genes was identified that could be used as markers of virulence as defined by the ability of *S.* Enteritidis to contaminate eggs. BLAST analysis of other *Salmonella* whole genome databases was performed to evaluate conservation of ORFs across other serotypes. Five genes appeared to be linked more specifically to the ability of *S.* Enteritidis to contaminate eggs. One of these appears to involve ß-lactam antibiotic resistance as confirmed by PM™. Another was D-serine dehyratase (dsdA), which converts L-serine to the D form. The ability to catalyze the production of ATP sulfurylase (APS) and pyrophosphate (PPi) from ATP and sulfate could be important, because spontaneous mutation of cysN correlated with suppressed utilization of many nitrogenous compounds in the PM™ panel. Two genes of *S.* Typhi that were absent in *S.* Typhimurium were linked to either egg contamination or to oral colonization, but not both. The ability to couple PM™ with high-throughput genomics thus helped identify unexpected genetic markers that could be linked to panoramic physiological profiles of *Salmonella enterica*. **SUMMARY:** Combinatorial convolutions occurring within *Salmonella enterica* are an indication that multiple markers of pandemic potential are needed when conducting epidemiological surveys. Phenotype Microarray analysis identified specific metabolic properties linked to genetic markers and also helped to reduce the use of animals in research.

PHENOTYPIC ANALYSIS OF MULTIDRUG EFFLUX PUMPS - NOT JUST FOR MULTIDRUG RESISTANCE

K. NISHINO[1, 2, 3]

Department of Cell Membrane Biology, Institute of Scientific and Industrial Research, Osaka University,[1] PRESTO, Japan Science and Technology Corporation,[2] Graduate School of Pharmaceutical Sciences,[3] Osaka University, Osaka, Japan.
Mailing address: Institute of Scientific and Industrial Research, Osaka University, 8-1 Mihogaoka, Ibaraki, Osaka 567-0047, Japan.
Phone: 81-6-6879-8546, fax: 81-6-6879-8549, e-mail: nishino@sanken.osaka-u.ac.jp.

A number of drug-resistant bacterial strains are appearing in clinical field, and bacterial infections have become one of the major problems. Multidrug efflux pumps cause serious problems in cancer chemotherapy and the treatment of bacterial infections. These efflux pumps produce multidrug resistance by exporting antibiotics from cells. In addition, many bacterial genome sequences have been determined in recent years, allowing us to trace the drug-resistance gene libraries of bacteria. Genomic analysis has resulted in the identification of many genes proposed to code for multidrug efflux pumps on bacterial genomes, but only a small number of the putative genes have been confirmed as actually involved in drug resistance.
Salmonella enterica is a pathogen that causes a variety of diseases in humans raging from gastroenteritis to bacteremia and typhoid fever. In the 1990s, the prevalence of multidrug-resistant *Salmonella* has increased dramatically. We determined that nine potential drug efflux pumps contribute to drug resistance of *Salmonella* and found that the *Salmonella*-specific MdsABC system conferred resistance to a variety of toxic compounds. Phenotypic analysis revealed that these efflux pumps are required for resistance against a wide range of antimicrobial agents, dyes and detergents.
We also found that these efflux pumps are required for *Salmonella* virulence. We inoculated BALB/c mice by the oral route with isogenic strains harboring deletions in drug efflux genes and followed mouse survival over time. Deletion of the *macAB* pump genes attenuated *Salmonella* virulence and a strain lacking all nine drug efflux systems was unable to kill mice. The promoter region of the *macAB* drug efflux system genes harbors a binding site for the response regulator PhoP. The PhoP/PhoQ two-component signal transduction system is a major regulator of *Salmonella* virulence. These results indicate that drug efflux genes are required for *Salmonella*'s ability to cause a lethal infection in mice.
The results that we obtained enable postgenome analysis in drug-resistance studies and showed that efflux pumps have greater clinical relevance than has previously been thought, because there is now accumulating evidence that certain classes of efflux pumps not only confer resistance to drugs used in therapy but also have a role in bacterial pathogenicity.

PHENOTYPIC ANALYSIS OF *ENTEROBACTER SAKAZAKII* (*CRONOBACTER* SPP.)

C. IVERSEN[1], B. D. TALL[2], S. FANNING[1]

[1]Centre for Food Safety & Food-borne Zoonomics, UCD Veterinary Sciences Centre, University College Dublin, Belfield, Dublin 4, Ireland.
[2]U.S. Food and Drug Administration, Laurel, MD 20708, USA.

Enterobacter sakazakii is an opportunistic pathogen of concern to infant formula manufacturers, health professionals and consumers because of its association with illness in infants fed contaminated, reconstituted powdered infant formula. *E. sakazakii* was defined as a new species in 1980 by Farmer *et al*, and since then 16 biogroups have been described. Based on results from a polyphasic phylogenetic study, *E. sakazakii* has been reclassified into the genus *Cronobacter*, which contains five species and one genomospecies. The DNA homology between strains within genomogroups was found to be between 70-100%, whereas homology values amongst strains belonging to different groups were all clearly below 70%. This indicated that at least 6 genomospecies were present; however using initial conventional phenotypic tests only 4 of these groups could be easily differentiated biochemically. The phenotypic descriptions of the proposed novel species were expanded using Biotype 100 and Biolog Phenotype MicroArray. For the Biolog Phenotype MicroArray cell suspensions were prepared from 24 h cultures grown on blood agar at 37°C and suspended in either IF-0 or IF-10 medium to the appropriate density. Metabolic panels PM1, PM2A, PM9 and PM10 were inoculated, the plates were incubated for 48 h at 37 °C and the data recorded using an Omnilog instrument. The areas under the growth curves were analysed and a value of greater than 15,000 tetrazolium dye reduction units was used to designate a positive result. The utilisation of malonate was also determined using Malonate Phenylalanine Broth and indole production was determined by adding Kovac's reagent to cultures grown in tryptone. A negative designation for a particular trait denoted consistent negative results for isolates across all methods. A positive designation denoted a positive result using one or more methods. The phenotypic profiles determined fourteen variable biochemical characteristics that differentiate species and subspecies. These were utilisation of dulcitol, lactulose, maltitol, palatinose, putrescine, D-melezitose, D-turanose, *myo*-inositol, *trans*-aconitate, *cis*-aconitate, 1-0-methyl-alpha-D-glucopyranoside, 4-aminobutyrate, malonate and production of indole. It was then possible to amend the taxonomic description of these organisms. A further study was undertaken using the complete 20 plates of the Phenotype MicroArray to examine the metabolic diversity among 95 strains, which were from different sources and representative of the six *Cronobacter* species. *Cronobacter muytjensii*, utilizing 42 C sources, was observed to be the most metabolically active group, while *Cronobacter* genomospecies, utilizing only 19 C sources, was the least active group. Clinical strains were generally more sensitive than environmental strains to concentrations of NaCl greater than 9% and a greater number of clinical strains were more acid-sensitive than environmental isolates. All of the strains grew at pH levels 5.5-10, but not at or below pH levels of 4.0. Resistance to over 25 antimicrobial agents was also seen. In summary, the results demonstrate that *Cronobacter* species are phenotypically diverse. Also, that pathoadaptation in *Cronobacter* species may be occurring as the organism moves from environmental into clinical niches and is associated with specific changes in phenotypes.

ANTIMICROBIALS INDUCING *E. COLI* BIOFILM FORMATION, IDENTIFIED BY PHENOTYPE MICROARRAYS

A. BÖHM, S. STEINER, U. JENAL

Biozentrum, University of Basel, Klingelbergstr. 50/70 CH-4056 Basel, Switzerland, e-mail: alexander.boehm@unibas.ch.

In response to various stress conditions bacteria can form surface attached and matrix embedded communities, called biofilms. Because cells in a biofilm can resist antibiotic chemotherapy, they contribute to recurring and persistent infections in human and animal hosts and present a major problem for public health. Thus, elucidation of the molecular factors underlying biofilm formation may aid the treatment of chronic infections. To understand the input signals that contribute to biofilm formation, an *Escherichia coli in vitro* biofilm model was used to screen Biolog™ phenotype microarrays for compounds that alter surface attachment. It was found that subinhibitory concentrations of translation inhibiting antibiotics, from at least five different chemical classes lead to strong biofilm induction. This induction phenomenon was shown to rely on two different small signaling molecules – bis-(3'-5')-cyclic di-guanosine-mono-phosphate (c-di-GMP) and guanosine-bis 3´, 5´(diphosphate) (ppGpp) – which together orchestrate upregulation of the polysaccharide adhesin poly-β-1,6-N-acetyl-glucosamine (poly-GlcNAc). A molecular model is presented that links ribosomal functioning to poly-GlcNAc production via ppGpp and c-di-GMP. These findings will be exploited to search for compounds that interfere with bacterial signal transduction essential for biofilm formation. Such compounds could be of potential use in the development of antibiofilm drugs.

COMPARATIVE PHENOTYPIC MICROARRAY ANALYSIS OF *LISTERIA MONOCYTOGENES* STRAINS INVOLVED IN INVASIVE AND GASTROENTERITIS LISTERIOSIS OUTBREAKS

A. DATTA, L. CARTER, L. BURALL

CFSAN, Food and Drug Administration, Laurel, Maryland, USA.

Listeria monocytogenes is the causative agent of human listeriosis, a foodborne illness affecting the elderly, immunocompromised and pregnant women. Although most of the major listeriosis outbreaks were characterized by septicemia, meningitis, abortion and death (invasive listeriosis), several human listeriosis outbreaks were reported to have febrile gastroenteritis as the only symptom. In an effort to understand the differences between the organisms associated with these diverse disease outcomes we have initiated analyses of these strains both at the genomic level and also at the phenotypic level using the Biolog Phenotypic Microarray system. The genomes of two invasive strains, F2365 and H7858, were compared to the genome of a gastroenteritis strain, HPB2262, using BLAST analysis of the proteins. This analysis identified 153 proteins as unique to the invasive strains. Two gene clusters with at least seven genes were largely missing from the gastroenteritis strain but present in both the invasive strains. These proteins are mostly unknown, though a few have annotated functions, including one with a putative regulatory role. Also, 97 proteins were identified as unique to the gastroenteritis strain. The majority of these proteins were hypothetical proteins (n=57) or phage-associated (n=38)**.** Two genes had a non-phage functional role, a DNA methyltransferase and an inorganic pyrophosphatase. Several strains from the known invasive and gastroenteritis outbreaks were characterized by using Phenotypic Microarray (PM) using Biolog PM1 and 2 which identifies the carbon utilization patterns and PM9 and PM10 which determines the sensitivity to various osmolytes and pH. A limited number of strains were also analyzed by PM3 (nitrogen pathways) and by PM4 (phosphorus and sulphur utilization pathways). The gastroenteritis and invasive strains included matched pairs of food and patient isolates belonging to serotypes 1/2b and 4b from well documented listeriosis outbreaks. The samples were prepared and inoculated in Biolog plates as per the manufacturer's instructions. The data were collected for 48 hours and analyzed in several different ways using Biolog software. The maximum reproducibility was obtained with the Max Slope data and therefore these data were mostly used to analyze the strains. The carbon utilization profile varied with the strains: both types of strains showed significant gains in selective carbon utilization profile over other type. Some of the differences were found to be associated with the particular serotype. While invasive isolates were in general more resistant to low pH, particularly in presence of several amino acids, the gastroenteritis strains were more resistant to osmolytes. PM3 (Nitrogen source utilization) and PM4 (phosphorus and sulphur utilization) also showed significant differences in terms of utilization of several nitrogen and phosphorus sources. In conclusion, our results from the genomic comparison data and from the PM data indicate that there are distinct physiological differences between invasive and gastroenteritis *L.monocytogenes.* Whether these differences are indicative of the difference in symptoms they produce is not clear. We think that a combination of genotypic and phenotypic characterization would ultimately provide important clues towards the understanding of the mechanism by which *L.monocytogenes* produce invasive and gastroenteritis listeriosis.

CARBON AND ENERGY METABOLISM OF *ESCHERICHIA COLI* IN THE INTESTINE

T. CONWAY, P. S. COHEN

University of Oklahoma, Advanced Center for Genome Technology, Norman, Oklahoma, 73019 USA.
University of Rhode Island, Department of Cell and Molecular Biology, Kingston, Rhode Island, 02882 USA.

The essence of how bacteria colonize their mammalian hosts is poorly understood. There have been many attempts in the past to construct laboratory models of the mammalian gastrointestinal tract for the purpose of studying the physiology of individual organisms and microbial interactions. These models suffered from an imprecise knowledge of intestinal chemistry and physical parameters in the intestine. In our laboratories, we have chosen instead to use the streptomycin-treated mouse model of colonization, which has provided a wealth of information regarding the physiological state of colonized bacteria. Our focus has been specifically on *E. coli* and primarily on the in vivo carbon and energy metabolism of this model organism. Using an approach of systematic mutational analysis, we identified metabolic pathways that are important for colonization. From the results of these experiments, we deduced many of the carbon sources and electron acceptors that are used by *E. coli* to support growth in vivo and are important for the competition of various *E. coli* strains with one another and with the microflora. Importantly, we obtained evidence that the expressed metabolic capacity of *E. coli* in animals differs from that of laboratory cultures. For the most part, these differences reflect the gene expression responses to complex nutrient availability in vivo, conditions that are not normally investigated in the laboratory. We found also that in vivo metabolism differs between *E. coli* strains. There possibly are a large number of differences between closely related members of the intestinal microflora that should be investigated in light of our most current understanding of the intestinal environment. Thus, it is important that methods be developed to rapidly screen the metabolic capacity of intestinal bacteria under conditions that simulate the intestinal environment. One approach to this problem would be to design custom Biolog plates based on the lessons learned from our in vivo *E. coli* experiments. To this end, we present an overview of recently published information and newly obtained results. From this information, we make a number of deductions with respect to growth parameters that could potentially influence microbial success in the intestine. This new knowledge of conditions in the intestinal environment can form the basis of discussions leading to design of a Biolog intestinal phenotype assay.

PHYSIOLOGICAL CHARACTERIZATION OF *PSEUDOMONAS PSEUDOALCALIGENES* KF707: BIOFILM DEVELOPMENT AND ADAPTATION TO ENVIRONMENTAL STRESS

S. FEDI[1], V. TREMAROLI[1], R. J. TURNER[2], H. CERI[3], C. VITI[4], E. TATTI[4], D. ZANNONI[1]

[1]University of Bologna, Department of Biology, General Microbiology Unit, 40126 Bologna, Italy.
[2]University of Calgary, Department of Biological Sciences, Calgary, Alberta, Canada.
[3]University of Calgary, Department of Biological Sciences, Biofilm Research Group, Calagry, Alberta, Canada.
[4]University of Florence, Department of Agricultural Biotechnology, 50144 Florence, Italy.

Growth in a biofilm provides distinct physiological traits that may enhance the degradation and/or removal of pollutants and increase adaptation/survival to environmental stresses. *Pseudomonas pseudoalcaligenes* KF707 is a polychlorinated biphenyls (PCBs)-degrader strain and a robust environmental bacterium. In order to extend what is known about the physiology of this organism, biofilm formation, nutritional requirements including BIOLOG Phenotype Microarray analysis and the role of CheA-regulated chemosensory pathway in the development of a mature biofilm structure were investigated. We observed that the formation of biofilm communities by this soil microorganism follows a general four step model. Biofilm development was found to be metabolically regulated, and, particularly, minimal media composition and high salinity triggered the production of dense, flat biofilms. These biofilms were less subjected to dispersal and more tolerant to antibiotics and toxic metals in comparison to structured biofilms formed in rich media. The analysis of biofilm formation in a *cheA* chemotactic mutant showed that mutant biofilms were blocked in a early phase of the growth process and that the number of viable cells attached to the surface was decreased by 95% in comparison to the parental strain. BIOLOG Phenotype Microarray analysis was curried out to evaluate alteration of carbon and nitrogen metabolism in the *cheA* chemotactic mutant when compared to the KF707 wild type strain. The data we present suggest that the cheA regulated signalling pathway is necessary for the production of stable cellular aggregates and for the progression in biofilm formation. This work adds evidence to the observation that the molecular requirements for biofilm formation are regulated in a species-specific manner.

Acknowledgements. *We are grateful to the Genexpress laboratory (Department of Agricultural Biotechnology, Università di Firenze, Italy).*

PHENOTYPIC ANALYSIS OF CHROMATE-SENSITIVE MUTANTS OF *PSEUDOMONAS CORRUGATA* STRAIN 28

E. TATTI, F. DECOROSI, A. MINI, L. GIOVANNETTI, C. VITI

Dipartimento di Biotecnologie Agrarie, Sezione di Microbiologia, Università degli Studi di Firenze, Piazzale delle Cascine 24, Firenze, Italy.

Hexavalent chromium is considered as a severe contaminant since differently from trivalent chromium is highly water soluble and toxic, mutagenic to most organisms and carcinogenic for humans. In bacteria Cr(VI)-resistance was found to be linked to plasmid and/or chromosome, nevertheless only strains with low chromate tolerance were examined. Thus the availability of Cr(VI)-hyper-resistant isolates is a useful tool in order to gain a better understanding of chromate resistance and reduction mechanisms. Therefore, the aim of this research was to deepen mechanisms underlying chromate resistance in Cr(VI)-hyper-resistant *Pseudomanas corrugata* strain 28.

This study was made possible by two complementary approaches, one was the isolation and identification of chromate sensitive mutants by transposon mutagenesis, and the other the examination of cellular phenotypes by a high-throughput technology defined Phenotype MicroArray. Obtained results permitted to correlate the functions of impaired genes to chromate resistance in the *P. corrugata* strain 28. Among these genes, *recG* and *crg8* genes played a crucial role in chromate stress counteraction. The *recG* involvement in Cr(VI) resistance was well reported, but our findings suggest the role of this gene also in solving PALs lesions due to Cr(VI)/Cr(III). The PM system allowed to attribute a function to the *crg8* gene, encoding a hypothetical small protein. Our data indicated that *crg8* is involved with the activation of SSR, and thus enables cells to resolve the sulphur starvation condition induced by both reduced uptake of sulphate and increased consumption of sulphur during oxidative stress response.

Moreover, two mutants impaired in metabolic genes suggested that regeneration of reducing power may be fundamental in cellular response to oxidative stress, since it is consumed by enzymes involved in protection and repairing processes, in biosynthetic pathways for rebuilding damaged cellular components, and also in Cr(VI) reduction. This is the first work which indicates that an enzyme playing a key role in supplying NADPH could be directly involved with cellular chromate resistance.

In conclusion the combination of genetic and phenotypic results suggested that chromate resistance in *P. corrugata* strain 28 is a complex system that depends on sulphur starvation response, on supply of NADPH required in repairing damage induced by chromate, and on DNA integrity maintenance.

Acknowledgements. *We are grateful to the Genexpress laboratory (Dipartimento di Biotecnologie Agrarie, Università di Firenze, Italy). This work was supported by MIUR (PRIN, 2004).*

INTEGRATING BIOLOG PHENOMIC ANALYSIS WITH GENOMIC APPROACHES TO EXPLORE THE DIVERSITY OF NATURAL *SINORHIZOBIUM MELILOTI* STRAINS

E.G. BIONDI[1], E. TATTI[2], D. COMPARINI[1], L. GIOVANNETTI[2], M. BAZZICALUPO[1], A. MENGONI[1], C. VITI[2]

[1]Departement of Evolutionary Biology, Univiversity of Florence, Italy
[2]Departement of Biotecnologie Agrarie, Sez. Microbiologia, University of Florence, Italy

Sinorhizobium meliloti is a soil bacterium able to penetrate in root tissues of leguminous plants, forming nodules where bacteria fix atmospheric nitrogen. *S. meliloti* natural populations show a high genetic diversity. At the genomic level, natural isolates differ from each other of 5-10 % of the whole genome. This massive genomic diversity therefore suggests also a high phenotypic and metabolic differentiation among different strains of the species which to date have never been investigated.
In this study we analyzed the phenotypic diversity of 5 different strains of *S. meliloti*, using Phenotype Microarray technique (Biolog): the laboratory strain Rm1021 and 4 natural isolates (AK58 and AK83 from Aral Sea region (Kazakhstan) and two Italian isolates, BL225C and BO21CC). All those natural strains were previously characterized using CGH to evaluate their genomic diversity in comparison with Rm1021. In this study a total number of 571 different growth conditions was tested (PM1, PM2, PM3, PM4, PM9 and PM10).
Results showed that *S. meliloti* strains exhibited metabolic activity on most of the PM3 (nitrogen) and PM4 (phosphorus and sulphur) wells. Moreover, about half of the tested carbon sources (PM1 and PM2) and 20% of osmolytes and pH (PM9 and PM10) conditions displayed an active metabolism. The highest differences in PM metabolic profiles of the 5 strains were retrieved on carbon source utilization (PM1 and PM2), osmolyte and pH (PM9 and PM10). In particular glycosides utilization and tolerance to sodium nitrite, high salt concentrations and high pH were the more variable conditions among the tested strains. Then, we attempted to explain phenotypic differences among strains on the basis of CGH data on genomic diversity. In particular we discuss an explanation for the high ammonium and nitrite tolerance of natural strains with respect to Rm1021.
Finally, we propose possible future applications of those Biolog data for rhizobial studies such as the creation of a selective medium specific for isolation of *S. meliloti* from natural environments and the design of a customized Biolog plate for analysis of *S. meliloti* phenotypic diversity.

Acknowledgements. *We are grateful to the Genexpress laboratory (Departement of Biotecnologie Agrarie, University of Florence, Italy).*

PHENOTYPIC MICROARRAY ANALYSIS OF ANAEROBES

T.C. HAZEN

Virtual Institute for Microbial Stress and Survival, http://vimss.lbl.gov and Lawrence Berkeley National Laboratory, Berkeley, CA. USA.

For the past 5 years we have developed a variety of techniques for Phenotypic Microarray™ analysis of obligate anaerobes. This technique can be useful to understand the growth changes of an organism when changing medium, temperature, or adding a stressor, or when testing mutant strains. The plates, which are commercially available from Biolog™ (Hayward, CA), consist of array of 20 plates. The first eight plates test a variety of metabolic agents, including electron donors, acceptors, and amino acids. Plates 9 and 10 cover a pH and osmotic stressors, while plates 11-20 contain a variety of inhibitors, including toxic agents and antibiotics. Techniques were developed to use these plates under anaerobic conditions to be able to culture obligate anaerobes. To accomplish this the plates were set up in an anaerobic chamber and heat sealed in polyethylene bags containing an anaerobic sachet. Under these conditions, anaerobic environments were maintained in the plates for up to a week. Growth of the cells was measured by the increase in turbidity of the cells, which was correlated with both optical densities at 600 nm and total cell counts. Preconditioning of the cells and specialized media preparation are required for the different types of plates in order to get a valid phenotype. The plates have been successfully used to characterize the phenotype of several anaerobes and are currently being applied to mutant strains to provide rapid screening of mutant phenotypic changes, for rapid pathway analyses and modeling. We have also developed automated upload and analysis of plates over time using heat-mapping techniques. We have used variations of the Omnilog for high throughput measurements of growth rates, minimum inhibitory concentration, optimum medium composition for yield, stress determination, co-culture syntrophy, etc. The Omnilog has become a critical instrument for our systems biology approach to the environment.

FUNCTIONAL DIVERSITY AND PRODUCTIVITY PEAK AT INTERMEDIATE DISPERSAL RATE IN EVOLVING BACTERIAL METACOMMUNITIES

P.A. VENAIL[1], R.C. MACLEAN[2], T. BOUVIER[3], M.A. BROCKHURST[4], M.E. HOCHBERG[1], N. MOUQUET[1]

[1]Université Montpellier 2, CNRS, Institut des Sciences de l'Evolution, CC064, Place Eugène Bataillon, 34 095 Montpellier cedex 05, France
[2]NERC Center for Population Biology, Imperial College London, Silwood Park Campus, Ascot SL5 7PY, UK
[3]Université Montpellier 2, CNRS, Ifremer, UMR 5119 Ecosystèmes Lagunaires, CC093, Place Eugène Bataillon, 34 095 Montpellier cedex 05, France
[4]School of Biological Sciences, Biosciences Building, University of Liverpool, Crown Street, Liverpool, L69 7ZB, UK

The relationship between species richness and ecosystems properties such as productivity is central to our understanding of biodiversity. A consensus has emerged that positive relationships can arise through complementarity or selection effects. However the joint effect on ecosystem properties of ecological and evolutionary mechanisms that control diversity has yet to be considered. Bacteria offer the unique opportunity to obtain rapid ecological and evolutionary responses using experimental designs that fulfill the assumptions of theoretical models. We allowed a single clone of Pseudomonas fluorescens SBW25 to evolve for ~500 generations in a highly spatially heterogeneous environment (i.e., Biolog® GN2 microplates containing 95 unique sources of carbon) under four levels of dispersal. We show that limited dispersal in a heterogeneous metacommunity leads to the evolution of maximal functional diversity and productivity at the regional scale. As predicted by models of niche differentiation, higher complementarity in resource use strategies allows greater coverage of the heterogeneous environment leading to higher productivity. Our results demonstrate for the first time that a positive relationship between functional diversity and productivity can evolve de novo through adaptive radiation into complementary types. Dispersal and environmental heterogeneity determine the evolution and maintenance of functional diversity that in turn determine ecosystem productivity levels.

METABOLIC NETWORK INFERENCE USING PATHWAY TOOLS AND METACYC

R. CASPI, H. FOERSTER, C.A. FULCHER, P. KAIPA, M. KRUMMENACKER, M. LATENDRESSE, S. PALEY, S.Y. RHEE, A.G. SHEARER, C. TISSIER, P. ZHANG, P.D. KARP

SRI International, Menlo Park, CA, 94025-3493, USA.

Significant metabolic insights can be obtained from the annotated genome sequence of an organism. To fully exploit a genome, especially in the context of having high-throughput metabolic data, requires not just annotated genes, but also the ability to infer a full metabolic network from those annotations. The Pathway Tools software package can generate a complete Pathway/Genome database including an inferred metabolic network for an organism using just an annotated genome and the MetaCyc reference database. Metabolic network inference is a multi-step process that begins with the matching of enzyme functions in the annotated genome to known reactions in MetaCyc. The MetaCyc database contains over 1,000 metabolic pathways from over 1,000 organisms, all derived directly from the experimental literature. Once reactions are assigned, metabolic pathways are in turn inferred from the presence of some or all of their constituent reactions. The Pathway Hole Filler tool subsequently applies a Bayes classifier-based method to predict proteins as possible enzymes for reactions without assignments in the original annotation. The end product is a complete inferred metabolic network for the organism of interest, which can then be used for visualization of high-throughput data such as metabolomics datasets, for metabolite tracing through the network, comparison to other metabolic networks, and many other applications in metabolic analysis. Our latest development is the addition of a “taxonomic pruning” tool to Pathway Tools, to improve the accuracy of the final inferred metabolic network.

ADAPTING BIOLOG PHENOTYPE MICROARRAY TECHNOLOGY TO REVEAL THE METABOLOMICS OF *MYCOBACTERIUM TUBERCULOSIS* AND *MYCOBACTERIUM BOVIS*, SLOW-GROWING PATHOGENS WITH d = 0.03 TO 0.05/h

B. UPADHYAY[1], M.D. FIELDER[2], P.R. WHEELER[1]

[1]Tuberculosis Research, Veterinary Laboratories Weybridge, Addlestone, Surrey, UK.

[2]School of Life Sciences, Kingston University, Kingston Upon Thames, Surrey, UK.

To date, Biolog phenotype microarray **(**PM) technology had been developed for use with a number or organisms including "rapid-growing" (d ~0.3 /h) mycobacteria such as *Mycobacterium smegmatis*. However, the major human and animal pathogens, such as *M. tuberculosis, M. bovis* and *M. avium* divide much more slowly (d = 0.03 to 0.05 /h), presenting a problem for PM which utilises growth dependant metabolic activity as a readout indicator.

In order to develop Biolog PM methodology for slow-growing mycobacteria, and demonstrate the value of the technique in strain comparison, experiments utilising attenuated vaccine strains of *M. bovis* BCG were carried out. The Pasteur and Russia strains of *M. bovis* are known to have phenotypic differences, notably in their secretion of a key protein antigen, mpb70. Russia and field strains of *M. bovis* secrete mpb70 constitutively while Pasteur, similar to the human pathogen *M. tuberculosis,* only secretes mpb70 *in vivo*.

The Biolog PM readout is based upon colour formation following formazan dye reduction as a consequence of oxidative bacterial metabolism of a different substrate added to each well of an ELISA plate. Preliminary assays showed a high level of colour even in the absence of substrate, giving unacceptably high background noise. As a consequence the suspensions of BCG were starved for 24h prior to use and the resultant background noise was negligible to acceptably low. Each experiment was validated by dye reduction in vessels containing establish growth substrates such as: for carbon utilisation (PM1 plate): glycerol, pyruvate and tween 80 (oleate ester) and for nitrogen utilisation (PM3 plate): L-asparagine. Interestingly, L-asparagine gave dye reduction when also used as a carbon source in BCG Pasteur. This finding was not observed with BCG Russia. Other carbon substrates that discriminated between the two strains included bromosuccinic acid, fumaric acid, D-alanine and L-alanylglycine on PM1 plates and L-glutamine on PM3 plates which were metabolised by BCG Pasteur, whilst cellobiose and D-lactose (on PM1), gentiobiose and amygdalin and salicin (on PM2) were only metabolised by BCG Russia. Both strains were seen to metabolise other carbon assimilation substrates namely, tween 20 and 40 (more fatty acyl esters), D-glucose, dihydroxyacetone (the product of glycerol dehydrogenase being used like glycerol) and unexpectedly, methyl-succinate.

A period of 5 days of incubation was required to detect substrate specific metabolism for the slow-growing mycobacteria.

This work represents an important novel step in the rapid characterisation of pathogenic mycobacteria. Further work is needed to improve protocols and reproducibility, for example BCG Russia suspensions varied in their metabolic capabilities. However, the use of PM may offer a great improvement in terms of both rapid diagnostic capability as well as isolation and culture in the human and veterinary clinical setting.

NOVEL CUES OF *L. PNEUMOPHILA* DIFFERENTIATION UNCOVERED BY PHENOTYPE MICROARRAYS

R.L. EDWARDS[1], Z.D. DALEBROUX[2], M.S. SWANSON[1,2]

[1]Cellular and Molecular Biology Graduate Program University of Michigan, Ann Arbor, MI 48109 United States.
[2]Department of Microbiology and Immunology University of Michigan, Ann Arbor, MI 48109 United States.

In aquatic environments, the gram-negative bacterium *Legionella pneumophila* typically parasitizes freshwater protozoa. Consequently, if humans inhale bacteria-laden aerosols, *L. pneumophila* can survive and replicate within alveolar macrophages to cause the severe pneumonia, Legionnaires' disease. Essential to the life cycle of *L. pneumophila* is its ability to exist in at least two distinct phenotypic phases: a non-infectious, replicative form required for intracellular growth and an infectious, transmissive form that is vital for dissemination. Using *L. pneumophila* as a tool, we seek to understand the environmental cues and biochemical pathways that elicit this developmental switch. By screening hundreds of metabolites via phenotypic microarrays, we determined that when replicative *L. pneumophila* encounter excess carboxylic acids, their growth is restricted and expression of transmissive phase phenotypes is quickly activated. In particular, short chain fatty acids trigger the expression of motility, cytotoxicity, lysosomal evasion and sodium sensitivity; all hallmarks of transmissive *L. pneumophila*. Interestingly, the phenotypes displayed by *L. pneumophila* following fatty acid supplementation are largely dependent on the two-component system LetA/LetS and the stringent response. However, rather than employing the conventional arm of the stringent response pathway governed by RelA, genetic data indicate that *L. pneumophila* utilizes a second ppGpp synthetase, SpoT, to trigger differentiation in response to fatty acid supplementation. From subsequent studies, we have deduced that the addition of excess fatty acids alters flux in the fatty acid biosynthetic pathway, which signals the bacteria to induce mechanisms that promote host transmission. We postulate that by coupling phase differentiation to its metabolic state, *L. pneumophila* can swiftly acclimate to environmental fluctuations and stresses encountered in host, thereby enhancing its overall fitness.

H-NS, A MASTER CONTROLLER OF WARM ADAPTATION IN *ESCHERICHIA COLI*: A TRANSCRIPTOMIC ANALYSIS

F. FABRETTI[1], T. CARZANIGA[1], F. FALCIANI[2], G. DEHÒ[1]

[1]DSBB, Università degli Studi di Milano, Milano, Italy.
[2]School of Biosciences, University of Birmingham, Birmingham, UK.

Temperature shifts of mesophilic bacteria to low and high (*e.g.* <20 and >45 °C, respectively) temperatures induce specific adaptive changes. Likewise, transition to the "warm" (37 °C) temperature of bacteria coming from the cold may involve specific mechanisms. Understanding the "warm response" may also provide insights into the early stages of bacterial infection of warm-blooded hosts. Growth curves of *Escherichia coli* (wild type and mutants in global regulators) upon temperature upshift from 10 to 37 °C suggested that "warm adaptation" involves specific regulatory mechanisms and that *hns* (which encodes the *h*eath stable-*n*ucleoid *s*tructuring H-NS protein, a global repressor) plays a central role in this process. We thus investigated global variations in mRNA abundance under the above conditions in wild type and *hns* deletion strains. The results of our transcriptomic analysis will be discussed. Overall our data underscore the essential role of H-NS both for growth at low temperature and for adaptation to 37 °C.

PART II

POSTERS

EXTENDED-SPECTRUM BETA-LACTAMASE PRODUCING STRAINS OF *ESCHERICHIA COLI* ISOLATES: PHENOTYTIC AND GENOTYPIC CHARACTERISATION

E.C. ADIBE[1], C.E. ILODIGWE[3], H.I. EKENEDO[2], L.C. OFORA[3], S.I. OKONKWO[2], S.A.OBI[1]

[1]Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, P.M.B.5025, Awka, Anambra State, Nigeria.
[2]Department of Geology/Geophysics, Nnamdi Azikiwe University, P.M.B.5025, Awka, Anambra State, Nigeria.
[3]Department of Environmental Management, Nnamdi Azikiwe University, P.M.B.5025, Awka,Anambra State, Nigeria.

A total of 253 clinical isolates of *Escherichia coli* were isolated from attendants and patients in Nnamdi Azikiwe University Teaching Hospital for a period of five months(July-October 2007). Clinical isolates from wound (48), blood (62), urine (67), sputum (33) and semen (43) were all characterized using standard procedures . A preliminary sensitivity study was carried out against the isolated organisms using disc diffusion method and the organisms were later screened for Extented Spectrum Beta-Lactamase(ESBL) production phenotypically using double disc synergy test (DDST). A preliminary molecular characterization of ESBL- producing isolates was carried out based on the evaluation of their plasmid profile through agarose gel electrophoresis. There after a conjugation experiment was carried out by growing ESBL-producing *E.coli* (donor strain) with a sensitive clinical *Salmonella paratyphi* A(recipient strain). The data generated in this study shows that 49 (19.37%) out of 253 clinical isolates of *E. coli* are putative ESBL producers and the prevalence is more in patient than in attendants. ESBL- producing isolates were obtained more from blood (7.1%) than urine (3.42%), wound (4.18%), semen (2.34%) and sputum (2.34%). The plasmid profile studies reveals the presence of low molecular weight plasmid DNA within the ranges of 21.3 kb-29.4 kb and the conjugation studies also showed that only four ESBL producers are able to transfer their plasmid DNA responsible for ESBL expression to the recipient *S. Paratyphi* A strain.

THE CELL SURFACE HYDROPHOBICITY IS AN IMPORTANT FACTOR IN VIRULENCE OF *STREPTOCOCCUS FAECALIS*

E.C. ADIBE[1], C.E. ILODIGWE[3], H.I. EKENEDO[2], C.L. OFORA[3], S.I. OKONKWO[2], S.A. OBI[1]

[1]Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, P.M.B.5025, Awka,Anambra State, Nigeria.
[2]Department of Geology/Geophysics, Nnamdi Azikiwe University, P.M.B.5025, Awka, Anambra State, Nigeria.
[3]Department of Environmental Management, Nnamdi Azikiwe University, P.M.B.5025, Awka,Anambra State, Nigeria.

The attachment of pathogenic microorganisms to surfaces is an important virulence factor and involves different types of interactions. *Streptococcus faecalis* is an opportunistic pathogen to humans. This is because it has the ability to adhere to surfaces. Silicone rubbers used by laryngectomy patients frequently become clogged with bacterial biofilms, with *S. faecalis* as one of the predominant species. Ten *S. faecalis* strains isolated from silicone rubber were investigated for the presence of specific biochemical factors involved in their adhesion: aggregation substances and the enterococcal surface protein. In addition, physico-chemical factors involved in adhesion (zeta potential and cell surface hydrophobicity) were determined, as well as the influence of ox bile on these properties. Three quarters of the silicone rubber isolates displayed culture heterogeneity in the pH dependence of their zeta potentials. Moreover, 32 out of 57 clinical isolates of *S. faecalis*, including laboratory 14 strains, were found to exhibit such heterogeneity. Results presented show that culture heterogeneity in zeta potential enhances adhesion to surfaces. A higher prevalence of culture heterogeneity in zeta potential in pathogenic as compared to non-pathogenic isolates could indicate that this phenomenon might play a role in virulence and putatively in pathogenesis.

PROFILE OF THE RESISTANCE TO SEVERAL ANTIBIOTIC FAMILIES OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* STRAINS ISOLATED AT CONSTANTINE UNIVERSITY HOSPITAL

H. AOUATI[1], A. BOULAHROUF[1], K. BENLABED[2], A. KHIDER[2], F. SMATI[2]

[1]Mentouri University of Constantine, Life Sciences Faculty. Department of Microbiology and Biochemistry.
[2]Laboratory of Bacteriology Constantine University Hospital. E-mail: han_banda@yahoo.fr.

The prevalence of the methicillin-Resistant *Staphylococcus aureus* strains (MRSA) was evaluated in an epidemiological study during the time from 31 January to 31 July 2007 at the microbiology laboratory of the Constantine University Hospital.
122 non repetitive strains of *Staphylococcus aureus* were isolated from different biological samples (hemoculture, pus and others) taken from both patients hosted in different services or consulting treatment. They were identified with conventional methods.
The disk diffusion method (especially measuring the inhibition diameter of Cefoxitin 30µg) allowed to identify 46 strains resistant to methicillin. The screening with Oxacillin (6µg/ml) corroborated this resistance to methicillin only with 40 strains.
Resistance to methicillin (Oxacillin) of these *Staphylococcus aureus* strains is crossed with all beta lactamines.
The profile of resistance to other classes of antibiotics of these methicilin-resistant *Staphylococcus aureus* strains was regularly determined by the diffusion method in jellified Muller-Hinton medium using the disk containing antibiotics (controlled with reference strain *Staphylococcus aureus* 25923).
The global analysis of the susceptibilities to antibiotics confirmed the multi-drug resistance character of MRSA strains consequently several different antibiotypes were determined and are as follows:
-Aminosid family: 24 strains were the phenotype KNm, 15 strains were the phenotype KTG and 01 strain was the phenotype KT.
-Macrolid family: 16 strains were the phenotype MLS_B inductible and 01 strain was constitutive phenotype MLS_B.
-Other antibiotic families: 31 were cyclin-resistant strains, 29 were Fusidic-acid resistant strains, 07 were strains resistant to Trimetoprim+Sulfamethoxazol, 06 were Ofloxacin resistant strains, 02 were Rifampicin-resistant strains and 01 Fosfomycin-resistant strain.
None of the 40 methicilin-resistant strains shows resistance to Chlorophenicol, and Glycopeptides (the MIC were evaluated for Glycopeptides).

USE OF A PHENOTYPE ARRAY TO STUDY AN ADAPTIVE RESPONSE IN *ENTEROBACTER SAKAZAKII*

B. ARKU[1,2], S. FANNING[2], T. QUINN[2], K. JORDAN[1]

[1]Teagasc, Moorepark Food Research Centre, Fermoy, Cork, Ireland.
[2]Centre for Food Safety, UCD Veterinary Sciences Centre, University College Dublin, Belfield, Dublin 4, Ireland.

Enterobacter sakazakii is an opportunistic pathogen that can cause fatal meningitis in premature, low birth-weight and immunocompromised infants. It is particularly associated with powdered infant milk formula. Like most bacteria, exposure of cells to a sub-lethal stress (adaptation) increases the survival potential during lethal stress. However, it is not clear if exposure of cells to sub-lethal stress increases the growth potential of cells under stress. The aim of this study was to determine if adaptation to a sub-lethal heat stress, which could occur on reconstitution of powder infant milk, increased the growth potential of *E. sakazakii*. Two strains of *E. sakazakii* (the type strain and another clinical isolate) were grown overnight in nutrient broth at 37ºC. After growth, a portion of the cells was adapted to a sub-lethal temperature of 46ºC for 30 min. This adaptation increased the survival potential of the cells by >2 log cycles at 52ºC. The adapted and unadapted cells were inoculated into 12 x 96-well plates in a Biolog phenotype microarray and incubated at 37ºC for 48 h. Of the 96-well plates used, 10 had stressful conditions, for example, pH, osmolarity, antibiotics etc. There was no difference in growth between adapted and unadapted cells in the vast majority of wells. The most significant conditions where adapted cells showed increased growth were in the presence of 5 antibiotics (including tetracycline and penicillin), although this was only with one strain. From these results, it appears that while adaptation to heat increased survival of lethal heat stress, it did not increase the growth potential of *E. sakazakii* under stress, except in the presence of 5 antibiotics.

CHARACTERIZATION OF H_2-PRODUCING PURPLE NON SULPHUR BACTERIA ISOLATED FROM THE TROPHIC LAKE OF AVERNO, ITALY

L. BIANCHI, A. ADESSI, R. DE PHILIPPIS

Department of Agricultural Biotechnology, University of Florence, Florence, Italy.

The purpose of this work was the isolation and the characterization of new strains of purple non sulphur bacteria (PNSB) with the aim of finding good hydrogen producers in a trophic lake characterized by high concentrations of organic substances, an environment considered to be suitable for harbouring this kind of microorganisms. The sampling site was the lake of Averno, Campania, Italy. This lake is situated in a volcanic basin in the area named *Campi Flegrei*, close to Naples; it is a trophic lake, occasionally polluted by urban waste waters coming from the overflows of the pipelines connecting the city sewer system to the local depuration plant.
The waters of Averno Lake were sampled at ten different depths (from -1 m to the bottom, at -33 m) and from the samples 17 PNSB strains were isolated, at least one per each depth, pointing out the presence of purple non sulphur bacteria all along the whole water column.
The strains, characterized by comparing the sequences of their 16S rDNA, were identified as: *Rhodobacter capsulatus*, *Rhodobacter sphaeroides*, *Rhodospirillum rubrum* and *Rhodopseudomonas palustris*. The more represented species is *Rhodopseudomonas palustris*, 13 of the 17 isolated strains belonging to this species.
The species distribution along the water column was shown to be not homogeneous: indeed, the strains isolated in the first three metres were shown to belong to the species *Rb. capsulatus*, *Rb. sphaeroides*, *Rs. rubrum*, while all the strains isolated at deeper levels (from -5m to - 33m) were shown to belong to the species *Rp. palustris*.
All the isolated strains were tested for their capability to produce hydrogen by utilizing four different organic substrates as electron donors, namely acetic, succinic, lactic and malic acids. The experiments, carried out by individually using each organic substrate, showed that all the strains are capable to produce hydrogen, ten of them utilizing three different substrates, six two substrates and one only utilizing malic acid.
In conclusion, all the strains of PNSB isolated from the waters of Averno Lake showed the capability to produce hydrogen, confirming the hypothesized suitability of that environment to harbour hydrogen producing purple non sulphur bacteria. The strains showed differences in their capability of using different organic substrates for hydrogen production, nevertheless all of them were able to produce H_2 at least with one of the four acids tested. However, the observed differences in the capability of the strains of using different kind of electron donors are not directly related to both their taxonomical position and the depth of their habitat.

Acknowledgements. *This research was supported by MIUR (Italian Ministry of University and Research) in the framework of the FISR Project "Innovative methodologies for hydrogen production from biological processes".*

IDENTIFICATION OF *SACCHAROMYCES CEREVISIAE* BY THE BIOLOG AUTOMATED MICROBES IDENTIFICATION SYSTEM

C. CHI, L. JIN-XIA, Y. SU, H. HAI-RONG

China National Research Institute of Food &Fermentation Industries, China Center of Industrial Culture Collection, Beijing 100027.

Biolog Automated Microbes Identification System was used to check 29 strains of *Saccharomyces cerevisiae* preserved in China Center of Industrial Culture Collection (CICC). Of the 29 strains, 27(93%) were correctly identified at the species level. The values of Probability (PROB) and Similarity (SIM) were satisfied. 70% SIM values of the strains were more than 0.9, while 89% SIM values were more than 0.8. Two strains were not given identification results, one is haploid and another is for genetics study. The test result indicates that *S. cerevisiae* can be identified correctly by Biolog System, but the yeasts of haploid and gene engineering may not be identified accurate.

BIOLOG MICROBIAL IDENTIFICATION SYSTEM-STUDY ON THE OPERATING REGULATION OF BACTERIA IDENTIFICATION

C. CHI, Y. MEI, L. JIN-XIA, Y. SU, H. HAI-RONG

China Center of Industrial Culture Collection, China National Research Institute of Food & Fermentation Industries, Beijing 100027, China.

Biolog Microbial Identification System, which is developed by Biolog Inc., is a new automated technology for rapid identification of microorganisms. The system is based around the assimilation of 95 carbon sources by bacteria on a microtiter tray. Up to 100 sets of Biolog system were introduced into China, but most of them are not extensively used. Based on the practice experience, and referred to Biolog User Guide 4.2 and Rules of National Infrastructure of Culture Resources, the operating regulation of bacteria identification was established, which is for operating the Biolog system correctly to obtain accurate results and improving the application level.

INCIDENCE OF *SALMONELLA* INFECTIONS IN LIZARD DROPPINGS IN IMO STATE UNIVERSITY ENVIRONMENT

A. CHINEDU WILLIAM

IMO State University, Owerri. P.O. BOX 6406, Aladinma Post Office HQ, Aladinma Owerri IMO State, Nigeria.

Lizard dropping were investigated for the carriage or presence of *Salmonella* in them. Isolation was done using the selective media *Salmonella* and *Shigella* agar, which selects for *Salmonella* and *Shigella*. Only Salmonella appeared as black colonies due to characteristic hydrogen sulphid production. Isolates were later characterized using a combination of morphological, biochemical and serological tests. Out of the 50 samples of Lizard droppings collected and examined, 19 were confirmed positive for Salmonella representing 38% incidence. The chi-square test was used to statistically analyze the results obtained and it showed that the incidence of *Salmonella* infections in Lizard droppings in Imo State University environment is high. The main objective of this work is to estimate the incidence of *Salmonella* in lizard droppings, which will go a long way in shedding some light on the epidemiological significance of the presence of *Salmonella* in lizard droppings and possible ways of controlling them.

VALIDATION OF A MOLECULAR APPROACH TO DETECT AFLATOXIGENIC STRAINS OF *ASPERGILLUS FLAVUS*

F. DEGOLA[1], E. BERNI[2], E. SPOTTI[2], I. FERRERO[1], F.M. RESTIVO[1]

[1]Department of Genetics, Biology of Microorganisms, Anthropology, Evolution, University of Parma, Parma, Italy.
[2]Stazione Sperimentale per l'Industria delle Conserve Alimentari (SSICA), Parma, Italy.

Aflatoxins are potent hepatocarcinogens and mutagens produced as secondary metabolites by several *Aspergillus* species. *Aspergillus flavus* and *Aspergillus parasiticus* represent the prominent contaminant of food commodities and, because of their adverse effect on human and animal health, they may cause economic problems for international trades and losses to grain producers. Moreover M1, a metabolic thermostable derivative of aflatoxin B1, may accumulate in the milk of cows fed with aflatoxin-contaminated feed, and it may be found still active in cheese and other dairy products. This is a relevant threat for the EU economy, in particular for our region where dairy production is one of the main industries in the social and economic framework. Nevertheless, not all the strains of *A. flavus* are aflatoxigenic. In fact, many isolates may be not mycotoxigenic because of mutation in one or more genes belonging to the biosynthetic gene cluster. Conventional methods of identifying these fungi rely on microbiological techniques and the detection of mycotoxins by immunological systems; both methods have drawbacks: the former is time consuming whereas the latter is prone to aspecificity and may result in false positives.
We have developed a multiplex reverse transcription-polymerase chain reaction (RT-PCR) protocol to discriminate aflatoxin-producing from aflatoxin-non producing strains of *Aspergillus flavus*. The protocol was first optimized on a set of strains obtained from laboratory collections and then validated on *A. flavus* strains isolated from corn grains collected in the fields of the Po Valley (Italy). Five genes of the aflatoxin gene cluster of *A. flavus*, either regulatory or structural, were targeted with specific primers to highlight their expression in mycelia cultivated under inducing conditions for aflatoxins production. We show that a good correlation exists between gene expression of the aflatoxin genes, analysed by multipex RT-PCR, and aflatoxin production (Degola *et al.* 2007). We have now extended our analysis to 14 genes (covering 80% of the aflatoxin gene cluster) and validated the protocol on new isolates coming from different regions of Northern Italy. The proposed protocol will be helpful in evaluating the risk posed by *A. flavus* in natural environments and might also be a useful tool to monitor its presence during the processing steps of food and feed commodities.

References

Degola F., Berni E., Dall'Asta C., Spotti E., Marchelli R., Ferrero I., Restivo F.M.R. (2007) A multiplex RT-PCR approach to detect aflatoxigenic strains of *Aspergillus flavus.* Journal of Applied Microbiology **103**: 409-417

Acknowledgements: *this research was funded by grants from SIQUAL (Laboratorio Regionale di Qualita`e Sicurezza degli Alimenti) and from Progeo. Francesca Degola is the recipient of a post graduated fellowship from Progeo.*

PRELIMINARY STUDIES ON A BACTERIAL DISEASE OF HEMP (*CANNABIS SATIVA* L.)

G. GRASSI[1], S. CASANO[1,2]

[1]CRA-CIN, Rovigo Branch Section, Italy.
[2]Department of Environmental and Territorial Agronomy, Faculty of Agricultural Sciences, University of Palermo, Italy.

Hemp (*Cannabis sativa* L.) is a herbaceous annual species that has been cultivated worldwide from the most ancient cultures to the XX century for its fibre, seed and resin. After decades of abandonment of its licit cultivation, there has been a change in recent years in public and private interest in this industrial and pharmaceutical crop, therefore the interest is increasing and there are plans to restart extensive licit cultivations. Despite claims that hemp does not have serious sanitary problems, some pests can cause considerable yield and economic loss. Some years ago, in the summer of 2000, we first reported a severe disease in fibre/seed cultivars grown in outdoor conditions in Northern Italy, and in the following years similar signs and symptoms have been observed in several accessions and breeding lines grown in indoor and outdoor conditions. The experiments conducted have allowed us to ascertain that the disease under study is infectious and that the causal agent is a bacteria.
Signs and symptoms can be observed starting from the seedling stage to the fruiting stage, and their expression is strongly influenced by the affected growing stage of the plant and by the environmental parameters of the growing conditions. The main symptom observed in infected seedlings is the curling of the first true leaves, moreover in the first portion of the lamina. At the vegetative stage plants start to show more frequently foliar blights and spots, which are irregular in shape and position, and are often surrounded by a greeenish-yellow halo. If favourable conditions are reached during the vegetative stage, the disease may become systemic and very severe, causing dwarfed internodes and general interveins yellowing and fall of the true leaves. At the flowering stage the tops assume more or less a rosette form and an abortion of floral structures can occur. Top leaves become curled with wider necrotic areas that become dried and brown or sooty in color.
General and selective bacterial broth and solid media have been used for the enrichment and isolation of bacteria. Seeds, leaf disks and different plant tissues derived from infected plants have been used for the broth enrichment. Isolation of bacteria have been performed by plating in solid media limited dilutions of the broth, in order to stock at -20°C mono-cultures for further works on characterization and identification. Some of them have been already characterised and identified through:
1.Gram-stain reaction by using the KOH method;
2. Pathogenicity tests by:
a) injection of bacterial suspensions into leaves of hemp and bean;
b) application of bacterial suspensions into stems of hemp artificially wounded;
3. Immunological evaluation by using a Adgen ELISA kit specific for the detection of *Pseudomonas syringae pv. phaseolicola*;
4. Fatty acid profiles evaluation by performing gas-chromatographic analysis in our lab and at the CSL at York (UK) comparing with two libraries: TSBA6.0 and NCPPB3;
5. Carbon-substrate assimilation tests by using Biolog GN2 MicroPlates and MicroLog database software.
Those bacteria that have received an univocus identification by using the last two systems are not related to *Pseudomonas cannabina*, *Pseudomonas amygdali pv. mori* or *Xanthomonas campestris pv. cannabis*, which are the only causal agents of bacterial diseases of hemp reported in the literature.

A PHENOTYPIC APPROACH TO DIFFERENTIATE STRAINS WITHIN *OENOCOCCUS OENI* SPECIES

S. GUERRINI, S. MANGANI, L. GRANCHI, M. VINCENZINI

Dipartimento di Biotecnologie Agrarie, P.le delle Cascine 24 50144 Florence, Italy.
Tel: 055 3288309, e-mail: simona.guerrini@unifi.it.

Oenococcus oeni, owing to its resistance to high ethanol concentrations and low pH values, is the species of lactic acid bacteria most frequently associated with the malolactic fermentation in wine (MLF). MLF consists of the conversion of L-malate into L-lactate and CO_2 and plays an important role in winemaking, improving the biological stability and organoleptic properties of the wine. *O. oeni* constitutes a phylogenetically homogeneus group, as supported by chromosomal DNA homology studies, 16S and 23S rRNA sequencing and sequence analysis of the 16S – 23S rDNA ISR. Results from several studies on genotypic diversity among the strains of *O. oeni* carried out utilizing DNA fingerprinting, Restriction Endonuclease Analysis (REA-PFGE), Polymerase Chain Reaction – Randomly Amplified Polymorphic DNA (PCR-RAPD) and ribotyping suggest that this species is also genomically homogenenous. Moreover, *O. oeni* strains of wine origin often differ in their tolerance towards environmental determinants, such as ethanol concentration, pH and SO_2, in their growth behaviour and their carbohydrate fermentation pattern. In order to investigate on the biodiversity degree within *O. oeni* species, 37 *O. oeni* isolates from Italian wines of different oenological areas were characterized determining their capability to release biogenic amines (BAs) and their fatty acid (FA) composition. These properties were chosen because of their roles in survival of *O. oeni* strains in the harsh environment of wines. Indeed, BA production is known to play a protective function against intracellular acidification and to furnish an energetic advantage to the bacterial cells by generating a trans-membrane proton motive force. On the other hand, the membrane, with its FA composition, is generally known as the primary site of an adaptive response of cells to ethanol and low pH. In this connection, recent studies demonstrated that *O. oeni* strains possessing higher percentages of oleic acid and its methylated derivate (dihydrosterculic acid) in their fatty acid profile showed higher cell viability in wine. Numerical analysis (cluster analysis) of the results (FA profiles and amounts of histamine, putrescine, and cadaverine released by the strains under standardized conditions) grouped the isolates into four clusters at a 0.3% of linkage distance (1 – Pearson's coefficient). The major cluster, including 65% of the total strains, was characterized by higher percentages of oleic acid and its methylated derivative, unability to produce both cadaverine and putrescine and capability to produce histamine up to more than 30 mg/L, depending on the strain. The second cluster included strains (16% of the total) possessing lower percentages of oleic acid and its methylated derivative and producing only low amounts of histamine. Finally, the third and fourth clusters included strains with the same FA composition as the major cluster, but possessing the capability to produce all the considered BAs. These findings confirm, in spite of the genotypic homogeneity, a high intraspecific biodiversity within *O. oeni* species, also in properties that have a quite different importance under a technological point of view, despite they enable the cells to cope the harsh conditions of wine. Consequently, the cluster analysis of some properties, such as FA composition and the capability to produce BAs, can be a useful approach to select *O. oeni* strains to be used as malolactic starter in winemaking.

PHENOTYPIC CHARACTERIZATION OF EXTENDED SPECTRUM BETA LACTAMASE ENZYME FROM CLINICAL ISOLATES OF *KLEBSIELLA PNEUMONIA* FROM A TERTIARY HOSPITAL IN EBONYI STATE NIGERIA

I.R. IROHA, C.O. ONWOSI

[1]Department of Applied microbiology Ebonyi State University, P.M.B. 058 Abakaliki, Ebonyi State Nigeria, e-mail: ifynero@yahoo.com.
[2]Biotechnology Research Center P.M. B. 5025, Awka Anambra State Nigeria.

The prevalence of extended spectrum beta- lactamase enzymes (ESBLs) of clinical isolates of *Klebsiella pneumonia* from a tertiary hospital in Abakaliki Ebonyi state Nigeria was evaluated. 200 clinical isolates of *klebsiella pneumonia* were isolated from 3 clinical specimens, which are blood (78), name (67) and wound (45). These organisms were identified and characterized using standard techniques recommended by the National Committee for Clinical Laboratory Standard (NCCLS). Each of the test organisms was subjected to sensitivity studies using the agar disc diffusion method of testing for antimicrobial susceptibility. The phenotypic characterization of the test organism for ESBL production was done using the double disc diffusion test (DDST). The overall results reveal that out of the 200 clinical isolates of *klebsiella pneumonia*, 64 were putative ESBL producers and the susceptibility studies shows that they were multi-drug resistant in nature.

DIFFERENTIATION OF NOVEL SPECIES USING THE PHENOTYPE MICROARRAY

C. IVERSEN[1], N. MULLANE[1], S. FANNING[1], B. McCARDELL[2], B. D. TALL[2], M.H. KOTHARY[2], L. CARTER[2], A. LEHNER[3], R. STEPHAN[3], H. JOOSTEN[4]

[1]Centre for Food Safety & Food-borne Zoonomics, UCD Veterinary Sciences Centre, University College Dublin, Belfield, Dublin 4, Ireland. [2]U.S. Food and Drug Administration, Laurel, MD 20708, USA. [3]Institute for Food Safety and Hygiene, Vetsuisse Faculty, University of Zurich, CH-8057, Zurich, Switzerland. [4]Quality and Safety Department, Nestlé Research Centre, Vers-chez-les-Blanc, CH-1000 Lausanne, Switzerland.

Enterobacter sakazakii was defined as a new species in 1980 by Farmer *et al*, however DNA-DNA hybridization gave no clear generic assignment for the organism as it was shown to be 53–54% related to species in two different genera, *Enterobacter* and *Citrobacter*. The species was placed in *Enterobacter* as it appeared phenotypically and genotypically closer to *E. cloacae* than to *C. freundii*. Since 1980 sixteen biogroups have been described and independent molecular methods, including f-AFLP, automated ribotyping, full-length 16S rRNA gene sequencing and DNA-DNA hybridization, were employed to clarify the taxonomic relationship of *E. sakazakii* strains. For each of the methods 4-6 groups could be delineated. DNA-DNA hybridizations were performed with strains from each of the groups. The DNA homology between strains within groups was found to be between 70-100%, whereas homology values amongst strains belonging to different groups were all clearly below 70%. This indicated that at least 6 genomospecies were present; however using conventional phenotypic tests only 4 of these groups could be easily differentiated. The phenotypic descriptions of the proposed novel species were expanded using Biotype 100 and Biolog Phenotype MicroArray. For the Biolog Phenotype MicroArray cell suspensions were prepared from 24 h cultures grown on blood agar at 37°C and suspended in either IF-0 or IF-10 medium to the appropriate density. Metabolic panels PM1, PM2A, PM9 and PM10 were inoculated, the plates were incubated for 48 h at 37 °C and the data recorded using an Omnilog instrument. The areas under the growth curves were analysed and a value of greater than 15,000 tetrazolium dye reduction units was used to designate a positive result. The utilisation of malonate was also determined using Malonate Phenylalanine Broth and indole production was determined by adding Kovac's reagent to cultures grown in tryptone. A negative designation for a particular trait denoted consistent negative results for isolates across all methods. A positive designation denoted a positive result using one or more methods. The phenotypic profiles determined fourteen variable biochemical characteristics that differentiate species and subspecies. These were, utilisation of dulcitol, lactulose, maltitol, palatinose, putrescine, D-melezitose, D-turanose, *myo*-inositol, *trans*-aconitate, *cis*-aconitate, 1-0-methyl-alpha-D-glucopyranoside and 4-aminobutyrate as sole carbon sources; utilisation of malonate as indicated by increase in pH in Malonate Phenylalanine Broth, and production of indole as revealed on addition of Kovac's reagent to cultures grown in tryptone.

It was then possible to amend the taxonomic description of these organisms. However, as they are a microbiological hazard occurring in the infant food chain with historic high morbidity and mortality in neonates it was important that reclassification of the species was not detrimental to health protection measures already in place. Therefore it was proposed that *E. sakazakii* be alternatively classified as 5 species, 1 genomospecies, and 3 subspecies in a new genus, *Cronobacter* gen. nov., within the *Enterobacteriaceae*. The creation of a new genus simplifies the inclusion of these potentially pathogenic organisms in legislation (the proposed genus *Cronobacter* being synonymous with *Enterobacter sakazakii*) and current identification schemes developed for *E. sakazakii* remain applicable for the *Cronobacter* genus.

GENOTYPIC AND PHENOTYPIC DIVERSITY OF *STREPTOCOCCUS THERMOPHILUS* STRAINS ISOLATED FROM THE CAUCASIAN YOGURT-LIKE PRODUCT MATSONI

I. MALKHAZOVA[1], M. MERABISHVILI[1], N. CHANISHVILI[1], L. BRUSETTI[2], D. DAFFONCHIO[2], D. MORA[2]

[1]Laboratory of Genetics of Microorganisms and Bacteriophage, G. Eliava Institute of Bacteriophage, Microbiology and Virology, Tbilisi, Georgia, e-mail: ianamalkh@mail.ru, n_chanish.ibmv@caucasus.net, m_merabish.ibmv@caucasus.net.
[2]Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche (DISTAM), Università degli Studi di Milano, Milano, Italy, e-mail: lorenzo.brusetti@unimi.it; daniele.daffonchio@unimi.it; diego.mora@unimi.it.

The goal of this research was to determine a correlation between the genetic diversity of *Streptococcus thermophilus* strains, their biotechnologically important phenotypic traits and geographic distribution.
Altogether 49 strains isolated from domestic samples of Matsoni have been analyzed for acidifying and urease activities, exopolysaccharide (EPS) production, casein degradation ability etc. The phenotypic characterization demonstrated significant phenotypic variability in *S. thermophilus* strains associated with their geographical origin.
Genetic diversity of the strains was analyzed by F-Rep-PCR (fluorescent repetitive extragenic palindromic – PCR) technique commonly used for biogeographical studies. F-BOX-PCR was performed with BOX-A1R primers labeled with 6-carboxyfluorecsein (6-FAM). Separation of the PCR products was done automatically in ABI-Prism 310 capillary electrophoresis system. Genetic grouping of the strains and evaluation of their endemic nature was based on application of the unweighted pair group method and arithmetic average cluster analysis of the F-BOX-PCR profiles. These techniques allowed easy typing and differentiation of 41 genotypes out of 49 *S. thermophilus* strains, highlighting a high degree of variability within this specie. Analyzed strains were clustered according to their geographic locations (East Georgia, West Georgia, Black Sea cost). Comparison of genetic and phenotypic/biotechnological data allowed finding the interesting correlations between technological features and genotypes that could be used for development of the collection of industrially valuable dairy strains. Moreover, the possibility to obtain strain-specific molecular markers by genotypic fingerprinting could be useful to identify strains with interesting biotechnological properties.

ASSESSMENT OF PHENOTYPIC FEATURES OF BACTERIA ISOLATED FROM DIFFERENT ANTARCTIC ENVIRONMENTS

S. MANGANO, C. CARUSO, L. MICHAUD, A. LO GIUDICE, V. BRUNI

Department of Animal Biology and Marine Ecology, University of Messina, Salita Sperone 31, 98166 Messina, Italy.

Nowadays, there is a renewal interest on the study of bacterial phenotypic traits, that is, in our opinion, a fundamental tool for the complete characterization of any microbial community, in addition to modern molecular approaches.
Antarctic ecosystems are among the less-explored environments on Earth and offer to researchers a unique opportunity for studying microbial diversity and evolution.
In this context, the aim of the present work was to investigate the phenotypic characteristics of Antarctic bacteria. Strains used in this study were isolated from different sources (seawater, lakes and sponge specimens) collected during three Antarctic expeditions.
Phenotypic characterization included morphological, biochemical and physiological analyses. Gram stain, cell morphology, presence of flagella and endospores, motility and presence of pigment were recorded to asses morphological features of Antarctic isolates. Among biochemical tests, the hydrolysis of macromolecules (e.g., agar, Tween 80, chitin and starch), the susceptibility to several antibiotics (e.g., ampicillin, penicillin G, polimixine B and vibriostatic agent O/129) and the presence of the enzymes oxidase and catalase were analyzed. Additional biochemical and enzymatic tests were performed using the miniaturized systems API 20E and API 20NE. Isolates were tested for the ability to grow on various solid media such as Trypticase Soy Agar with or without 3% (w/v) NaCl, and TCBS. pH range for growth was tested by using separate batches of Marine Broth medium with pH values adjusted to 4, 5, 6, 7, 8 or 9. Salt tolerance tests were performed on Nutrient Agar with NaCl concentrations ranging from 0 to 13% (w/v). Temperature range for growth was assessed incubating Marine Broth cultures between 4°C and 37°C.
Results obtained put on evidence a general predominance of psychrotrophic, Gram-negative, pigmented (yellow, orange, red, brownish), rod-shaped bacteria. Significant differences in physiological traits were observed among the sources investigated. Bacteria isolated from lake samples grown in a wide pH range between 4 and 9; with respect to salt tolerance, the majority of strains tolerated concentrations of NaCl from 0.5% to 2%. On the contrary, bacteria isolated from seawater and sponge specimens grown in a narrow pH range, mainly between 6 and 8; in addition, as expected for marine organisms, they showed a natural higher salt tolerance, growing also at NaCl concentrations around 11-13%. No significant differences between the environments investigated were recorded for macromolecule hydrolysis and antibiotic susceptibility.
Results here reported contribute to the better knowledge of Antarctic bacterial community. In addition, isolation and characterization of Antarctic microorganisms represent a fundamental approach in order to achieve the comprehension of their physiology and their ecological role.

ROLES OF MULTIDRUG EFFLUX PUMPS IN ANTIMICROBIAL PEPTIDE RESISTANCE OF *SALMONELLA ENTERICA*

T. NAKANO[1, 3], K. NISHINO[1, 2, 3], A. YAMAGUCHI[1, 3]

Department of Cell Membrane Biology, Institute of Scientific and Industrial Research, Osaka University,[1] PRESTO, Japan Science and Technology Corporation,[2] Graduate School of Pharmaceutical Sciences,[3] Osaka University, Osaka, Japan.
Mailing address: Institute of Scientific and Industrial Research, Osaka University, 8-1 Mihogaoka, Ibaraki, Osaka 567-0047, Japan. Phone: 81-6-6879-8546. Fax: 81-6-6879-8549. E-mail: nishino@sanken.osaka-u.ac.jp.

Recently, drug-resistant pathogenic bacteria have become a growing concern in clinical field. The multidrug efflux system transport antibiotics from the inside to the outside of a cell, and is the factor responsible for multidrug resistance. *Salmonella* is the causative agent of food poisoning. The high incidence rate of multidrug-resistant *Salmonella* has become a problem. We are investigating the function and regulation of multidrug efflux systems in *Salmonella*. Our study showed that the multidrug efflux system not only contributes to the drug resistance, but also its virulence. To identify how the multidrug efflux system contributed to *Salmonella* virulence, we investigated the substances that were recognized by the efflux system.
The defence system in our body resists attacks from micro-organisms. This can be divided into acquired and innate immunity. Antimicrobial peptides are important factors of innate immunity. Polymyxin B is a cationic antimicrobial peptide that forms a channel in the bacterial membrane and then displays its bactericidal action. One possible mechanism by which the multidrug efflux system enhances bacterial virulence is by inhibiting the antibacterial peptide attack on the bacteria. This study investigates the susceptibility of wild-type *Salmonella* and a strain, which is genetically deficient in multidrug efflux system, to polymyxin B.
Post-genome analysis showed the existence of nine different multidrug efflux systems in *Salmonella*. To investigate the effect of *Salmonella* multidrug efflux systems on antibacterial peptide resistance, we constructed a strain that was genetically deficient in all the nine multidrug efflux systems. We found that this mutant had 100 times more susceptible to polymyxin B compared with the wild-type *Salmonella*.
It has been clarified that the multidrug efflux system is involved in the antibacterial peptide resistance of *Salmonella*. Because the multidrug efflux system is involved in virulence and bacterial defence against antibacterial peptide, we believe that it plays a role in allowing bacteria to elude the innate immunity of the host. As mentioned above, considering that the efflux systems contribute to antibacterial resistance and bacterial virulence, it can be an attractive new target for new drugs.

USE OF MICROFLUIDIC TECHNOLOGY FOR THE CHARACTERIZATION OF DIFFERENT LACTIC ACID BACTERIA ISOLATED FROM ASS'MILK

F. NAZZARO[1], M. ANASTASIO[2], F. FRATIANNI[1], P. ORLANDO[3]

[1]Institute of Food Science and Technology-CNR, Via Roma, 52 Avellino, Italy, e-mail: mena@isa.cnr.it.
[2]EURECO S.p.A.- European Environmental Company, Piana di Monte Verna, (CE), Italy.
[3]Institute of Protein Biochemistry-CNR, Via P. Castellino, Napoli, Italy.

Laboratory-on-a-chip (LOC) devices, also recognized as 'micro total analysis systems (mTAS)' or 'biological microelectronic mechanical systems (BioMEMS)' have became known as important technology platforms for researches in genetics, proteomics, as well as for clinical and forensic analyses [1], but to a lesser extent in food microbiology. Use of innovative molecular biology and biochemistry technologies has revealed remarkable microbial diversity in many ecosystems [2]. Raw milk or fermented dairy products are useful in the diet for the reintegration of gut microbiota. Ass milk is very similar in composition to human one and has been utilized for feeding of children with severe allergy to cow milk proteins [3]. Identification of probiotic strains in such product allows to hypothesize their use to formulate ass yoghurt, which fermentation with natural occurring probiotic Lactic Acid Bacteria (LAB) strains could have noticeable effects on its sensorial and texture properties and could constitute the basis for a new line of health-functional dairy products. Our work was aimed to a finer characterization of some probiotic *Lactobacillus* spp previously isolated from raw ass milk [4]. The strains were characterized by biochemical and genotype identification. Biochemical identification was validated by strain-genotyping. DNA purification, DNA-DNA hybridization and RAPD-PCR fingerprint were performed as previously described [5]. Amplimers from RAPD-PCR and protein profile of the different strains were analysed by microchip-electrophoresis (2100 Bioanalyzer, Agilent). Protein profile was evaluated through LOC by using the Experion analyzer (BioRad). Three of the *Lactobacillu*s strains resulted related to the *L. plantarum* spp. The microchip electrophoresis carried on the whole LAB proteins supported the other biochemical and genetic data. LOC technology for food microbiology studies can result of immediate impact for the study of food microbial pathogen and beneficial communities.

References

1) Sanders, G.H.W. and Manz, A. (2000) Chip-based micro systems for genomic and proteomic analysis. TrAC Trends Anal. Chem. 19, 364–378.
2) Pace, N.R. (1997) A molecular view of microbial diversity and the biosphere. Science 276, 734–740.
3) Chiofalo, B., Salimei, E., Chiofalo, L.(2003): Acidi grassi del latte d'asina:proprietà bio-nutrizionali ed extranutrizionali. Large Animals Review, 5,1-6.
4) Nazzaro F, Anastasio M, Fratianni F, Orlando P. (2007). Isolamento di batteri lattici ad attivita' probiotica nel latte di asina. Atti del II Convegno Nazionale sul latte di asina, Roma, pp. 88-91.
5) Poli, A., Romano, I., Caliendo, G., Nicolaus, G., Orlando, p., Falco, A., Lama, L., Nicolaus, B. (2006) *Geobacillus toebii* subsp. *decanicus* subsp. *nov*., a hydrocarbon-degrading, heavy metal resistant bacterium from hot compost. J Gen Appl Microbiol. 52, 223-34.

REGULATORY NETWORK OF AcrAB MULTIDRUG EFFLUX PUMP IN *SALMONELLA* AND ITS ROLE IN RESPONSE TO METABOLITES

E. NIKAIDO[1,2], K. NISHINO[1,2,3], A. YAMAGUCHI[1,2]

[1]Department of Cell Membrane Biology, Institute of Scientific and Industrial Research, Osaka University, Japan.
[2]Graduate School of Pharmaceutical Sciences, Osaka University, Japan.
[3]PRESTO, Japan Science and Technology Corporation, Japan.
Mailing address: Institute of Scientific and Industrial Research, Osaka University, 8-1 Mihogaoka, Ibaraki, Osaka 567-0047, Japan. Phone: 81-6-6879-8546, fax: 81-6-6879-8549, e-mail: nikai37@sanken.osaka-u.ac.jp.

Multidrug efflux pumps cause one major form of bacterial multidrug resistance. *Salmonella enterica* has at least nine multidrug efflux pumps. Recently, multidrug efflux pumps appear to be induced by environmental signals. Investigation of the regulatory network and the nature of the signal that induce the expression of multidrug efflux pumps are needed in order to understand the biological significance of these pumps. Here we report that metabolites of *Escherichia coli* induce multidrug efflux pumps in *Salmonella*. Indole is one of a major metabolite produced by *E. coli*. We found that indole induced four efflux pumps in *Salmonella*: *acrAB*, *acrD*, *mdtABC* and *emrAB*. Because AcrAB is especially effective in generating resistance, we investigated the regulatory pathway of *acrAB*. We constructed the deletion mutants of known regulators of *acrAB* and revealed that indole induction of *acrAB* is dependent of the RamA regulator. RamA is specifically present in *Salmonella* and *Klebsiella*, not in *E. coli*. Quantitative real time PCR showed that indole induces the expression of *ramA*. Furthermore, we found that metabolites of *E. coli* induce the expression of *acrAB* in *Salmonella* and this induction is also dependent of the RamA regulator. These results indicate the possibility that multidrug resistance of *Salmonella* is induced by extracellular signals, such as metabolites, and RamA is responsible for this induction by regulating *acrAB* in *Salmonella*.

PHENOTYPIC CHARACTERIZATION OF THERMOPHILIC BACILLI ISOLATED FROM THE SITES WITH GEOTHERMAL AND GEO-CHEMICAL ANOMALIES OF ARMENIA

H. PANOSYAN

Yerevan State University, 0025, Yerevan, Armenia.

The areas natural geothermal and geochemical anomalies are widely distributed around the globe but they are. The information on microbial diversity of several habitats, such as the areas natural geothermal and geochemical anomalies mainly associated with tectonically active zones, where high temperatures are combined with other factors such as alkaline or acidic pH, high salinity, is one of the major tasks of environmental microbiology.

Distribution and diversity of thermophilic bacilli in sites with geothermal and geo-chemical anomalies on the territory of Armenia was studied. Several termophilic bacilli strains from the different sampled substrates (soil, water, silt) were isolated and identified up to species level. Enrichment and isolation of colonies was operated aerobically on a complex organic culture medium. Physiological studies were realized to describe the effect of temperature, pH and salinity on growth. Identification of strains was carried out based on morphological, physiological and biochemical traits. Based on phenotypic analyses dominate species were *Bacillus licheniformis, B. subtilis* and *B. stearothermophilus.*For detailed identification of some isolates the phenotypic analyses methods were combined with chemo- and genotypic analyses, including of fatty acids composition, as well as 16S rDNA sequence. They were mainly Gram-positive endospore-forming rods and analysis of 16S rDNA indicated they were closely related to *Bacillus licheniformis*. To obtain more information on microbial diversity in this environment we are planning to develop more phenotypic and molecular techniques. Using the combination of several approaches of traditional microbiology with state-of-the-art molecular biology techniques will successfully access to study structural and functional diversity of microbial community, than by using either method alone

THE METABOLIC FINGERPRINT OF FILAMENTOUS FUNGI RESPONSIBLE FOR DAMAGE AND BIODEGRADATION IN CULTURAL HERITAGE: APPLICATIONS AND PERSPECTIVES

F. PINZARI, V. CIALEI

Istituto Centrale per la Patologia del Libro - Laboratorio di Biologia. Via Milano, 76. 00184 Roma, Italy. Tel: +39-06-48291215, e-mail: flavia.pinzari@beniculturali.it.

Most of the fungal species that are commonly associated with biodeterioration phenomena in objects of cultural heritage stored in closed environments are present as spores in the air, both indoors and outdoors. When mould spores land on a damp surface, they can germinate, grow, and digest the material they have settled on, or alternatively they can survive in a dormant state. Biodegradation of materials by moulds can compromise their solidity as a result of the penetration of hyphae within the substrate, or through enzymatic action. Moulds can produce a broad range of enzymes (proteinases, gelatinase, cellulases) that are able to destroy the component materials found in collections of cultural heritage. Although a knowledge of the functional diversity and the metabolic characteristics of moulds is important in both the prevention and treatment of biodeterioration of cultural heritage, very few in-depth studies have been carried out on the subject until now. In this study Biolog FF microplates were used to obtain the metabolic fingerprint of filamentous fungi responsible for biodegradation in library materials. As part of a survey made in the historical library at Sant'Anselmo Benedictine monastery in Rome, several samples of moulds were collected from the bindings of volumes showing evidence of attack by fungi. Fungal strains were also isolated from the air using an impaction sampler (SAS), and from the walls, where colonies were clearly visible. The fungal strains were purified of bacterial contaminants, separated and identified. Biolog FF microplates were then inoculated with each strain according to Biolog's Manual protocol. The plates were read using a Biolog MicroLog microplate spectrophotometer at 490 and 750 nm. Raw data (optical densities) were transferred to an Excel (Microsoft™) sheet according to sample (fungal strain), replicate (3 replicates each), and reading time (10 reading points, one every 24 hours), although for each strain only the values at the relevant plateau of the colour development curve were chosen for statistical analysis. Following a background correction, average values for six categories of substrates were calculated (polymers, carbohydrates, carboxylic acids, amines and amides, amino acids, miscellaneous). Discriminant Analysis was employed to investigate the differences in the metabolic fingerprint among fungi according to two classifying categories: the sampled material (leather, fabric, air, plaster), and the sampled area of the library (upstairs, first floor, ancient books section). When using polymers, carbohydrates, carboxylic acids as variables in the analysis the fungal strains displayed significant classification clustering. The results showed that the fungal strains present in the air and on plastered surfaces were functionally similar to those that attacked the volumes with bindings made of fabric, while the fungal strains that developed on bindings made of leather consisted in a particular group of fungi possessing a different metabolic profile. A statistically significant separation of fungal strains was also obtained for the sampled areas, indicating that different zones of the conservation environment under examination were colonised by fungal communities with a different functional profile. If the fungal communities that develop where organic-based materials are stored (libraries, archives, etc.) are analysed based on their functional aspects in addition to their structural nature, they can be better evaluated for their potential harmfulness to materials. Further studies should be focused on the analysis of fungal phenetic characteristics which directly relate to important processes that take place in cultural heritage biodeterioration, and to the identification of a set of substrates that can better indicate the potential of fungal strains to decompose specific materials.

INFLUENCE OF *PSEUDOMONAS AERUGINOSA* ON BIOFILM FORMATION AND INTERNALIZATION OF *BURKHOLDERIA CENOCEPACIA* STRAINS

L. PIRONE[1,2], C. AURICHE[2], C. DALMASTRI[1], F. ASCENZIONI[2], A. BEVIVINO[1]

[1]Department of Biotechnologies, Agroindustry and Protection of Health, ENEA C.R. Casaccia, Roma, Italy.
[21]Department of Biologia Cellulare e dello Sviluppo, Università "La Sapienza", Roma, Italy.

Burkholderia cenocepacia is an important opportunistic pathogen in cystic fibrosis (CF) patients and has also been isolated from natural environments. Two *in vitro* virulence factors that seems linked to clinical virulence of *B. cenoecpacia* are biofilm formation and invasion of lung epithelial cells, both of which may contribute to its persistence in the CF lung. The purposes of the present study were (i) to compare the ability of different clinical and environmental *B. cenocepacia* strains to form biofilm and to invade respiratory epithelial cells (A549) *in vitro*, and (ii) to evaluate the influence of *P. aeruginosa*, the major pathogen in CF, on these virulence factors. Interestingly, clinical and environmental strains formed similar amounts of biofilm, although, on average, clinical isolates performed slightly better than environmental ones. From experiments performed by using dual co-cultures, i.e. *P. aeruginosa* PA01 and *B. cenocepacia* LMG16656, we found a significantly higher amounts of biofilm in both minimal and rich media compared to those observed in single culture. By using the A549 pulmonary epithelial cells, we found that clinical *B. cenocepacia* strains were significantly more invasive than environmental ones ($P<0.01$). In the co-infection assays, we found a higher level of invasion by *B. cenocepacia* in the presence of *P. aeruginosa* as compared to *B. cenocepacia* alone, while the internalization rate into human respiratory epithelial cells of *P. aeruginosa* was lower in the presence of *B. cenocepacia* as compared to *P. aeruginosa* alone. These results strongly indicate that the co-presence of the two bacteria at the moment of infection may have a profound effect on the expression of their virulence factors.

This work was supported by the Italian Cystic Fibrosis Research Foundation (grants FFC#11/2004 and FFC#7/2006) with the contribution of "Associazione Laziale e Associazione Lombarda per la lotta alla fibrosi cistica eDelegazione FFC di Latina".

TRANSCRIPTIONAL RESPONSE OF *DESULFOVIBRIO VULGARIS* HILDENBOROUGH TO OXYGENATION: AN INDIRECT METABOLIC ANALYSIS BY RESTRICTION FRAGMENT FUNCTIONAL DISPLAY (RFFD)

M. SANTANA, J. GONZÁLEZ

INRAS-CSIC, Av. Reina Mercedes, 10, 41012 Sevilla, Spain.

The complete genome sequence of *Desulfovibrio vulgaris* Hildenborough (Heidelberg *et al.*, 2004) revealed peculiar features in this anaerobic sulphate-reducing bacterium, a member of the delta subdivision of proteobacteria group. Sulfate-reducing bacteria (SRB) use sulfate as a terminal electron acceptor for the heterotrophic oxidation of organic compounds, or hydrogen, thereby producing sulfide. SRB are metabolically diverse however utilization of carbohydrates by sulfate-reducers has been rarely shown. To date, utilization of sugars by *D. vulgaris* Hildenborough has not been reported. In this context, the presence in *D. vulgaris* genome of putative PTS genes encoding proteins that should be involved in transport of sugars of the mannose class with subsequent metabolism through the Emdden-Meyerhof-Parnas (EMP) pathway was not expected. Also surprising, is the presence of a high-affinity *bd*-type terminal oxidase and an additional low affinity aa_3-type terminal cytochrome *c* oxidase, considering the anaerobic character of *D. vulgaris* Hildenborough. *D. vulgaris* cells consume oxygen at rates comparable to those of aerobic bacteria, although oxygen-dependent growth was never demonstrated. In *Escherichia coli*, it has been shown that mutational adaptation leading to improved PTS transport is dependent on the availability of molecular oxygen (Manche *et al.*, 1999). Therefore, it is relevant to hypothesize that *D. vulgaris* Hildenborough may be able to utilize mannose using oxygen as a terminal electron acceptor.

We propose a differential analysis of genome-wide gene expression by Restriction fragment functional display (RFFD) in order to infer the metabolic changes during the aerobic metabolism in this microorganism. RFDD is a modification of the traditional differential display technique that profits from the knowledge of total genome sequence, thus avoiding the need of additional reamplification and sequencing. Using this technique, we were able to detect band pattern differences corresponding to PTS genes and EMP enzymes in both wild-type and a *bd* mutant of *D. vulgaris* Hildenborough during aeration. Complementary physiological tests on carbon source used by *D. vulgaris* were also performed in order to evaluate the puzzling metabolism of this bacterium under aeration.

PHENOTYPIC MICROARRAY ANALYSIS OF *CRONOBACTER* SPP. (FORMERLY *ENTEROBACTER SAKAZAKII*)

B.D. TALL[1], S. FANNING[2], C. IVERSEN[2], N. MULLANE[2], M.H. KOTHARY[1], A. DATTA[1], L. CARTER[1], S.K. CURTIS[1], B.A. McCARDELL[1]

[1]Food and Drug Administration, Laurel, MD, USA.
[2]University College Dublin, Dublin, Ireland.

Cronobacter spp., formerly *Enterobacter sakazakii* (Es), are a major concern for infant formula manufacturers, health professionals and consumers because of its association with illness in infants fed contaminated, reconstituted powdered infant formula. Since 1980, 16 biogroups of Es have been described. Based on results from phylogenetic and DNA-DNA hybridization studies, Es has been reclassified into the genus *Cronobacter*, which contains five species and one genomospecies. The present study was undertaken to examine the metabolic diversity among 95 strains which were from different sources and are representative of the six *Cronobacter* species by using phenotypic microarray (PM) analysis. To inoculate metabolic panels PM1to PM20, cell suspensions were prepared from 24 h cultures grown on Trypticase soy agar at 37°C according to the manufacturer's instructions. Plates were incubated for 48 h at 37°C during which time the data was recorded using an Omnilog® instrument. The areas under the growth curves were analyzed for each strain and a value of greater than 15,000 tetrazolium dye reduction units (TDRU) was used to designate a positive result for each phenotypic test. The cut-off value was determined by comparing quantitative plate counts with TDRU over a 48 h incubation period using *Cronobacter* type strain, ATCC 29544. PM analysis of a subset of 42 known strains showed that the organisms could be separated into six groups on the basis of utilization of 89 different carbon (C) sources. *Cronobacter muytjensii,* utilizing 42 C sources, was observed to be the most metabolically active group, while *Cronobacter* genomospecies, utilizing only 19 C sources, was the least active group. PM analysis could also differentiate *Cronobacter* species from *Enterobacter cloacae*. Seven auxotrophs deficient in pyrimidine and purine, vitamin K3, Tween 80, L-citrulline, and chorismic acid utilization were observed among the *Cronobacter* species, of which five were clinical strains. Results also showed that only two of the 15 environmental strains, compared to 31 of the 38 clinical strains could utilize dextrin. Forty two of 43 known strains showed a high level of NaCl tolerance. However, 11 of 17 known clinical strains, compared to three of eight known environmental strains did not grow in media containing greater than 9% NaCl. All of the strains grew at pH levels 5.5-10, but none survived at or below pH levels of 4.0. Twenty-three strains were unable to grow at a pH level of 4.5, but amino acids, such as arginine, asparagine, glutamine, glycine, and ornithine rescued the growth of these pH-sensitive strains. A greater number of clinical strains were more acid-sensitive than environmental isolates. PM analysis also showed that all of the *Cronobacter* species were sensitive to Enoxacin, 2, 2`-Dipyridyl, Cefotaxime, and could not utilize D-Serine. Moreover, resistance to over 25 antimicrobial agents was also seen; most notable was the resistance to Ampicillin, Spectinomycin, Streptomycin, Nitrofurantoin, Cetoperazone, Cefsulodin, Vancomycin, and Cefazolin. In summary, these results demonstrate that *Cronobacter* species are phenotypically diverse, and thus can be differentiated from one another as well as from other *Enterobacter* species. Also, these results suggest that pathoadaptation in *Cronobacter* species is occurring as the organism moves from environmental into clinical niches and is associated with specific changes in phenotypes.

ECOLIHUB INFORMATION RESOURCE FOR EXPERIMENTATION AND MODELING

B.L. WANNER[1], S. ESS[1], K.A. DATSENKO[1], H. MORI[2]

[1]Purdue University, Biological Sciences, West Lafayette, IN 47907 USA.
[2]Nara Institute of Science and Technology (NAIST), Ikoma, Nara, JAPAN.

Efforts will be described on development of a biology-driven, integrated "one-stop-shopping" *E. coli* community information resource to permit full use of our existing knowledge and to enable new discoveries leading to deeper understanding of life processes. Moreover, since most cellular processes are universal, these tools and the advances they allow will have important impact on human health, through their application to pathogenic bacteria, especially enteropathogenic *E. coli*, *Shigella*, and *Salmonella* species.

The resource is being designed for seamless and transparent bi-directional connections among cooperating and interoperable *E. coli* informatics databases (EcoCyc, GenExpDB and Genobase), as well as with major biological database like ERIC, NCBI's Entrez Gene, UniProt, and KEGG.

EcoliHub has recently released on the web at www.ecolicommunity.org. Two main search engines are being developed for use by the *E. coli* community. EcoliWebsearch is a web-crawler engine that searches *E. coli* websites and websites with *E. coli*-specific information. Our major effort has been on development of the EcoliDatabase search engine, which is currently available only as a demo. EcoliDatabase search is being developed with a web services protocol that will allow seamless searching across multiple databases simultaneously. Registered EcoliHub users can also save their search results in personal (private) "workspaces" and retrieve them on subsequent visits to the site. EcoliHub is developing a community a wiki (www.EcoliWiki.org) for community annotation. Our current release also includes: EcoliPredict (a database of computational predictions of the *E. coli* genome), E. coli-specific BLAST, E. coli in the News, and an Events calendar of meetings/conferences. A community forum is available for discussion and feedback to EcoliHub and the user community, as well as current research topics and publications.

Supported by NIH U24 GM077905.

LONGITUDINAL STUDY OF *PSEUDOMONAS AERUGINOSA* ISOLATES FROM CYSTIC FIBROSIS LUNGS

L. YANG[1], S. K. HANSEN[1], L. JELSBAK[1], H. JARMER[2] , J.A.J. HAAGENSEN[1], H.K. JOHANSEN[3], N. HØIBY[3], S. MOLIN[1]

[1]Infection Microbiology Group, Biocentrum, Technical University of Denmark, Kgs. Lyngby, Denmark, email: lya@biocentrum.dtu.dk.
[2]Centre for Biological Sequence Analysis, Biocentrum, Technical University of Denmark, Kgs. Lyngby, Denmark.
[3]Department of International Health Immunobiology and Microbiology, Panum Institute, University of Copenhagen; Copenhagen CF Centre, Department of Clinical Microbiology, Rigshospitalet, Copenhagen, Denmark.

Cystic Fibrosis (CF) is one of the most common genetic diseases in Caucasians caused by a mutation in cystic fibrosis conductance regulator (CFTR) gene. Majority of CF patients suffer from chronic lung infection by the opportunistic bacterial pathogen *Pseudomonas aeruginosa.* It is an example of long-term persistent infections, which is normally impossible to eradicate despite intensive antibiotic treatment. In contrast to acute infections which damage the hosts by virulence factors, chronic infections involve considerable genetic adaptation of the pathogens during their coexistence with the host. However, little is known about the evolutionary strategies pathogens employ during their persistence. Here we focused on the changes in transcriptome during the adaptation of *P. aeruginosa* strains in CF patients using Affymetrix GeneChip microarrays. Phenotype chips (Biolog) and other assays were also used in parallel to confirm the transcriptome data.

We chose a *P. aeruginosa* early/late strain pair from a CF patient in the Danish CF center, Copenhagen. The early strain was isolated one year after the patient was diagnosed to be chronically infected. The late strain was isolated 14 years later. Genotyping showed that this patient had a stable infection by a non-mocoid clone during the 14 years. This particular clone is a transmissible and competitive clone that also persists in many other patients in Danish CF center. The two CF strains showed 2 to 3 folds reduction in growth rate compared to wild type PA01. The late isolate grows even slower than the early one. The patient has an ideal clonal pattern to study the evolution of *P. aeruginosa* population. The two CF isolates and PAO1 were grown in LB medium aerobically at 37°C. Samples for RNA extraction were harvested at middle exponential phase. All the experiments were repeated for three times.

Compared to PAO1, the early CF isolate has many genes associated with antimicrobial susceptibility and LPS modification which are up-regulated, while genes related to motility and quorum sensing are down-regulated. Some of these results have been confirmed by phenotype analysis. We also found that genes related to amino acid metabolism are expressed significantly different between late and early CF isolates. Genes related to leucine, valine and phenyalanine are up regulated in the late isolate, which means these three amino acids are preferably used in CF lung by *P. aeruginosa*. This suggests certain genetic modifications in metabolic pathways and growth physiology are important for persistence in the CF lung.

The results indicate that a successful *P. aeruginosa* persistent strain has to go through a two-step adaptation. The first step is the result of changes from the environment to a much more stressed condition. This allows the bacterium to hide from host immune attack and be more resistant to antibiotics. The second step of evolution is the optimization of metabolic pathways to best utilize the nutrition. This is a step which is normally ignored by researchers. Our model constitutes a new perspective on long term adaptation of *P. aeruginosa* populations in CF patients. It has important consequences for designing new therapies of CF and understanding chronic microbial infection in general.

PHENOTYPES OF *SALMONELLA ENTERICA* SEROVAR TYPHI AND *ACINETOBACTER* SPP.: REFLECTIONS OF DIFFERENT LIFESTYLES?

L. DIJKSHOORN, J.T. VAN DISSEL

Dept. of Infectious Diseases, Leiden Univ. Med. Ctr., P.O. Box 9600, 2300RC, Leiden, Netherlands

Salmonella enterica serovar Typhi (*S. typhi*) is an obligatory pathogen with humans as its unique host. It survives and multiplies in the host inside macrophages but is able to survive in polluted environmental surface waters as well. *Acinetobacter* species include environmental and opportunistic human pathogens. Only few species including *Acinetobacter baumannii* are associated with hospital infection and epidemic spread among severely ill patients. These organisms are usually multidrug resistant.
The aim of our study was to compare a set of *S. typhi* and of *Acinetobacter* spp. strains for their metabolic capacity and stress resistance potential. Five strains of *S. typhi* and three *Acinetobacter* spp. strains were profiled for nearly 2000 characters using Phenotype MicroArray^{TM} (PM) analysis. Four *S. typhi* strains (clinical isolates from typhoid patients) were from Indonesia and belonged to different genotypes as determined by pulsed field gel electrophoresis. One *S. typhi* strain (LUH 9529) was originally isolated in Ghana and was used as reference . The *Acinetobacter* strains included one *A. baumannii* strain (RUH 875, test strain), one genomic species (gen. sp.) 10 strain (RUH 2222) and one gen. sp. 11 strain (RUH 2234).
Methods. The phenotypic comparison was performed by Biolog Inc. Hayward, CA, USA.
Results and conclusion. Phenotypic differences between the *S. typhi* test strains when compared with the reference strain were limited. For example, only one or no C-source differences and a few or no N-source differences were observed when the respective test strains were compared with the reference strain. No differences or a poor signal was observed for nutrient stimulation. Differences were greatest for sulphur and phosphate sources. The reference strain had a high signal at high pH. All *S. typhi* strains were sensitive to folate antagonists, chloramphenicol, and thiamphenicol. As compared to the *Acinetobacter* strains, the number of positive tests for metabolic and stress response activity of the *S. typhi* strains was low. The *Acinetobacter* strains showed a strikingly high number of positive metabolic and stress response tests. The differences between RUH 875 an epidemic *A. baumannii* strain and RUH2222 of gen sp 10 (of which the clinical importance does not seem great), were limited. RUH2234 belonging to gen. sp. 11 which is closely related to gen. sp. 10 showed a lower metabolic potential than RUH2222, in particular regarding the stimulation by nutrients (plate PM5). The noted differences between *S. typhi* and *Acinetobacter* strains might be explained by their different lifestyles as *S. typhi* is highly adapted to the human host, with habitats limited to their intracellular niche, faeces and limited environmental circumstances, while acinetobacters as opportunistic pathogens, have to survive in many different environments and conditions and cope with a multitude of variations in substrate availability.

Author index

Participant list

Manal Abuoun
Veterinary Laboartories Agency
Surrey - United Kingdom
m.abuoun@vla.defra.gsi.gov.uk

William Agbakwuru Chinedu
Imo State University
Owerri - Nigeria
acwilliam2002@yahoo.com

Sarmad Mohammed Ali Abed
Ministry of Agricolture
Iraq
agro_ministry@yahoo.com

Ahmed Hussein Alwan
Ministry of Health
Baghdad - Iraq
heaith.ministry@yahoo.com

Obi Samuel Amala
Nnamdi Azikiwe University
Awka - Nigeria
obisamuelamala@yahoo.com

Franck Andrieux
AES-Chemunex

Muna Anjum
Veterinaru Laboratiories Agency
Surrey - United Kingdom
m.anjum@vla.defra.gsi.gov.uk

Matthew Anochirionye
Imo State University
Owerri - Nigeria
mathewcalisto@yahoo.com

Hanane Aouati
Mentouri University of Constantine
Algeria
han_banda@yahoo.fr

Benedict Arku
TEAGASC
Fermoy - Ireland
Benedict.Arku@teagasc.ie

Alan Bakalinski
Food Scinece and Technology
Corvallis - USA
alan.bakalinski@oregonstate.edu

Marco Bazzicalupo
University of Firenze
Italy
marcobazzi@dbag.unifi.it

Lucia Bianchi
University of Firenze
Italy
lucia.bianchi@unifi.it

Emanuele Biondi
University of Firenze
Italy
emanuele.biondi@unifi.it

John Bisset
Agriculture and Agri-Food Canada
Ottawa- Canada
bissettj@agr.gc.ca

Barry Bochner
CEO & CSO, Biolog, Inc.
USA
bbochner@biolog.com

Alexander Boehm
Basel University
Switzerland
alexander.boehm@unibas.ch

Sara Borin
University of Milano
Italy
sara.borin@unimi.it

Francesca Borsetti
University of Bologna
Italy
francesca.borsetti@emmebox.net

Margarida Maria Cabral Santana
Dionisio Azevedo Santana and Belmira Cabral Santana
Spain
msantana@netc.pt

Alessandro Camussi
University of Firenze
Italy
alessandro.camussi@unifi.it

Francesco Canganella
University of Tuscia, Viterbo
Italy
canganella@unitus.it

Martina Cappelletti
University of Bologna
Italy
marty.cap@gmail.com

Enrico Casalone
University of Firenze
Italy
enrico.casalone@unifi.it

Salvatore Casano
University of Palermo
Italy
ecobise@gmail.com

Duccio Cavalieri
University of Firenze
Italy
duccio.cavalieri@unifi.it

Thomas A. Cebula
Food and Drug Administration
Laurel - USA
thomas.cebula@fda.hhs.gov

Giulia Cheloni
University of Firenze
Italy
giulia-cheloni@hotmail.it

Cheng Chi
China Center of Industrial Culture Collection
Beijing - China
Chengc100027@163.net

Ofora Lawrence Chidozie
Nnamdi Azikiwe University
Awka - Nigeria
oforachidozie@yahoo.com

Amadi Chima
China Center of Industrial Culture Collection
Beijing - China
am2chima@yahoo.com
Adibe Chukwuemeka Emmanuel
Nnamdi Azikiwe University
Awka - Nigeria
chukwuemekaadibe@yahoo.com

Elise Clerc
AES-Chemunex

Abu Conteh
Sierra Leone Farmers
Sierra Leone
gibrill2004@yahoo.com

Tyrrel Conway
University of Oklaoma
Norman - USA
tconway@ou.edu

Daniele Daffonchio
University of Milano
Italy
daniele.daffonchio@unimi.it

Atin R. Datta
US Food and Drug Administration
Laurel - USA
atin.datta@fda.hhs.gov

Francesca Decorosi
University of Firenze
Italy
francesca.decorosi@unifi.it

Francesca Degola
University of Parma
Italy
francesca.degola@libero.it

Gianni Deho'
University of Milano
Italy
gianni.deho@unimi.it

Lenie Dijkshoorn
Leiden University Medical Centre
Leiden - The Netherlands
l.diykshoorn@lumc.nl

Nicolas Dorange
AES-Chemunex

Irina Druzhinina
Vienna University of Technology
Austria
druzhini@mail.zserv.tuwien.ac.at

Ekenedo Henry Ebere
Nnamdi Azikiwe University
Awka - Nigeria
ekenedohenry@yahoo.com

Emmanuel Eberendu
Imo State University
Owerri - Nigeria
Emma22ndu@yahoo.com

Rachel L. Edwards
West Medical Center Drive
Ann Arbor - USA
rlschwar@umich.edu

Renato Fani
University of Firenze
Italy
r_fani@dbag.unifi.it

Stefano Fedi
University of Bologna
Italy
stefano.fedi@unibo.it

Luciana Giovannetti
University of Firenze
Italy
luciana.giovannetti@unifi.it

Giancarlo Grassi
C.R.A.- CIN Rovigo
Italy
g.grassi@isci.it

Jean Guard-Bouldin
US Department of Agriculture
Athens - USA
jean.guard.bouldin@ARS.USDA.GOV

Simona Guerrini
University of Firenze
Italy
simona.guerrini@unifi.it

Yong Han Tek
University Putra of Malaysia
Malaysia
abbieyong84@gmail.com

Raino Kristian Hansen
University of Copenhagen & Bridge
Bioresearch Int. Ltd
Copenhagen - Denmark
info@raino.dk

Terry Hazen
Lawrence Berkely National Laboratory
USA
TCHazen@lbl.gov

Carol Iversen
University College Dublin
Ireland
carol.iversen@ucd.ie

Mantie Kalokoh
Sierra Leone Farmers
Sierra Leone
gibrill2004@yahoo.com

Petras Kavaliauskas
INTERLUX CO LTD
Vilnius - Lithuania
sandra@interlux.lt

Keith Lampel
U.S. Food and Drug Administration
College Park - USA
keith.lampel@fda.hhs.gov

Haider Sabah Lateef
Ministry of Agriculture
iraq
agro_ministry@yahoo.com

Aleksandra Magic-Knezev
HetWaterLaboratorium, NL
Haarlem - The Netherlands
aleksandra.magic@hetwaterlaboratorium.nl

Isabel Maida
University of Firenze
Italy

Iana Malkazova
G. Eliava Institute of Bacteriophage
Tbilisi -USA
ianamalkh@mail.ru

Marina Mameli
INAIL-Firenze
Italy
m.mameli@inail.it

Santina Mangano
University of Messina
Italy
carusoc@unime.it

Valentina Masciale
University of Firenze
Italy

Alessio Mengoni
University of Firenze
Italy
alessio.mengoni@unifi.it

Anna Messini
University of Firenze
Italy
anna.messini@unifi.it

Michaela Michaylova
LB Bulgaricum PLC, R&D Dpt. Heas of Probiotics
Bulgaria
michalylova.m@lbbulgaricum.bg

Stefano Mocali
C.R.A.
Roma - Italy
stefano.mocali@entecra.it

Hirotada Mori
Nara Institute of Science and Tecnology
Japan
hmori@ttck.keio.ac.jp

Tomoyuki Nakano
University of Osaka
Japan
nakano37@sanken.osaka-u.ac.jp

Filomena Nazzaro
Istituto di Scienze dell'Alimentazione - CNR
Italy
mena@isa.cnr.it

Eiji Nikaido
University of Osaka
Japan
nikai37@sanken.osaka-u.ac.jp

Kunihiko Nishino
University of Osaka
Japan
nishino@sanken.osaka-u.ac.jp

Onwosi Chukwidi Ogbonnaya
Nnamdi Azikiwe University
Awka - Nigeria
onwosicoach@yahoo.com

Anders Omsland
National Institute of Health
Hamilton - USA
omslanda@niaid.nih.gov

Hovik Panosyan
Yerevan State Univesrity
Armenia
hpanosyan@yahoo.ca

Maria Cristiana Papaleo
University of Firenze
Italy
cristiana.papaleo@unifi.it

Ian Paulsen
Macquarie University
Sydney - Australia
ipaulsen@cbms.mq.edu.au

Peter Penn
Biolog Europe
ppenn@biolog.com

Brunella Perito
University of Firenze
Italy
brunella.perito@unifi.it

Flavia Pinzari
Istituto Centrale di Patologia del libro
Milano - Italy
ic-pl.biologia@beniculturali.it

Luisa Pirone
University La Sapienza- Rome
Italy
luisapirone@hotmail.com

Mario Polsinelli
University of Firenze
Italy
mario.polsinelli@unifi.it

Charles Rio
AES-Chemunex

Hydn Rubelmann
University of South Florida
USA
rubelman@mail.usf.edu

Anwar Sleem Salh
Ministry of Health
Baghdad - Iraq
heaith.ministry@yahoo.com

Alexander Shearer
SRI international Menlo Park
Menlo Park - USA
shearer@ai.sri.com

Jana Sioufi
AES-Chemunex

Yao Su
China Center of Industrial Culture Collection
Beijing - China
milly@china-cicc.org

Giovanni Taccetti
Meyer Hospital
Firenze - Italy
g.taccetti@meyer.it

Lucilla Taddei
University of Firenze
Italy

Barbara Tarabella
University of Firenze
Italy

Enrico Tatti
University of Firenze
Italy
enrico.tatti@unifi.it

Bhagwati Upadhyay
Veterinary Laboartories Agency
Surrey - United Kingdom
b.upadhyay@vla.defra.gsi.gov.uk

Coen van der Weijden
DSM Food Specialties
Delft- The Netherlands
Coen.Weijden-van-der@dsm.com

Patrick Venail
University of Montpellier
France
pvenail@univ-montp2.fr

Carlo Viti
University of Firenze
Italy
carlo.viti@unifi.it

Astra Vitkauskiene
Kaunas Univeristy of Medicine Hospital
Kaunas - Lithuania
sandra@interlux.lt

Barry L. Wanner
Purdue University
West Lafayette - USA
blwanner@purdue.edu

Paul Wheeler
Veterinary Laboartories Agency
Surrey - United Kingdom
p.wheeler@vla.defra.gsi.gov.uk

Laurence Yang
University of Toronto
Canada
Laurence.yang@gmail.com

Lei Yang
Technical Univesity of Denmark
Kgs. Lyngby - Denmark
lya@biocentrum.dtu.dk

Severino Zara
University of Sassari
Italy
szara@uniss.it

Fabio Zenna
AES-Chemunex
Italy
f.zenna@aesit.191.it