

Conference Report

FLORENCE CONFERENCE ON PHENOTYPE MICROARRAY ANALYSIS OF MICROORGANISMS – THE ENVIRONMENT, AGRICULTURE, AND HUMAN HEALTH 19-21 March 2008, Florence, Italy

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Approaches used to describe biological differences between cells typically lack speed, simplicity, and sensitivity and have progressed very little. On the other hand, cellular biochemical analyses such as DNA Microarrays and proteomics technologies have advanced rapidly. In the last decade they have enabled an explosion of studies aimed at identifying novel messenger RNA transcripts, or patterns of transcripts, or protein expression in association with a particular physiological state of cells. However a limitation of biochemical approaches is that there is no assurance that observed changes are really significant to the cell. Only an integrated approach of genomics and proteomics technologies, and phenotypic characterization can provide, in a more comprehensive manner, a cell-wide perspective.

Phenotypes are the last major area of analysis to become amenable to efficient overall analysis. Cellular phenotypes have traditionally been analyzed one at a time and, because phenotypes are often qualitative and vaguely defined, it has not been obvious how one could devise an efficient method with adequate scope and sensitivity for global analysis. Since 2001 a high-throughput technology has been available that permits testing of thousands of phenotypes at once. This technology, sold by Biolog, Inc., is called a Phenotype MicroArray (PM) and, following on to proteomics and genomics technologies, it can be categorized as "phenomics".

The primary aim of the "Florence Conference on Phenotype MicroArray Analysis of Microorganisms-The Environment, Agriculture, and Human Health" held at University of Florence, Italy, over 3 days, from Wednesday, March 19, to Friday, March 21 was to provide a forum for discussing the state of the art concerning applications and data analyses of PM, as well as future perspectives.

The following people served as members of the

Organizing and Scientific Committee for the Florence Conference: Carlo Viti, Luciana Giovannetti and Alessandro Camussi, from the Department of Biotecnologie Agrarie, University of Florence; Barry R. Bochner, from CEO & CSO, Biolog, Inc.; Fabio Zenna, from AES-Chemunex. The event had the patronage of the Italian societies of microbiology (Società Italiana di Microbiologia, Società Italiana di Microbiologia Agro-Alimentare e Ambientale, Società Italiana di Microbiologia Generale e Biotecnologie Microbiche). With the generous support from the Ente Cassa di Risparmio di Firenze, the Scientific Committee sponsored the attendance of 15 students and young researchers.

The conference offered a first, unique opportunity to bring together, microbiologists, medical doctors, bioinformaticists, geneticists, microbial ecologists and biotechnologists in order to present their works and exchange their ideas with an audience knowledgeable in the use of PM technology. The Conference attracted more than 110 researchers, postdoctoral associates, students and others from 23 countries. During the Conference over 60 scientists presented their research in lectures, oral presentations and posters.

The Conference opened with welcoming speeches by Carlo Viti, Chairman of the Organizing and Scientific Committee, Guido Chelazzi, Vice-Rector of the University of Florence, and Luciana Giovannetti, Director of the Dipartimento di Biotecnologie Agrarie, University of Florence. Then there was the opening lecture from Thomas Cebula (Laurel, US FDA, USA), who gave an outstanding presentation expounding that the "-omics" era of research has brought new technologies to bear in identifying and tracking the causative agents in food borne outbreaks. Cebula demonstrated the use of the PM system, exploiting novel phenotypes within *Salmonella* and *Escherichia coli*, which has led to specific assay

development that enhances stains attribution.

On the next day there were three sections: i) *Principles, practice and data analysis*, ii) *Phenotypic analysis of eukaryotes*, iii) *Phenotypic analysis of human and animal pathogens*.

In the section *Principles, practice and data analysis*, co-chaired by Alessandro Camussi (University of Florence, Italy) and Francesco Canganella (University of Tuscia, Viterbo, Italy), there were four lectures. Barry Bochner (Biolog Inc., Hayward, USA) provided an overview on how PM technology works and how it can be used in both basic and applied research. Laurence Yang (University of Toronto, Canada) showed two examples of how large scale growth profiling data can be used to inform genome scale models. In the first, he illustrated the use of PM data to obtain a highly accurate and expanded metabolic model of *Bacillus subtilis*. In the second example, PM profiling of data from growth competition experiments between strains of different genetics backgrounds was used to refine metabolic models of *Escherichia coli*. The talk of Hirota Mori (Nara Institute of Science and Technology and Kieo University, Japan) was devoted to the application of PM technology to elucidate metabolic network of *E. coli*, through the analysis of single gene deletion mutants of 107 genes related to glycolysis, the TCA cycle and pentose phosphate pathway. The section was closed by Ian Paulsen (Macquarie University, Australia), who, in working on the function of transporter genes of *Pseudomonas aeruginosa*, emphasized that rapid phenotypic assays based on the PM are an invaluable tool for confirming and extending bioinformatic predictions.

The second section was chaired by Duccio Cavalieri (University of Florence, Italy) and focused on the *Phenotypic analysis of eukaryotes*. The lecture of Enrico Casalone (University of Florence, Italy) noted that the characterization of *Saccharomyces cerevisiae* mutants in the *Leu4* gene with PM technology permitted elucidation of new possible metabolic connections of the leucine biosynthetic pathway. The contribution of Irina Druzhinina emphasized experimental prerequisites in using PM technology to study carbon source utilization and growth physiology of industrially important fungi such as *Trichoderma* and *Fusarium*. In the last lecture, Barry Bochner showed that PM technology has now been extended to work for mammalian cells from different animals and from different organs and tissues. This section ended with an oral presentation, selected by the Scientific Committee, on characterization of a porcine cell line by PM technology (Manal Abuoun, VLA, Addlestone, United Kingdom). This work is a precursor to studies of attachment and invasion by *Salmonella enterica*.

The last section of the 20th had six lectures and

two oral presentations. The section, co-chaired by Giovanni Taccetti (Mayer Hospital, Florence, Italy) and Renato Fani (University of Florence, Italy), was devoted to *Phenotypic analysis of human and animal pathogens*. The main challenge is to gain greater insight in to the behavior of pathogenic organisms: the possibility to identify differences in virulence properties and antibiotic resistance properties of pathogenic bacteria, as well as the capability to investigate among and between virulent and non-virulent isolates from environmental or clinical sources, and correlate phenotypic traits to genetics markers. Anders Omsland (National Institutes of Health, Hamilton, USA) reported that PM technology is a useful tool for the study of metabolic activity of *Coxiella burnetii*, the agent of human Q fever, a pathogen whose replication is strictly limited to colonization of a viable eukaryotic host cell. The talk given by Muna Anjum dealt with the use of PM data to validate an *in silico* genome-scale metabolic network for the sequenced *Salmonella typhimurium* LT2 strain. Anjum concluded her talk setting an overall goal to validate the model to characterize serovar, phage, and type variability, in order to understand how pathogenic strains differ fundamentally and adapt to different niches. In *Salmonella enterica* subsp I 1400 serovars are known, but only about 20 of these pose a threat to the public health. Until now it has been difficult to identify genetic markers associated with the pandemic potential of *Salmonella enterica* subsp I. Jean Guard-Bouldin (US Department of Agriculture, Athens, USA) coupling PM approaches with high-throughput genomics, identified unexpected genetic markers that could be linked to panoramic physiological profiles of *Salmonella enterica*. In the 1990s the prevalence of multidrug-resistance *Salmonella* has increased dramatically and genomic analysis has resulted in the identification of many genes that code for multidrug pumps, but only a small number of putative genes have been confirmed as actually involved in drug resistance. Kunihiro Nishino (Osaka University, Osaka, Japan) showed by PM analysis that certain classes of efflux pump not only confer resistance to drugs but also have a role in pathogenicity. *Enterobacter sakazakii*, reclassified into the new genus *Cronobacter*, is an infant opportunistic pathogen that includes at least 6 genomospecies. With conventional phenotypic tests only 4 of these groups could be easily differentiated. All *Cronobacter* genomospecies were distinguished using PM phenotypic testing (Carol Iversen, University College Dublin, Ireland). Elucidation of the molecular factors underlying bacterial biofilm formation may aid the treatment of chronic infections because cells in a biofilm can resist antibiotic chemotherapies. Alexander Böhm (University of Basel, Switzerland) has discovered new input signals that contribute to biofilm forma-

tion. This was accomplished by creatively using an *E. coli* biofilm model with PM technology to identify external compounds that lead to biofilm induction by triggering intracellular signals.

The section *Phenotypic analysis of human and animal pathogens* ended with two oral presentations entitled: "Comparative phenotypic Microarray analysis of *Listeria monocytogenes* strains involved in invasive and gastroenteritis listeriosis outbreaks" (Atin R. Datta, Food and Drug Administration, USA), and "Carbon and energy metabolism of *Escherichia coli* in the intestine" (Tyrrell Conway, University of Oklahoma, USA)

The last section of the Conference (21st March), *Phenotypic analysis of environmental microorganisms*, co-chaired by Daniele Daffonchio (University of Milano, Italy) and Marco Bazzicalupo (University of Florence, Italy), began with the lecture of Stefano Fedi (University of Bologna, Italy) on the use of PM to evaluate alterations of carbon and nitrogen metabolism in a *cheA* chemotactic mutant strain when compared to the *Pseudomonas pseudoalcaligenes* KF707 wild type strain, which is a polychlorinated biphenyls-degrading strain. Enrico Tatti (University of Florence, Italy) using a combination of genotypic and PM analyses studied mechanisms underlying chromate resistance in a Cr(VI)-hyper-resistant *Pseudomonas corrugata* strain. Data obtained suggested that chromate resistance in this strain is a complex system that depends on the sulphur starvation response, on the supply of NADPH required in repairing damage induced by chromate, and on the overall maintenance of DNA integrity. Emanuele Biondi (University of Florence, Italy) analyzed the phenotypic diversity of 5 different strains of *Sinorhizobium meliloti* previously characterized by Comparative Genomic Hybridization, and proposed possible future applications of PM results for the development of a selective medium for isolation of *S. meliloti* from environments and the design of a customized phenotypic assay for analysis of *S. meliloti* diversity. The last lecture in this series, presented by Terry Hazen (Lawrence Berkeley National Laboratory, USA), described the development of protocols to use PM technology under anaerobic conditions for characterization of the phenotypes of several anaerobic bacteria and mutant strains. This provides for rapid screening of mutant phenotypic changes and for rapid pathway analyses and modeling.

The following oral presentations were selected for this section: Patrick Venail (University of Montpellier, France) "Functional diversity and productivity peak at intermediate dispersal rate in evolving bacterial metacommunities", Alexander Shearer (SRI International, Menlo Park, CA, USA) "Metabolic network inference using Pathway Tools and MetaCyc", Bhagwati Upadhyay (Veterinary Laboratories Weybridge, Addlestone, United

Kingdom) "Adapting Biolog Phenotype MicroArray technology to reveal the metabolomics of *Mycobacterium tuberculosis* and *Mycobacterium bovis*, slow-growing pathogens with $d=0.03$ to $0.05/h$ ", and Rachel Edwards (University of Michigan, Ann Arbor, USA) "Novel cues of *Legionella pneumophila* differentiation uncovered by Phenotype MicroArrays".

The conference ended with a very valuable round table on bioinformatics approaches and tools for analyzing PM data and then a demonstration of the GeneExpress laboratory at the Dipartimento di Biotecnologie Agrarie (University of Florence).

Luciana Giovannetti, during her closing remarks, after having thanked everyone for their participation to the Florence Conference, expressed an enthusiastic appreciation for the high scientific level of all contributions and for the important role of all participants in lively and productive discussions. Without doubt, the Conference was informative, stimulating and, from the excellent experience of scientists from around the world, very effective in assessing current applications, data analysis strategies, and future developments of PM technology. Finally, she pointed out that, because the Florence Conference had proven to be both an important forum for information exchange on the potentials of PM and also had encouraged many new interactions and possible collaborations, there was a need to meet again and schedule a second conference on "Phenotype MicroArray Analysis of Microorganisms – The Environment, Agriculture, and Human Health" in 2010.

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