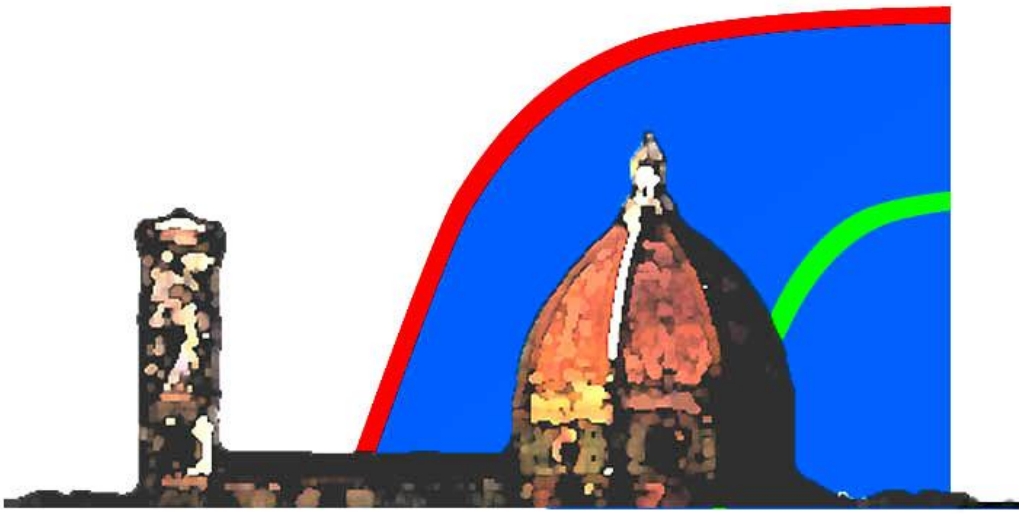
2nd Florence Conference on Phenotype MicroArray Analysis of Microorganisms

The Environment, Agriculture, and Human Health



Firenze-Italy, September 13-15, 2010

Programme and Abstracts

Università degli Studi di Firenze

2nd Florence Conference on Phenotype MicroArray Analysis of Microorganisms

The Environment, Agriculture, and Human Health

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Programme and Abstracts

Edited by: F. Decorosi, L. Santopolo, E. Tatti

Contents

Welcome to the 2nd Florence Conference on Phenotype MicroArray Analysis of Microorganisms: the Environment, Agriculture, and Human Health

On behalf of the scientific and organizing committee, we would like to thank everyone who have joined us today, for coming to this conference.

We are very pleased that the 2nd "Florence Conference on Phenotype MicroArray Analysis of Microorganisms: the Environment, Agriculture, and Human Health" has attracted more speakers and participants than the first, indicating the growing interest in this exciting field.

The key topic of the conference is on the use of phenotypic analysis and phenotypic data to gain deeper understanding of the biology of microorganisms. Within the conference there are sessions focused on topics such as environmental microorganisms, and pathogenic microorganisms that are transmitted to humans via the environment, animals, and food. One session includes communications on traditional uses of Biolog plates (ecoplates, GNs, etc.).

We are grateful to the University of Florence, for hosting also the second edition of conference and we would also like to take the opportunity to thank all of the Partners and Sponsors of the conference.

I believe that we have assembled a very interesting mix of international contributors and hope that you enjoy the conference.

Carlo Viti
Chair of the conference

Luciana Giovannetti
Organizing committee

Organizing and Scientific Committee

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Luciana Giovannetti, Department of Agricultural Biotechnology, University of Florence, Italy
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Gail M. Preston, Department of Plant Sciences, University of Oxford, United Kingdom
Joana Falcão Salles, Microbial Ecology, Centre for Ecological and Evolutionary Studies, University of Groningen, the Netherlands
Carlo Viti, Department of Agricultural Biotechnology, University of Florence, Italy

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VACCINES


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Programme

Monday, 13th
Afternoon

15.00 16.45	***Registration***	
16.45-17.00	The Authorities **Carlo Viti** and **Luciana Giovannetti**	Welcome to University of Florence Opening of the Second Florence Conference on Phenotype MicroArray
	Opening lecture	
17.00-18.00	**Roberto Marcello La Ragione** Veterinary Laboratories Agency Surrey, UK	Exploring the phenome of human and animal enteric pathogens
18.00-18.30	**Terry C. Hazen** LBNL, Berkeley, CA, USA	Deep water horizon oil spill - intrinsic bioremediation or Mother Natures's abilities to cleanup our messes
18.30-19.30	***Welcome cocktail***	

Tuesday, 14th
Morning

8.00-8.30 ***Registration***

Section: *Principles, practice and data analysis*
Chair: Terry Hazen

	Lectures	
8.30-8.55	**Barry Bochner** CEO & CSO, Biolog, Inc., USA	Phenotype MicroArray technology: history and recent advances
8.55-9.20	**Marcin Joachimiak** University of California, USA	Associative biclustering of functional genomics data
9.20-9.45	**Gail M. Preston** University of Oxford, United Kingdom	Integrating PM technology with *in situ* and *in silico* analyses of microbial activities
9.45-10.00	**Wenling E. Chang** The MITRE Corporation, USA	PheMaDB: a web-based system for storage, retrieval, visualization, and analysis of OmniLog phenotypic microarray data
10.00-10.15	**Lars E. Barquist** Wellcome Trust Sanger Institute, United Kingdom	Experimental design and analysis of Phenotype Microarray dataset
10.15- 10.30	**Lay Ching Chai** University Putra Malaysia, Malaysia	Quantitative antimicrobial susceptibility assay of extracts and natural products: a potential application of Omnilog for high-throughput screening

10.30- 11.30 Coffee break and poster section

Section: *Genomic analysis combined with PM analysis*
Chair: Gail M. Preston

	Lectures	
11.30-11.55	**Akira Ishihama** University of Hosei, Japan	Prokaryotic genome regulation: multifactor promoters and multitarget regulators
11.55- 12.20	**Duccio Cavalieri** University of Florence, Italy	Yeast as a model in nutritional systems biology: a whole genome parallel phenotyping approach
12.20- 12.45	**Renato Fani** University of Florence, Italy	Genomic, transcriptomic, and phenomic analysis of *Burkholderia cepaci*a mutants impaired in HAE efflux pumps

12.45-14.45 Lunch

Tuesday, 14th
Afternoon

Section: *Interaction between microbial metabolism and toxic chemicals*
Chair: Gail M. Preston

Lectures

14.45-15.10	**Jose L. Martinez** Centro Nacional de Biotecnología, Madrid, Spain	Metabolic modulation of *Pseudomonas aeruginosa* virulence and antibiotic resistance
15.10-15.35	**Larry Gallagher** University of Washington, USA	Phenotypic and genomic analysis of antibiotic resistance in *Pseudomonas aeruginosa*
15.35-16.00	**Roselyne Ferrari** University of Paris-Diderot, France	Toxicity of free zinc ions *vs* zinc oxide nanoparticles on *E. coli* MG 1655 grown in Luria Bertani or in Seine river water
16.00-16.25	**Francesca Decorosi** University of Florence, Italy	Chromate resistance in *Pseudomonas corrugata* strain 28

16.25- 17.00 Coffee break

Section: *Phenotypic analysis of human and animal pathogens*
Chair: Marco Oggioni

Lectures

17.00-17.25	**Alessandro Bidossi** University of Siena, Italy	A genomic view on carbohydrate uptake in *Streptococcus pneumonia*e
17.25-17.50	**Kari Belin** Food and Drug Administration, USA	Utilizing the Biolog PM assay to identify phenotypic changes in *Shigella flexneri* post-eukaryotic cell invasion
17.50-18.15	**Jean Guard** U.S. Department of Agriculture, Agricultural Research Service, USA	Linking phenotype to genotype of epidemiologically prevalent *Salmonella* by comparative microarray analysis
18.15-18.40	**Soeren Molin** Technical University of Denmark, Denmark	Metabolic profiling of adapted strains of *Pseudomonas aeruginosa* isolated from chronically infected airways of cystic fibrosis patients
18.40-18.55	**Gemma Langridge** Wellcome Trust Sanger Institute, United Kingdom	Host restricted *Salmonella*: linking pseudogenes and metabolic ability

18.55-19.45 **Poster vision**

19.45 ***Departure for the historic centre of the town***
20.30 ***Conference Dinner***

Wednesday, 15th
Morning

Section: *Environmental and food microorganisms I*
Chair: Joana Falcão Salles

Lectures

8.30-8.55	**Ines Mulec Mandic** University of Ljubljana, Slovenia	Genetic and metabolic diversity of *Bacillus subtilis* at microscale
8.55-9.20	**Terry C. Hazen** LBNL, Berkeley, CA, USA	Isolation of aerobic lignin- and cellulose- degrading bacteria from tropical soils from biofuel feedstock deconstruction
9.20-9.45	**Jason Holder** Massachusetts Institute of Technology, USA	Metabolic model refinement via phenomic analysis of *Actinomycetales* catabolism
9.45-10.10	**Stefano Mocali** CRA Rome, Italy	Phenotype MicroArray supporting comparative genomic analysis in *Sinorhizobium meliloti*

10.10-11.00 Coffee break and poster vision

11.00-11.25	**Jesse Dillon** California State University, USA	The use of Phenotype Microarrays to characterize substrate usage by halophiles isolated from solar salterns
11.25-11.50	**Diego Mora** University of Milan, Italy	Phenotype MicroArray as a tool to study the physiology of dairy bacteria
11.50-12.15	**Marco Gobbetti** University of Bari, Italy	Biolog System: potential application within food related lactic acid bacteria
12.15-12.40	**Otakuye Conroy** University of Utah, USA	Using PM chemical sensitivity assays to determine new estrogenic and androgenic endocrine disrupting chemicals

12.40-14.20 Lunch

Wednesday, 15th
Afternoon

Section: *Environmental and food microorganisms II*
Chair: **Marco Bazzicalupo**

	Lectures	
14.20-14.45	**Joana Falcão Salles** University of Groningen, the Netherlands	Ecological significance of resource utilization in microbial ecology
14.45-15.10	**Raymond L. Legge** University of Waterloo, Canada	Some applications of a one-dimensional metric for tracking bacterial community divergence using sole carbon source utilization patterns
15.10-15.35	**Roberto Ambrosoli** University of Turin, Italy	Possible use of Biolog methodology for monitoring yeast presence in alcoholic fermentation for wine-making
	Closing lecture	
15.35-16.05	**Marco Oggioni** University of Siena, Italy	Biocide resistance associated phenotypes: a hot topic in light of the claimed interconnection between biocide and antibiotic co- or cross-resistance

Poster contributions

ABSTRACTS

PART I

LECTURES AND ORAL PRESENTATIONS

EXPLORING THE PHENOME OF HUMAN AND ANIMAL ENTERIC PATHOGENS

R.M. LA RAGIONE, M. ABUOUN, L. MAPPLEY, M. SAUNDERS, Z. PAN, M. J. WOODWARD

Department of Bacteriology, Veterinary Laboratories Agency, Weybridge. KT15 3NB

The interaction between host and pathogen is a complex and intricate process. The host and pathogen both contribute to the outcome of disease, and each factor or response produced by one induces responses and factors produced by the other. It is recognized, that research to understand bacterial pathogenesis is incomplete without studying host-pathogen interactions. In addition, many commensal bacteria use strategies for attachment and colonisation that are similar to those in use by their pathogenic counterparts. To date most studies have focused on specific virulence determinants rather than studying the global contribution/effects of metabolism on pathogenesis and host microbe interactions. With the availability of genome sequences for numerous pathogens the opportunity exists for more detailed studies of the contribution of bacterial metabolic capability to pathogenesis.

Our studies have focused on a number of emerging pathogens including atypical attaching and effacing *E. coli*, *Brachyspira* and novel emerging *Salmonella* pathotypes. In these studies we have focused on comparisons between highly prevalent types with those that are less so, our premise being that prevalent types may have genetic and phenotypic characteristics that provide them with distinct fitness advantages.

In our studies whole genome sequencing of the pathogens of interest readily identified a few virulence related factors in the prevalent types whilst others differences related more to metabolic capabilities. To confirm the genotype at a phenotypic level, mass phenotyping was performed using the Biolog system. The use of this system to characterise new and emerging *Salmonella*, *E. coli* and *Brachyspira* strains will be presented. In addition the development of host cell assays to evaluate the responses of eukaryotic cells to different conditions and infection with bacterial pathogens will also be discussed.

DEEP WATER HORIZON OIL SPILL - INTRINSIC BIOREMEDIAITON OR MOTHER NATURES'S ABILITIES TO CLEANUP OUR MESSES

TERRY C. HAZEN,

LBNL, Berkeley, CA, USA

The biological effects and expected fate of the vast amount of oil in the Gulf of Mexico from the Deepwater Horizon blowout are unknown due to the extreme depth and magnitude of this event, but also the unprecedented quantity of oil dispersant released and injected directly at the wellhead (1,544 m). We found that the dispersed hydrocarbon plume stimulated deep-sea indigenous bacteria that are closely related to known petroleum-degraders. Hydrocarbon-degrading genes coincided with the concentration of various oil contaminants. Changes in hydrocarbon composition with distance from the source, environmental isolates, and microcosms demonstrate faster then expected hydrocarbon biodegradation rates even at 5°C. This explains why intrinsic bioremediation of the oil plume in the deep-water column without substantial oxygen drawdown is observed.

PHENOTYPE MICROARRAY TECHNOLOGY: HISTORY AND RECENT ADVANCES

B.R. BOCHNER

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

Phenotype MicroArray technology was developed as a tool to permit a detailed analysis of biological phenotypes of cells and also to provide a basis for a detailed comparison of cells. The technology allows scientists to efficiently 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 200 scientific publications and presentations that have used PM technology. The most common use of the technology has been to compare cell lines that differ by a single gene mutation, thereby analyzing gene function. Other uses have been to analyze naturally occurring microbial strains, to analyze the biological properties of strains, to analyze the effects of chemicals on cells, and to employ it as a tool in bioprocess optimization. In this presentation I will describe details of how PM technology works and show examples of how it has been used in both basic and applied research.

DISCOVERY AND VALIDATION OF BIOLOGICAL HYPOTHESES BY ASSOCIATIVE SEARCHES OF FUNCTIONAL GENOMICS DATA

M.P. JOACHIMIAK[1, 2], C. TUGLUS[3], M. VAN DER LAAN[3], A.P. ARKIN[1, 2, 4]

[1] LBL
[2] VIMSS
[3] Dept. of Biostatics, UC Berkeley
[4] Dept. of Bioengineering, UC Berkeley

Functional genomics confronts researchers with a deluge of data from high throughput technologies aimed at understanding biological function at the level of whole cells and genomes. For example, the Ecosystems and Networks Integrated with Genes and Molecular Assemblies Project (ENIGMA) generates gene expression, gene fitness, proteomic, metabolomic, and protein-protein interaction data among others. In light of this it has become advantageous to: a) simultaneously query multiple data types, b) jointly determine confidence across data layers, and c) systematically form hypothesis from multiple types of evidence. Two dimensional clustering, i.e., biclustering, of gene expression data aims to reveal experimentally verifiable gene co-regulatory associations or regulons. A small number of recent bicluster search methods overcome limitations of basic biclustering, such as assigning one module per gene, however a statistical framework for integration of diverse gene and protein data remains a challenge.

We have developed a random-walk algorithm to search for gene associations that maximize a probabilistic combination of statistical criteria across data types. The algorithm models data archetypes: gene-by-experiment, gene-by-gene, experiment-by-experiment, gene-by-feature, and experiment-by-feature. Row and column correlations patterns in continuous data are discovered by a combination of a faster pre-criterion and a slower full criterion applied to the top pre-criteria value candidates. Different criteria functions are placed on an equivalent scale by using empirical null distributions.

The algorithm is amenable to high performance computing and results consist of multiple search trajectories obtained in parallel. In contrast to existing methods, multiple searches are pooled into a total result, giving global orderings of the data and a single threshold for tuning specificity and sensitivity. We embedded the algorithm in a flexible computational environment with customizable algorithm parameters and interactive visualizations for search trajectories and pooled results. The flexibility of this environment allows for rapid customization for different input datasets and search strategies.

We evaluate module discovery using a series of simulated 'biclustering challenge' datasets. We show that the algorithms' performance surpasses existing methods by more accurately identifying multiple, potentially overlapping biclusters while providing statistical associations across data types. To ascertain biological significance we use a diverse *Saccharomyces cerevisiae* functional genomics data compendium including many gene expression conditions and genome-wide data on transcription factor binding sites.

Our method provides a novel framework for enhanced discovery of the regulation and consequences of gene transcription. This framework can be used to search for data associations within other biological contexts.

INTEGRATING PM TECHNOLOGY WITH *IN SITU* AND *IN SILICO* ANALYSES OF MICROBIAL ACTIVITIES

A. RICO, A. MITHANI, A. STEINBRENNER, J. HEIN, G. M. PRESTON

Department of Plant Sciences, University of Oxford, South Parks Road, Oxford, OX1 3RB, UK.

Endophytic plant pathogens such as *Pseudomonas syringae* colonise the intercellular spaces of plant tissues, drawing nutrients from apoplastic fluid and using toxins and secreted proteins to manipulate and suppress plant defence responses. To understand *Pseudomonas*-plant interactions we need to analyse and interpret the interactions of both pathogen and host cells with a shared pool of apoplastic metabolites. A number of techniques can be applied to this problem, including metabolomic analyses of apoplastic fluid, bioinformatic analyses of bacterial genome sequences and transcriptomic analyses of gene expression by bacterium and host. However, to complete the picture these analyses require bridging technologies that can identify which metabolites are used and modified by plant and parasite. One approach that can be used to address this question is phenoarray technology. Microplate-based phenoarrays provide high-throughput profiles of cellular nutrient utilization and stress responses, providing data on metabolic activity in a wide range of defined media. We have used commercial Biolog phenoarrays and custom arrays to profile and compare the metabolic capacity and stress responses of pathogenic and non-pathogenic *Pseudomonas*. We have also developed a variation on phenoarray technology that allows us to profile bacterial metabolism in response to specific environmental conditions, and have used this technique to analyse *P. syringae* metabolism during growth in apoplastic fluid. We discuss how phenoarray data can be integrated with metabolomic data, reporter gene technology and comparative bioinformatic analyses to provide insight into the evolution and biology of plant-pathogen interactions.

PheMaDB: A WEB-BASED SYSTEM FOR STORAGE, RETRIEVAL, VISUALIZATION, AND ANALYSIS OF OMNILOG PHENOTYPIC MICROARRAY DATA

W.E. CHANG, K. SARVER, B.W. HIGGS, T.D. READ, N.M.E. NOLAN, K.A. BISHOP-LILLY, S. SOZHAMANNAN

The MITRE Corporation, McLean, VA, USA

PheMaDB is a web-based system that is standardized for OmniLog phenotypic microarrays (PM) data. It is used to store, visualize, and analyze large collections of time-series PM data. PheMaDB has seven analytical modules: outlier analysis, negative control analysis, phenotype barplot, correlation matrix, phenotype profile search, k-mean clustering, and heatmap analysis. These tools provide users with a number of ways to analyze the PM data to better understand the growth patterns among different strains within one or multiple organisms as they respond to various environmental conditions such as different metabolites, drugs, etc. The web-based system allows data to be shared between the registered users more easily than the OmniLog platform which is limited to a single attached computer in the laboratory. The system also provides three access levels to control the permissions for viewing, uploading, deleting, and modifying the data.

EXPERIMENTAL DESIGN AND ANALYSIS OF PHENOTYPE MICROARRAY DATASETS

L.E. BARQUIST[1], G.C. LANGRIDGE[1], S. REUTER[2], T. FELTWELL[1], A. McNALLY[2], N.R. THOMSON[1]

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[2]School of Science and Technology, Nottingham Trent University, Clifton Lane, Nottingham, NG11 8NS

While modern high-throughput sequencing and bioinformatic techniques have been widely successful in providing insight in to the function and evolution of microbial genomes, they are only capable of telling us part of the story. Translating raw sequence in to predictions of phenotype can be difficult. Often, we may only have a vaguely defined function of a predicted gene. In addition, gene products often interact in complex networks, many of which are poorly understood. Phenotype MicroArrays (PMs), which allow for the efficient querying of thousands of cellular phenotypes simultaneously, are well positioned to help us bridge this gap between modern genomics and phenotyping. Unfortunately, the current generation of PM analysis software is largely dependent upon subjective judgement of difference, and is not easily incorporated into high-throughput analysis pipelines. Borrowing from a body of statistics originally developed in the context of DNA microarrays, we propose a new statistically rigorous method of analyzing PMs. We present preliminary results of comparative analysis performed across multiple bacterial strains. Finally, we present an initial R package for the analysis of PM datasets.

QUANTITATIVE ANTIMICROBIAL SUSCEPTIBILITY ASSAY OF EXTRACTS AND NATURAL PRODUCTS: A POTENTIAL APPLICATION OF OMNILOG FOR HIGH-THROUGHPUT SCREENING

L.C. CHAI[1], C.L. OOI[2], R. SON[1]

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Identifying new classes of antimicrobial drug is imperative due to the growing emergence of multi-resistance pathogens globally. Traditionally, susceptibility testing based on minimum inhibitory concentration (MIC) has been the golden protocol in drug discovery. However, the rate of new drug discovery using the conventional approach is not satisfactory. In this paper, we describe an automated, high-throughput screen of drugs and herb extracts for potential antimicrobial activity with Phenotypic Microarray (PM) from BIOLOG. Using the standard 96-well microtiter plate, *E. coli* (the bacterial model used in this study) in various concentrations was tested against a series of herb extracts and drugs. The PM system is able to produce 4800 high quality kinetic growth models of *E. coli* in various drugs and extracts per run. The 16-hour growth models obtained were then combined and analyzed to yield accurate dose-response models. A modified Gompertz function was used to fit the data, from which a more exact value can be obtained for the MIT. The mechanism of action (MOA) of potential extract was further assessed by performing cluster analysis of the isobolograms obtained from the synergistic and antagonistic study using the same PM approach. The present study suggests Omnilog system as a highly potential approach for an accurate, rapid and quantitative high-throughput screening for antimicrobials.

PROKARYOTIC GENOME REGULATION: MULTI-FACTOR PROMOTERS AND MULTI-TARGET REGULATORS

A. ISHIHAMA

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The pattern of genome expression in *Escherichia coli* is controlled by regulating the distribution of a limited number (about 2,000 molecules) of RNA polymerase among a total of about 4,500 genes on the genome (Ishihama 2000). For survival in nature, stress-response genes are expressed in various combinations but at present, little is known how specific sets of the genes on the genome are selected for expression. We proposed such a model that a total of 300 species of DNA-binding transcription factor are involved in the gene selectivity control of RNA polymerase. Even in this best characterized model prokaryote, however, the regulatory function remained unidentified for more than 100 transcription factors. To get insights into the genome regulation, we have then initiated a systematic search of the whole set of regulation target promoters, genes and operons under the control of each of 300 transcription factors (Ishihama 2009; 2010). For this purpose, we developed 'Genomic SELEX', in which DNA sequences recognized by a test transcription factor are isolated *in vitro* from mixtures of genome DNA fragments and mapped along the genome using tilling DNA arrays. Here we report the progress of on-going 'Genomic SELEX' screening of regulation targets of *E. coli* transcription factors, focusing on the following topics: 1) The whole sets of regulation targets hitherto identified for both uncharacterized and characterized transcription factors; 2) the multiplicity of transcription factors involved in regulation of specific promoters for adaptation to environment such as the *csgD* promoter of a master regulator of biofilm formation; 3) the multiplicity of regulation targets by global regulators such as such as CRP and Cra, both being involved in regulation of transport and metabolism of carbon sources; and 4) the complex hierarchical networks formed by combination of multi-factor promoters and multi-target regulators. In this study, PM is used mainly in two series of experiment: 1) Identification of biological activities of uncharacterized genes under the control of each transcription factor; and 2) search for effectors and conditions affecting the activity of test transcription factors.

Ishihama, A.: Prokaryotic genome regulation: Multi-factor promoters, multi-target regulators and multi-factor networks. *FEMS Microb. Rev.*, in press (2010).

Ishihama, A.: Chapter 2.6, The Nucleoid: an Overview. In: EcoSal—*Escherichia coli* and *Salmonella:* cellular and molecular biology. (A. Bëák, R. Curtiss III, J. B. Kaper, P. D. Karp, F. C. Neidhardt, T. Nystrëë, J. M. Slauch, C. L. Squires, and D. Ussery, eds.), http://www.ecosal.org. ASM Press, Washington, DC.

Ishihama, A.: Functional modulation of *Escherichia coli* RNA polymerase. *Ann. Rev. Microbiol.* **54**, 499-518 (2000).

YEAST AS A MODEL IN NUTRITIONAL SYSTEMS BIOLOGY: A WHOLE GENOME PARALLEL PHENOTYPING APPROACH

D. CAVALIERI

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The combination of genetic analysis and genome-wide patterns of gene expression makes *Saccharomyces cerevisiae* the cradle of Systems Biology. Recently this microorganism is considered a promising model also in nutritional research. The way *S. cerevisiae* uses carbon sources has long been a landmark for studies related to cellular responses to environmental conditions. Yeast *S.cerevisiae* is a facultative anaerobe, which preferentially ferments glucose with the final production of ethanol and other secondary products of fermentation. Yet this microorganism conserves the entire machinery for utilization of fatty acids with a different compartmentalization of the catabolic reactions with respect to the mammalian counterparts. We propose *S.cerevisiae* as a model to understand how perixosomal compartmentalization of Oleate metabolism co-evolved with the transcriptional regulatory networks of FA metabolism. We performed growth competition experiment on the pooled barcoded collection of all viable yeast null mutants (24h and 72h of incubation) in different media, varying the carbon source from glucose to galactose to fructose to oleic acid 0.1% and 5%. We scored growth rate by hybridization to a phenotype microarray containing probes for every barcode (Affymetrix). We then probed the gene expression phenotype, performing whole genome transcriptional analysis using DNA microarrays. We then applied a cross-species meta-analysis approach to integrate twenty microarray datasets studying high fat diet and PPARa signal perturbations in different organisms. The integration at the pathway and transcriptional network level the results of gene expression with measurement of differential fitness identified two genes previously not directly associated to fatty acid metabolism: *OAR1* and *FET3*, and a network of 20 new potential candidate genes regulating differential Mitochondrial-peroxisomal localization of FA metabolism in yeast vs mammals. The results presented demonstrate the power of integrating *in vivo* and *in silico* analyses integrating gene expression and fitness measurements.

GENOMIC, TRANSCRIPTOMIC, AND PHENOMIC ANALYSIS OF *BURKHOLDERIA CEPACIA* MUTANTS IMPAIRED IN RND EFFLUX PUMPS

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[1]Lab. of Microbial and Molecular Evolution, Dept. of Evolutionary Biology, and [2]Dept. Of Agriculure Biotechnology, University of Florence, Italy
[3]Cardiff School of Biosciences, Cardiff University, Cardiff, Wales, United Kingdom
[4]Tree and Timber Institute - National Research Council, San Michele all'Adige (TN), Italy
[5]Lab. Molecular Microbiology, Dept of Genetics and Microbiology, University of Pavia

The genus *Burkholderia* includes a variety of species with opportunistic human pathogenic strains, whose increasing global resistance to antibiotics has become a public health problem. Usually, *Burkholderia* species are not considered pathogens for the normal human population, even though some of them are serious threats for specific patient groups. These species include *B. gladioli*, *B. fungorum* and all *B. cepacia* complex (BCC) bacteria. The BCC is a group of genetically distinct but phenotypically similar bacteria that up to now comprises seventeen closely related bacterial species, and they are important opportunistic pathogens that infect the airways of cystic fibrosis (CF) patients. In this context, it seems more and more evident that the intrinsic resistance of many bacteria to antibiotics depends on the constitutive or inducible expression of active efflux systems. It has been suggested that the main function of these systems is likely not conferring resistance to antibiotics (used in therapy) and that they might play other roles relevant to the behaviour of bacteria in their natural ecosystems. Among the potential roles, it has been demonstrated that efflux pumps are important for detoxification processes of intracellular metabolites, bacterial virulence in both animal and plant hosts, cell homeostasis and intercellular signal trafficking.
This class of proteins includes a very interesting group, referred to as the RND (Resistance-Nodulation-Cell Division) superfamily, which allows bacterial cells to extrude a wide range of different substrates, including antibiotics. A preliminary analysis of the role of these proteins in BCC has been carried out on *B. cenocepacia* strain J2315, representing one of the most problematic strains isolated from CF patients. The genome of this bacterium harbours 16 paralogous *rnd* genes, a finding that witnesses the importance of these pumps in the physiology of bacterial cells. In this study we have tried to decipher the role of RND efflux pumps in BCC bacteria using the strain J2315 as a model system. Three different approaches were used : i) the genomic/evolutionary approach may allow to identify *rnd* genes in the 21 available completely sequenced *Burkholderia* genomes, to analyze their phylogenetic distribution, define the putative function(s) that RND proteins perform within the *Burkholderia* genus, and to trace the evolutionary history of some of these genes in *Burkholderia*; ii) the transcriptomic and genetic analysis was performed on the wild type strain and two single mutants, impaired in different *rnd* genes and in the relative double mutant, allowing to identify sets of under- or over-expressed genes in the three mutants; iii) the phenomic analysis allowed to identify sets of metabolic traits altered in the three mutants.
All data were then integrated, allowing to shed some light on the role that RND proteins play in the complex metabolic efflux system.

METABOLIC MODULATION OF *PSEUDOMONAS AERUGINOSA* VIRULENCE AND ANTIBIOTIC RESISTANCE

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Nowadays, infected patients are regularly treated with antibiotics, al least in the developed countries, in such a way that the capability of bacterial pathogens for keeping proficient infections relies in both their virulence and their resistance to antibiotics. The entry into a new host is a situation that demands bacteria adapting their physiology to their new environment, making use of newly available nutrients in such a way that bacteria suffer strong metabolic shifts. These new physiological circumstances might impact on the expression of phenotypes relevant for microbial resistance and virulence, because some scattered pieces of evidence suggest that these two characteristics can be influenced by bacterial metabolism. To explore the potential crosstalk between bacterial metabolism, antibiotic resistance and virulence, we have use as a model the opportunistic pathogen *Pseudomonas aeruginosa*. This bacterial species is one of the most important nosocomial pathogens and presents a characteristic phenotype of reduced susceptibility to several antibiotics. To study this potential crosstalk, we have analyzed the contribution of Crc, a post-transcriptional global regulator that controls the metabolism of carbon sources and catabolite repression in *Pseudomonas*, to the intrinsic antibiotic resistance and virulence of *P. aeruginosa*. Using proteomic analyses, high-throughput metabolic tests (BIOLOG) and functional assays, we have determined that the virulence and the antibiotic resistance of this pathogen is linked to its physiology. The global regulator Crc plays a relevant role in liking antibiotic resistance and virulence to the metabolism of *P. aeruginosa*. A *crc* mutant strain made a more efficient use of dipeptides as nitrogen sources, and was impaired in its utilization of phosphorylated sugars as phosphate donors and of sulfur-containing amino acids as sulfur sources. Besides, this mutant strain presented defects in the expression of several virulence determinants, including Type III secretion, motility and quorum sensing-regulated virulence factors. Consistent with this observations, the mutant lacking Crc was less virulent in a *Dictyostelium discoideum* model. In addition, this mutant strain was more susceptible to antibiotics belonging to different structural families, inlcuding beta-lactams, aminoglycosides, fosfomycin and rifampin.
Since the Crc mutant is less virulent and presents a higher susceptibility to several antibiotics, Crc might be a good target in the search for new antibiotics.

GENOMIC AND PHENOTYPIC ANALYSIS OF ANTIBIOTIC RESISTANCE IN *PSEUDOMONAS AERUGINOSA*

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Natural resistance to antibiotics is a common feature of bacterial pathogens which frequently diminishes clinical treatment options. Bacterial gene products contributing to intrinsic resistance therefore represent attractive drug targets whose inhibition could enhance the efficacy of established antibiotics. To identify such drug targets, we have developed a new technique for negative selection screening based on second-generation sequencing of saturation-level populations of transposon mutants. The method was employed to identify *P. aeruginosa* genes required for full resistance to tobramycin, a clinically important aminoglycoside antibiotic. The results identify numerous functions involved in intrinsic resistance, including regulators, proteases, metabolic functions and hypothetical functions. We have subsequently used phenotype microarray analysis to more deeply characterize several of the most promising genes identified. The findings from this combination of genome-wide screening and broad phenotypic characterization support a model in which membrane stress response mechanisms contribute to intrinsic aminoglycoside resistance.

TOXICITY OF FREE ZINC IONS *VS* ZINC OXIDE NANOPARTICLES ON *E. COLI* MG 1655 GROWN IN LURIA BERTANI OR IN SEINE RIVER WATER

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New properties of nanoparticles have received considerable attention from an industrial perspective, nevertheless, few studies reporting the toxicity of nanoparticles to organisms have been published. This study reports the effects of manufactured coated nanoparticles of zinc oxide (ZnO) (Brayner *et al*., 2006), on the model bacterium, *Escherichia coli*, MG 1655. We investigated first the bacteriostatic and bactericidal effects of ZnO nanoparticles on *E. coli* and then the impact of toxic metals by using Phenotype MicroArrays technology.
Cells were grown in an organic nutritive medium (Luria Bertani), or in a Seine river water sample, representative of aquatic environmental conditions, in presence of zinc oxide or nanoparticles. A set of experiment with free zinc ion has been performed as control. The cultures were grown in shaking condition at 37°C for 3 hours.
In the organic medium, the two zinc forms induced similar toxic effects, suggesting a partial solubilization of ZnO nanoparticles, accordingly to thermodynamic modelling predictions. In the natural water sample a strong bactericidal effect was observed and was directly linked to the persistence of ZnO nanoparticles in agreement with the thermodynamic modelling predictions.
In the aim of a better knowing of the sensitivity of *E coli* to different kind of metals in presence of Zn nanoparticles , microplates were set up with different metal concentrations (from 1 to 5000 ppm) and the activity of *E. coli* was tested by PM technology. The cells grown in presence of zinc free ions or zinc were used to inoculate microplates with different concentrations of metals (PM metals) and incubated for 48 hours in the OMNILOG apparatus. The kinetics of cultures were analyzed and compared. Lag time as well as plateau time was plotted versus the conditions of preincubation of the cells. Increasing the concentration of Zn^{2+} in the PM metals also increased lag time for cells grown in Seine river water plus ZnO 2.5 mM. On the other hand, plateau time was higher in Luria Bertani for cells grown in presence of Zn^{2+} or ZnO. Plateau time was higher in Seine river water for the treated and untreated bacteria. Our results suggest that PM metals could be applicable to evidence differences in sensitivity of *E. coli* to toxic metals when the cells are grown different conditions.

R. Brayner, R. Ferrari-Iliou, N. Brivois, S. Djediat, M. F. Benedetti and F. Fiévet, Nano Lett.**4**, 866-70 (2006).

CHROMATE RESISTANCE IN *PSEUDOMONAS CORRUGATA* STRAIN 28

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Hexavalent chromium is a severe contaminant since, unlike trivalent chromium, it is highly water-soluble, toxic, mutagenic to most organisms, and carcinogenic to humans. Most micro-organisms are sensitive to Cr(VI), but some microbial groups are resistant and can tolerate high levels of chromate. In bacteria, Cr(VI) resistance is linked to plasmids and/or chromosomes (Viti & Giovannetti, 2007).
Pseudomonas corrugata 28 is a Cr(VI)-hyperresistant bacterium isolated from a soil artificially contaminated with K_2CrO_4 deeply characterized at phenotypical level and used as a model for the study of Cr(VI)-resistance systems.
Mutants of *P. corrugata* 28 have put in light some key processes involved in Cr(VI)-resistance in this strain: the DNA repair mediated by RecG helicase; the activation of sulfur starvation response by the *oscA* gene that is required to solve sulfur starvation induced by oxidative stress in cells; the supply of NADPH, required for repairing damages induced by chromate, mediated both by the malic enzyme and by soluble pyridine nucleotide transhydrogenase..
Consolidated knowledge and our most recent achievements will be shown and discussed.

Viti C., & Giovannetti L. (2007). Bioremediation of soils polluted with hexavalent chromium using bacteria-a challenge. In: *Environmental Bioremediation Technologies*. S.N. Singh e R.D. Triphati (eds.), Springer, Berlin, pp. 57-70 (Berlin, Germany).

A GENOMIC VIEW ON CARBOHYDRATE UPTAKE IN *STREPTOCOCCUS PNEUMONIAE*

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Pneumococci, an important cause of invasive disease and commensals of the human nasopharynx, are characterised by a high substrate flexibility in their exclusively fermentative metabolism. For carbohydrate uptake pneumococci possess over thirty carbohydrate import systems including per pneumococcal genome between 15 and 20 phosphotransfer systems (PTS) and six to seven ABC transporters belonging to the carbohydrate uptake family 1 (CUT1) and one CUT2 transporter.
Textbooks and biochemical identification schemes list less than ten sugar substrates that can be efficiently metabolised. Using the Phenotype Microarray and in house fermentation assays we have been able to identify 52 sugars used by *S. pneumoniae* both as carbon source or nitrogen source. So far only five of these transporter were characterised including the two PTS for beta-glucosides and sucrose and the three ABC transporters for sucrose, maltose and raffinose. Here we provide a functional genomic annotation of carbohydrate transporters combining phenotypic and genotypic analysis of many sequenced pneumococcal strains and by constructing mutants for all these uptake systems in one model strains. Altogether data provide a first complete functional overview of carbohydrate transport systems in a human pathogen with this extent of substrate and transport variability.

UTILIZING THE BIOLOG PM ASSAY TO IDENTIFY PHENOTYPIC CHANGES IN *SHIGELLA FLEXNERI* POST-EUKARYOTIC CELL INVASION

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Shigella flexneri's physiological response during the host-pathogen cell invasion process is complex. The association of a bacterial pathogen's virulence is linked to their ability to grow and survive in an eukaryotic host. Phenotypic Microarrays can be used to explore the metabolic activities of microbial cells in the host milieu. In this study, we developed a method to isolate intact bacteria from host mammalian cells in tissue cultures and screen for the biochemical attributes subsequently gained or lost by the bacteria post invasion using the Phenotypic Microarray (PM) system. *Shigella flexneri* 2457T, a causative agent of shigellosis, served as the model organism in this study. This bacterium contains both plasmid- and chromosomal-encoded genes that regulate the invasion process. After interacting with human HeLa epithelial cells, bacteria that were inside the cells were recovered and analyzed using the PM system. Phenotypic expression profiles were compared between *Shigella flexneri* 2457T pre- and post-invading cells. Differences in the ability of each to utilize carbon, nitrogen, phosphorus and sulfur sources were detected by the PM system and analyzed using the PM software. HeLa cells both invaded and not invaded, with *S. flexneri* 2457T, were also analyzed to determine potential phenotypic changes in carbon utilization. For example, by analyzing absorbance readings of PM plates 1 and 2A at 36 hours with a 2-way ANOVA, the expression of four phenotypes were shown to be statistically different between the samples (p value < 0.05). The substrates were maltose, alpha-methyl-D-galactoside, L-alaninamide, and N-acetyl-beta-D-mannosamine. The identification of metabolic activity changes in bacterial pathogens, in conjunction with virulence-associated genetic determinants, may provide a broader picture of the mechanism of bacterial pathogenesis. This method may be extended to other bacterial pathogens, whether in similar host cell environment, or as we envision, to a broader context such as the successful colonization when internalized in plants (produce commodities such as lettuce) or in their respective fruit, e.g. tomatoes. These studies should provide a more complete view of virulence, growth and survival of bacterial pathogens in varied environmental conditions.

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

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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 MicroarrayTM (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: 16 ORF-disrupting genes were identified that could be used as markers of virulence as defined by the ability of *S. Enteritidis* to contaminate eggs or to form biofilm. BLAST analysis of other Salmonella whole genome databases was performed to evaluate conservation of these ORFs across other serotypes. These genes were mostly absent in *E. coli* and varied in their presence across other *Salmonella* serotypes with sequenced whole genomes. The ability to couple PM with high-throughput genomics 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 to conduct epidemiological surveys. Phenotype Microarray analysis identified specific metabolic properties linked to genetic markers identified by whole genome analysis and hen infection experiments for *S. Enteritidis*.

EVOLUTIONARY DYNAMICS OF BACTERIA IN A HUMAN HOST ENVIRONMENT

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Laboratory evolution experiments have led to important findings relating organism adaptation and genomic evolution. However, continuous monitoring of long-term evolution has been lacking for natural systems, limiting our understanding of these processes *in situ*. We characterize the evolutionary dynamics of a lineage of a clinically important opportunistic bacterial pathogen *Pseudomonas aeruginosa* as it adapts to the airways of several individual cystic fibrosis patients over 200,000 generations through genome sequencing, transcriptomic profiling and Biolog catabolomics. In contrast to predictions based on *in vitro* evolution experiments we document limited diversification of the evolving lineage in spite of a highly structured and complex host environment. Notably, the lineage went through an initial period of rapid adaptation caused by a small number of pleiotropic mutations and loss of catabolic activity followed by a period of genetic drift with limited phenotypic change and a genomic signature of negative selection, suggesting that the evolving lineage has reached a major adaptive peak in the fitness landscape. The evolved phenotype of the infecting bacteria further suggests that the opportunistic pathogen has transitioned to a primary pathogen for cystic fibrosis patients.

HOST RESTRICTED *SALMONELLA*: LINKING PSEUDOGENES AND METABOLIC ABILITY

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Over 2,500 serovars have been characterised in the Gram negative bacterium *Salmonella enterica* and some, such as serovar Typhimurium, are capable of causing a range of diseases in a wide range of animal hosts. Other serovars, such as *S. enterica* serovar Gallinarum and *S.* Typhi are restricted to single hosts, chickens and humans respectively, where they cause systemic infection. In the past, biochemical testing has been used to differentiate between *Salmonella* isolates, exploiting differences in metabolic capability and suggesting that host-restricted serovars utilise a narrower range of substrates than host-generalists. Now, high-throughput sequencing has revealed that the genomes of host-restricted *Salmonella* serovars contain high proportions of pseudogenes. The aim of this study was to investigate whether loss of gene function through pseudogene formation could be linked to the apparent reduced metabolic ability. A metabolic pathway database was created under the Pathway Tools schema and used to identify the pathways in each serovar that contained pseudogenes. Using Biolog Phenotype MicroArrays, we investigated which of 380 metabolic substrates could or could not be used by *Salmonella* serovars adapted to different hosts. We present our findings linking genotypic changes with metabolic phenotypes.

GENETIC AND BIOLOGICAL DIVERSIFICATION OF *BACILLUS SUBTILIS AT* MICRO AND MACRO-GEOGRAPHICAL SCALES

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A genetic micro-diversity within *Bacillus* species is known to exist but ecological significance of this diversity for niche differentiation remains largely a mystery. The ComQXPA quorum sensing loci of *Bacillus subtilis* show a dramatic within species diversity, which results in formation of different communication groups or pherotypes. Members of one pherotype can exchange signals and consequently induce each other to produce surfactin, form biofilms and become competent, while members of different pherotypes can not. The ecological significance of this diversity for survival of the species is not known. We address this question by comparing various traits (DNA sequences, physiology) of *B. subtilis* river bank soil isolates obtained from millimeter scales and *Bacillus* strains from desert soils, isolated across the macro-geographical scales. Higher genetic (*gyrA, rpoB, recA, comQXPA* genes) and physiological diversity (Biolog Phenotype MicroArrays, pherotype specificity) was evident for macroscale as compared to microscale isolates. To our knowledge, this is the first study addressing pherotype diversification in connection to physiological and genetic within species diversity at micro and macro-geographical scales.

ISOLATION OF AEROBIC LIGNIN- AND CELLULOSE- DEGRADING BACTERIA FROM TROPICAL SOILS FROM BIOFUEL FEEDSTOCK DECONSTRUCTION

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Plant biomass deconstruction poses a large challenge to the biofuel industry. Hence, there is a need to find better methods that will more easily breakdown the plant's two main structural components - cellulose and lignin. The goal of this project was to identify cellulose- and lignin-degrading microorganisms from Puerto Rican tropical forest soils, where high plant litter decomposition rates have been observed in previous studies. Our efforts focused on bacterial cultivation since there are few bacterial taxa known to degrade lignin. Microbes were selectively cultivated using minimal media agar with lignin or cellulose as a sole carbon source. The cellulolytic activity rates of our microbial isolates were measured using 4-methylumbelliferone (MUB)-linked substrate assays where cellulase activity is proportional to the amount of fluorescence from free MUB after the substrate is degraded. The lignolytic activity rates were measured using colorimetric assays for the oxidation of the model lignin compound L-3,4-dihydroxyphenylalanine (L-DOPA). We cultivated 54 isolates that were able to utilize cellulose or lignin as a sole carbon source. The most cellulolytic isolates ranged from 200-500 µmol of MUB released per hour per OD of microbial cells. The most lignolytic isolates produced over 1 OD of L-DOPA per hour per OD of microbial cells. Based on 16S rRNA gene sequencing, our top performing isolates were members of the *Proteobacteria* phylum --*Burkholderia*, *Massilia*, *Vogesella*, and *Sphingomonas.* These taxa merit further investigation in order to discover their possible application in the biofuel industry. Their enzymes may aid the plant deconstruction process, consequently making future biofuels less costly and more energy efficient.

METABOLIC MODEL REFINEMENT VIA PHENOMIC ANALYSIS OF *ACTINOMYCETALES* CATABOLISM.

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The bacterial order *Actinomycetales* are remarkable for metabolisms rich in biosynthetic capabilities that impact humans in many ways ranging from numerous antibiotics produced in *Streptomycetales* and close related species, sophisticated lipid biosynthesis leading to intracellular storage central to the oleaginous lifestyles of *Mycobacteria* and *Rhodococcus*, and agricultural-scale amino acid production in *Corynebacteria* to name a few. Phenomic analysis of actinomycete catabolism was investigated to connect the genes with the pathways for the biochemical breakdown of chemical compounds found in the environments these bacteria dwell in. Screening 190 compounds for growth led to refinements in our genome-based metabolic models for 11 *Streptomycetes*, 1 *Saccharopolyspora*, 2 *Rhodococcus*, 1 *Corynebacteria* and 2 outlyers: 1 *Pseudomonad* and 1 *Ralstonia* species. This growth phenotype data was integrated with a DNA-sequence analysis pipeline; wherein genes were assigned to metabolic pathways using the protein sequences derived from genome annotation and to specific biochemical reactions via Enzyme Commision # assignment using KEGG's pipeline and an EficaZ2 algorithm in attempt to net all pathway genes and assign a confidence score for their relationship to these pathways. Manual curation is still required to complete some pathways that are predicted to be incomplete by EC assignments but in fact have the required genes and catabolic-phenotype. The genomes of *Actinomycetales* also contain gene redundancies predicted for many biochemical reactions; to gain further resolution on the specific-activity of particular enzymes we used comparitive genomics to analyze paraloguous and orthologuous enzymes with a set of phylogenetically related species using tribe- and ortho-MCL algorithms. This phenomic analysis allowed for more complete and accurate enzyme assignments that resulted in refined metabolic-models for each species tested that facilitates extrapolation from empirical data obtained from these and related species for metabolic model refinements. This combined approach increases our predictive power from genome sequences that sheds important light on how this order of bacteria consume organic compounds and produce molecules of interest.

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PHENOTYPE MICROARRAY SUPPORTING COMPARATIVE GENOMIC ANALYSIS IN *SINORHIZOBIUM MELILOTI*

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Sinorhizobium meliloti is a soil bacterium able to penetrate in root tissues of leguminous plants, forming nodules where bacteria fix atmospheric nitrogen.
We used extensive Phenotype Microarray analysis in order to assign functions to the accessory genome of the *S. meliloti* species. In fact, two strains of the alfalfa symbiont *Sinorhizobium meliloti* (AK83 and BL225C) were sequenced in collaboration with the Joint Genome Institute (DOE, USA) and assembled using the reference *S. meliloti* genome (RM1021) as template. Comparative genomic tools were then used to find the core and accessory genomes as well as high variable regions across the *S. meliloti* genome. Results obtained were integrated with Phenotype Microarray data in order to highlight the molecular basis of the strains variability.
Altogether the combination of Phenotype Microarray, various bioinformatic tools and experiments shed light on the metabolic network of *S. meliloti*, especially regarding those features related to plant association and nitrogen metabolism. Finally, this approach and the associated bioinformatic tools could be extended to other strains and different species with phenotypic variability.

THE USE OF PHENOTYPE MICROARRAYS TO CHARACTERIZE SUBSTRATE USAGE BY HALOPHILES ISOLATED FROM SOLAR SALTERNS

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The physiological and metabolic characterization of extreme halophiles by standard methods is challenging because of the very high salt content required for growth. The goal of this project was to characterize halophilic cultures isolated from a range of habitats in a solar saltern. We isolated 35 halophilic cultures including bacteria and archaea from 7 different evaporation ponds, flow through channels and crystallizers ranging from ~15% salinity to >30% (saturation) in the ESSA saltworks, Guerrero Negro, Baja CA, MX. 16S rRNA sequence analysis identified bacterial isolates from the genera *Salicola*, *Halovibrio*, *Halomonas* and *Salinibacter* and archaeal isolates from *Halorubrum*, *Haloarcula*, and a previously uncharacterized haloarchaeal genus. These cultures displayed variable salinity growth ranges and optima. Preliminary studies with BIOLOG Ecoplates failed due to a lack of clear signal in the tetrazolium dye-based assays. BIOLOG Phenotype Microarrays (PM1 & PM2) based on cell turbidity were successfully employed with statistical replication. The PM assays showed that bacterial strains tended to use more substrates than archaea and bacterial substrate usage patterns were more similar to each other than to archaea. Archaeal substrate usage varied widely even among isolates of the same genus (e.g. *Halorubrum*), indicating physiological variability among close relatives and suggesting niche differentiation. Additionally, differences were seen within a strain assayed at different salinities, suggesting that tubidity (growth)-based substrate utilization assays may be influenced by salinity. This study demonstrated that the Phenotype Microarray system can be used to characterize metabolic capabilities of extremely halophilic isolates, although further work is needed to relate these culture-based substrate usage patterns to the ecological setting from which isolates are obtained. Ongoing studies are evaluating substrate usage by isolates from other salterns.

PHENOTYPE MICROARRAY AS A TOOL TO STUDY THE PHYSIOLOGY OF DAIRY BACTERIA

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Phenotype microarray analysis of dairy and non-dairy streptococci revealed that the cell bioenergetics machinery of this group of microorganisms is optimize to work at alkaline pH. Using as a model, the dairy lactic acid bacterium *Streptococcus thermophilus*, we investigate the role of alkalizing reactions within a background of an acidogenic metabolism. While the induction of alkalizing enzymatic systems, such as urease, by several microorganisms has been predominantly considered a stress-response to counteract a low environmental pH, here we demonstrate a new role of these enzymes in the optimisation of cellular bioenergetics. By modulating the intracellular pH and thereby increasing the activity of β-galactosidase, glycolytic enzymes and lactate dehydrogenase, urease increases the overall change in enthalpy generated by the bioenergetic reactions. The physiological role of a single enzymatic activity demonstrates a novel and unexpected view of the non-transcriptional regulatory mechanisms that govern the bioenergetics of a bacterial cell, highlighting a new role for cytosol-alkalizing biochemical pathways in acidogenic microorganisms.

BIOLOG SYSTEM: POTENTIAL APPLICATION WITHIN FOOD RELATED LACTIC ACID BACTERIA

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Notwithstanding the usefulness of genetic tools, the phenotype characterization is always the ultimate goal to exploit the potential of strains during colonization of different environments. Phenotypic profile is an useful form of information that complements the current knowledge derived from global and molecular analysis. Often, phenotypic/fermentative profiles of lactic acid bacteria are determined by the API 50 CH System and/or by assays on culture media containing different carbon sources. The fermentative capacity of lactic acid bacteria may vary from a very few carbon sources (e.g., *Lactobacillus sanfranciscensis*) (De Angelis et al., 2007. *Int J Food Microbiol* 114, 69-82) to a very broad range of substrates (e.g., *Lactobacillus plantarum*) (Hammes and Vogel 1995. In The Lactic Acid Bacteria. Vol. 2, UK, Chapman and Hall. pp. 19-54). One of the striking property of lactic acid bacteria is concerning their enormous flexibility and potential with respect to catabolic substrates as a mechanism of response to the continuous changes in the surrounding environment (Gobbetti et al., 2005. *Trends Food Sci Technol* l16, 57–69). The semi-automated computer-linked technology of the Biolog System, consisting of a Microstation computer, turbidimeter (optical density at 590 nm) and MicroLog software, allow to get information regarding a very large number of carbon sources belonging to different chemical classes.

The Department of Biologia e Chimica Agro-Forestale ed Ambientale of the University of Bari has developed some researches on the use of the Biolog System in food related lactic acid bacteria (Di Cagno et al., 2006. *Appl Environ Microbiol* 72, 4503-4514; De Angelis et al., 2007; Di Cagno et al., 2007. *J Appl Microbiol* 114, 69-82; Di Cagno et al. 2009a. *Int J Food Microbiol* 128, 473-483; Di Cagno et al. 2009b. *Food Microbiol*, doi: 10.1016/j.fm.2009.11.012; Siragusa et al., 2009. *Appl Envir Microbiol* doi:10.1128/ AEM.01524-08; Minervini et al., 2010. *Food Microbiol* doi:10.1016/j.fm.2010.05.021).

Biolog System analysis helped to understand some aspects of the molecular heat stress response of *Lactobacillus helveticus* PR4, the primary natural whey-starter for the manufacture of Swiss and Italian hard cheese varieties, when cultured in whey-cheese under a temperature gradient from 55 to 20°C which mimic the manufacture of natural whey-starter (Di Cagno et al., 2006). Cells harvested at 40°C showed the complete pattern of fermentation which allowed Biolog System to identify *L. helveticus* at species level. Only glucose, lactose and mannose were fermented by cells exposed to 55°C for 35 min. The molecular typing of *L. sanfranciscensis*, and *Lactobacillus rossiae* was complemented using the Biolog System (De Angelis et al., 2007; Di Cagno et al., 2007). Phenotypic traits combined to genotypic analysis could be considered as an example of a computerized analysis to reliably and rapidly differentiate strains isolated from sourdough or raw vegetables and fruits. Community Level Catabolic Profiles (CLCP) of sourdough microbial communities during propagation were also assessed by using BIOLOG 96-well Eco-Microplates (Siragusa et al., 2009; Minervini et al., 2010). The metabolic profiles of the sourdough lactic acid bacteria are likely to vary according to changes of the microbial population during propagation and regardless of the starter persistence. Catabolic profiles of sourdoughs containing persistent starter cultures of *L. sanfranciscensis* or *L. plantarum* behaved similarly during propagation and clearly differentiated from the other sourdoughs. Based on the above studies, it was clear that changing the target of the analysis, the study of phenotypic profile of lactic acid bacteria by Biolog System may opens new frontiers from an ecological and applicative point of view.

USING PM CHEMICAL SENSITIVITY ASSAYS TO DETERMINE NEW ESTROGENIC AND ANDROGENIC ENDOCRINE DISRUPTING CHEMICALS

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Endocrine disruption in aquatic animals has long been attributed to anthropogenic and natural sources such as release of treated sewage or pulp/paper mill effluent into the environment. Endocrine disruption involves the normal endocrine (estrogen, androgen, or thyroid) functions due to exposure by endocrine disrupting chemicals (EDCs). Estrogenic chemicals include natural and synthetic estrogens, phytoestrogens, plasticizers, flame retardants, and non-ionic surfactants. Androgenic chemicals include natural and anabolic steroids. Here, we attempt to identify new endocrine disrupting chemicals by employing Biolog's Chemical Sensitivity Assays. Approximately 360 chemicals supplied in Biolog's Phenotype Microarray Chemical Sensitivity Assays were tested on the yeast estrogen screen (YES) and the yeast androgen screen (YAS). The Yeast Estrogen Screen (YES), developed by Glaxo, is a reporter gene system that responds to estrogens or estrogen mimics by producing □-galactosidase. This enzyme will then cleave specific substrates into chromogenic products. Simple modification in the assay will allow for the detection of anti-estrogen, anti-androgens, and synergistic chemicals.

The application of Biolog's Chemical Sensitivity Assays reveals three classes of endocrine disrupting activity: agonism (estrogenic or androgenic), antagonism (anti-estrogenic or anti-androgenic), and synergism caused by various classes of antibiotics, other pharmaceuticals, metals, and anions. Not many new estrogenic chemicals were identified, which is not surprising since this system is well-studied. The androgen screen revealed two novel classes of EDCs not previously reported. Several anti-estrogens and anti-androgens were identified. Of interest was the ability to detect synergism, in which sub-active combinations of chemicals produce a super-additive effect. This is important as many low-level chemicals are released into environments where endocrine disruption is observed. The results show that Biolog's PM Chemical Sensitivity Assays can be used as a rapid screening method to test for estrogenic and androgenic endocrine disrupting activity.

ECOLOGICAL SIGNIFICANCE OF RESOURCE UTILIZATION IN MICROBIAL ECOLOGY

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In most of the environments, microorganisms exist in communities of astonishing diversity and abundance; it has been estimated that the number prokaryotic cells in one kilogram of pristine soil exceeds the number of stars in our galaxy. This astronomical dimension of the prokaryotic world can be explained by the heterogeneity of the habitats where microbes are found. From microbial perspective, even one single gram of soil contains a great number of potential ecological niches, each one supporting an array of prokaryotic species and ecotypes. According to ecological theory, species coexistence in a given ecological niche can be explained by the way coexisting species share the resources available. Resource partitioning has also been seen as major mechanism explaining why more diverse communities perform better than less diverse ones. Thus, unraveling resource-use characteristics of the species present in a given community might provide a framework that will bring us one step forward in understanding the functioning of prokaryotic communities. During my talk I will discuss two experimental approaches in which we used Phenotype MicroArrays™ to address patterns of resource utilization by soil bacterial communities, which were then analyzed in the context of bacterial diversity and community stability. The first approach relates to the dynamics of bacterial communities in the rhizosphere of potato plants. By using different molecular approaches, we observed that bacterial diversity and community structure were strongly affected by plant growth stage, but also by the potato cultivar, which differed in the amount of starch stored in their tubers. Our hypothesis was that the plant physiological changes associated with the cultivars led to changes in the quality of root exudates, which then led to changes in bacterial communities. By using the carbon panels of the Phenotype MicroArrays™ we observed great variation in the metabolic profiles of the communities associated with the cultivars: whereas low starch cultivar selected for communities with preference for carbohydrates, alcohols and amines, the bacterial community associated with high starch cultivar preferred carboxylic and amino acids. Thus, differences in growth rates and root development associated with potato cultivars differing in tuber starch content likely influenced root exudation patterns, which selected for rhizosphere-associated bacterial communities that utilize different classes of organic compounds.
The second approach relates to the insurance hypothesis, in which diversity increases the stability of ecosystems by buffering the effect of environmental fluctuations or disturbances. The higher stability observed for diverse communities would also make them less prone to invasion than simpler communities. By using artificially constructed bacterial communities, we observed that soil microbial diversity was indeed a key factor in controlling the survival of the invading species (*E. coli* O157:H7), as microbial richness was negatively correlated with the survival of *E. coli*. Our hypothesis is that diverse communities are capable of exploring the resources available in the soil more efficiently than communities with low number of species. As a result, low resource availability hinders the establishment of the invader. We are currently using Phenotype MicroArrays™ to determine the niche occupied by each of the constructed bacterial communities. These data will allow us to demonstrate the role of complementarity for resource use in determining the stability of microbiologically-diverse soil communities to invasion, a mechanism that was recently shown to play a key role on the functioning of bacterial communities in a short-term perspective.

SOME APPLICATIONS OF A ONE-DIMENSIONAL METRIC FOR TRACKING BACTERIAL COMMUNITY DIVERGENCE USING SOLE CARBON SOURCE UTILIZATION PATTERNS

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Community level physiological profiling (CLPP) has become a popular method to characterize and track changes in heterotrophic bacterial communities. Although the CLPP method is a straight forward laboratory protocol which yields large amounts of functional information, the amount of data obtained can become overwhelming and often requires some type of multivariate analysis method for ordination and interpretation. A new approach to analyzing CLPP data and sole carbon source utilization data will be presented and illustrated. A one-dimensional (1-D) community metric has been developed and derived using Euclidean distances and shifts in sole carbon source utilization patterns (CSUPs) gathered using BIOLOG™ ECO plates[1]. Four case studies will be presented to illustrate the potential of this approach when applied to CLPP data. The case studies to be presented will include the use of the one-dimensional metric to: (I) assess functional bacterial community development during the start-up phase for wetland mesocosms; (II) assess functional bacterial community divergence in wetland mesocosms in response to exposure to the antibiotic ciprofloxacin; (III) assess and quantify the functional bacterial community differences at different depths in the supporting substrate of wetland mesocosms and (IV) assess and quantify the functional bacterial community changes during a corn silage retting process for the production of biofibre.
The 1-D metric has also been used to quantify shifts in banding patterns from denaturing gradient gel electrophoresis (DGGE) during case study (II) and will be compared to the 1-D metric results for the CLPP data collected over the same time period [Figure 1].

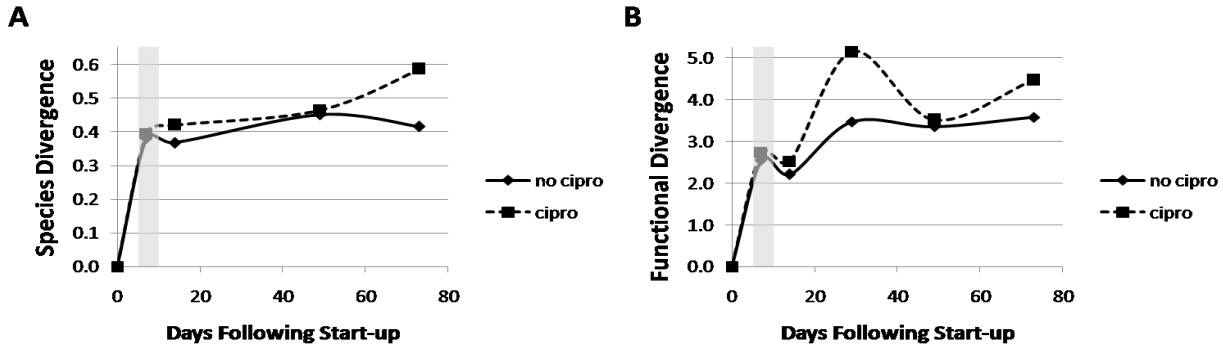

Figure 1: Bacterial community divergence in wetland mesocosms based on the Euclidean distances between DGGE banding patterns (A) and CSUPs (B) during microbiological development for two wetland mesocosms exposed to 2 mg/L ciprofloxacin for 5 days (cipro) and to mesocosms not exposed to ciprofloxacin (no cipro). Vertical grey bars represent the ciprofloxacin exposure period.

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POSSIBLE USE OF BIOLOG METHODOLOGY FOR MONITORING YEAST PRESENCE IN ALCOHOLIC FERMENTATION FOR WINE-MAKING

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The Biolog system is a well known methodology for the metabolic fingerprinting of microorganisms, based on the evaluation of their capacity of attacking a standard array of substrates contained in the micro-wells of ELISA plates, where the absorbance changes induced by microbial activity on a redox indicator added to the substrates are spectrophotometrically recorded. So far, the system has been used for pure culture identification and/or mixed populations' characterization, but for its specific features could also be employed to follow the evolution of microbial populations during bio-technological processes.
An application of Biolog system was developed to be used for monitoring yeast population evolution during alcoholic fermentation for wine-making. Such application allowed the elaboration of yeast growth curves (each one characteristic for each yeast population taken into consideration) that resulted suitable to discriminate between different oenological yeast species in pure culture and/or mixed consortia, in various cell concentrations. In particular, this application was found able to detect, in mixed populations, the presence of active dry yeasts used as starters in guided fermentation processes, suggesting the possibility to check the regular progress of the fermentation evaluating their capacity to overcome autochthonous yeasts.
The experimental curves obtained from Biolog application were expressed by suitable mathematical models. Three mathematical models that are normally used in predictive microbiology to express microbial growth have been tested and the one with best fitting properties (Lindstrom's equation) chosen. The results indicate the possibility of a predictive use of such model for the monitoring of yeast populations during alcoholic fermentation, in view of an early evaluation of an expected yeast evolution.

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BIOCIDE RESISTANCE ASSOCIATED PHENOTYPES: A HOT TOPIC IN LIGHT OF THE CLAIMED INTERCONNECTION BETWEEN BIOCIDE AND ANTIBIOTIC CO- OR CROSS-RESISTANCE

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Biocides have been in use for hundreds of years for antisepsis, disinfection and preservation. Despite this widespread and ever increasing use most bacterial and fungal species remain susceptible to biocides. The dramatic increase and spread of resistance to antibiotics linked to reports of co- and cross-resistance between antibiotics and biocides has raised speculation about the potential hazard of biocide use. The overarching question which BIOHYPO aims to address is: has the use of biocides contributed to the development and spread of clinically significant antibiotic resistance in human pathogens? Screening of over 1000 clinical Staphylococcus aureus isolates has been conducted for susceptibility to chlorhexidine, benzalkonium chloride, trichlosan and hypochloride. In parallel in vitro isolation of mutants with decreased susceptibility to these compounds was performed. Preliminary data of isogenic strain pairs, analyzed by standard antimicrobial susceptibility testing and phenotype microarray indicate no evidence for cross-resistance between biocides and antibiotics in S. aureus. Identification and tracking of defined phenotypes and molecular markers will provide a statistically significant dataset to evaluate if the hazard of co- and cross-resistance can be considered a risk for increased of clinically-relevant antibiotic resistance.

* the BIOHYPO consortium includes MR. Oggioni, G. Orefici, I. Morrissey, L. Baldassarri, J. Almeida, AT. Freitas, Y. Ulku, HJ. Roedger, P. Visa, L. Moce, JL. Martinez, A. Kalkanci, D. Mora, S. Leib, C. Viti, M. Elli

PART II

POSTERS

VARIABILITY OF *FUSARIUM* OXYSPORUM F. SP. *CICERIS* (F.O.C.) ISOLATES CAUSING WILT DISEASE ON CHICKPEA

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Ten isolates of *Fusarium oxysporum* f. sp. *ciceris (F.o.c.).*were isolated from infected chickpea plants collected from different Egyptian governorates. The tested isolates were proven to pathogenic on chickpea and highly susceptible cultivars of other legumes crops under favorable greenhouse conditions. Only chickpea plants were vulnerable to infection by tested *Fusarium oxysporum* isolates at different levels, this result indicated that all tested isolates were *Fusarium oxysporum f. sp. cicreis.* Morphological characterization and BIOLOG system were used to assess variability within the *Fusarium oxysporum* isolates, and used to determination the relationships between morphological characters and metabolism fingerprint for tested isolates. Presented data indicated that there was no correlation between morphological characters and metabolism fingerprint for tested isolates. However, there was correlation between pathological characterization and metabolism fingerprint for all isolates.

IDENTIFICATION AND CHARACTERIZATIONS OF DIFFERENT *TRICHODERMA* SPP. USING BIOLOG TECHNIQUE

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The ascomycete *Hypocrea* (*Trichoderma*) is the most common biological control agents in different soil type. Here we used the Biolog Phenotype MicroArrays technique to identification, characterization and evaluate the growth of *Trichoderma* isolates on 95 carbon sources. For this purpose, we compared between several isolates by morphological charactrization. The carbon utilizations of *Trichoderma* species were determine if there are differences in the carbon utilizations of selected isolates of these species. Analysis of biochemical characters revealed that *T. hamatum, T. harzianum, T. aureoviride, T. atroviride, T. viride and T. strictipile* formed clearly defined clusters, thus exhibiting species-specific metabolic properties, and although overlapping clusters, indicating that some species as *T. harzianum and T. hamatum* may share overlapping metabolic characteristics.

DEVELOPMENT OF A DNA MICROARRAY TO DETECT PROKARYOTES AND ITS APPLICATION TO THE ENVIRONMENTAL WATER IN JAPAN

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Metagenomic analysis that utilizes DNA sequencing still needs huge cost and specific facilities. To perform a simple metagenomic analysis that can be applicable to evaluate global warming in Japan, we have designed a DNA microarray to detect prokaryotes by utilizing 16S rRNA sequences from the DNA database of Japan (DDBJ) (ftp://ftp.ddbj.nig.ac.jp/ddbj_database/16S). One to three different 29 mer probes were designed against each of 97,927 sequences according to the method by Roche Diagnostics, and total of 258,697 probes were mounted to the array chip with control and random probes in duplicate. This prokaryotes microarray is expected to identify about 64,000 bacteria and archea species.

One liter of environmental water samples were collected from nine places in Japan and passed through a 0.22 micron filter. Whole DNA was extracted from the filter and uniformly amplified using a whole genome amplification (WGA) method. The amplified DNA was then labeled with Cy3 or Cy5 and the labeled DNA was hybridized with the array. The chip was scanned to detect array signals and the results were normalized and subjected for analysis. When 16S rRNA-specific primer and random primer were compared for labeling, the former showed lower background and higher signal to noise ratio.

We could successfully identify several bacterial species specific to the seawater in low temperature, which can be used as index species for global warming. We have also determined pathogenic bacteria that are known to cause human infectious diseases and to produce marine toxins.

The prokaryotes including bacteria and archea in the environmental water compose the basis of the food chain and the changes of their biomass or composition influence the entire water ecosystem. According to the estimation by Census of Marine Life, microbes including prokaryotes constitute 50-90% of all ocean biomass in the density of one billion cells per liter. The DNA microarray described herein may be an effective tool to evaluate the diversity of prokaryotes and to assess global warming.

THE BIOLOG PLATES TECHNIQUE AS A TOOL IN STUDIES OF *LACTOCOCCUS LACTIS* MUTANTS

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Lactococci belong to lactic acid bacteria, which are commonly used for various processes in the agro-food industry. In lactococci genes of biotechnological importance are mainly plasmid-located, however, some important technological features may be also chromosomally-encoded.
The CcpA and YebF proteins of the plasmid-free *L. lactis* IL1403 strain are transcriptional regulators involved in global catabolite repression and activation of transcription, respectively. Two mutants in the *ccpA* or *yebF* genes and one double *ccpA;yebF* mutant were constructed. In order to study the phenotypic variability of mutants obtained the Biolog analysis was performed on plates containing carbon, nitrogen, phosphorus or sulfur sources. As a control the wild-type IL1403 strain was used.
Additionally, during this study the *L. lactis* IL594 strain was used, which is the parental strain of IL1403 and contains seven plasmids. In order to verify if plasmidic genes have any influence on chromosomal genes expression the phenotypic analysis with the Biolog instrument was performed with IL594 strain as well as with strains containing some plasmidic genes.

This work is partly supported by the grant from the Polish Ministry of Science and Higher Education No. N N302 0154 33.

ADAPTATION OF *PSEUDOMONAS AERUGINOSA* TO CHANGING ENVIRONMENTAL CONDITIONS BY THE FORMATION OF ALANYL-PHOSPHATIDYLGLYCEROL

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Bacterial lipid homeostasis is a cellular process with special importance under infectious conditions or in the presence of antimicrobial agents. For the opportunistic pathogen *P. aeruginosa* modification of the phospholipid phosphatidylglycerol (PG) into an aminoacylated phosphatidylglycerol (alanyl-PG) has been described in response to acidic conditions (S. Klein *et al.*, 2009). Therefore the tRNA-bound amino acid alanine is transferred onto the 2' or 3' hydroxyl group of PG by an aminoacyl-PG synthase. This enzyme is located in the inner bacterial membrane and consists of an N-terminal transmembrane domain and a C-terminal domain which is proposed to be essential for the transesterification process.
It was shown that the overall lipid content of *P. aeruginosa* contains up to 6 % alanyl-PG when the cells are cultivated at a pH of 5.3. Furthermore phenotype microarray analysis revealed resistance against the cationic antimicrobial peptide protamine sulfate, the β-lactame cefsulodin, the osmolyte lactate and against Cr^{3+} based on alanyl-PG formation.
Resistance phenotypes due to aminoacylated phosphatidylglycerol have been generally ascribed to the overall modification of the net negative charge of the bacterial membrane (Staubitz *et al.*, 2004; Roy, 2009). Furthermore mechanisms including the alteration of the membrane fluidity or the permeability of the bacterial membrane are discussed in the literature (Roy *et al.*, 2009).

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INVESTIGATING THE IMPACT OF PGPB *ENTEROBACTER RADICINCITANS* ON PHENOTYPIC AND GENETIC CHANGES IN TOMATO PLANTS

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Plant colonizing bacteria like *Enterobacter radicincitans* can exert beneficial effects on plant development and growth via direct or indirect mechanisms. Fixation of biological N_2, *in vitro* production of phytohormones such as auxines and cytokinins, and solubilisation of P-compounds have all been demonstrated for *E. radicincitans*. The bacterium shows chemotactic affinity to plant roots, and successfully competes with the native microflora. Inoculation experiments revealed the ability of the bacterium to colonize plant roots and leaves without inducing plant defence mechanisms and to migrate within the plant.
To test whether and how the bacteria effects plant performance, we inoculated tomato seeds with *E.radicincitans*. Non-inoculated control plants and inoculated plants were grown in a greenhouse approach. Results showed a direct correlation for colonization with *E. radicincitans* and a better performance of tomato plants. We determined significantly increased stalk length, leaf fresh weight, dry weight, earlier flowering and fruit development for *E. radicincitans* treated plants compared to non-treated plants. Expression studies of genes involved in the nitrogen metabolism and phytohormone production in non-inoculated and inoculated leaf and root tissue of the plant and in the bacteria itself are on the focus of further research.

POLYPHASIC APPROACH FOR DIFFERENTIATING *P. NORDICUM* FROM *P. VERRUCOSUM*

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Surface moulding of meat products by environmental contaminating species, mainly belonging to the genera *Penicillium* and *Aspergillus*, should be always avoided, as some of such species proved to be toxigenic. In particular, on such products the presence of moulds such as *Penicillium verrucosum, Penicillium nordicum* and *Aspergillus ochraceus* must be kept under control, since they can produce ochratoxin A (OTA), a secondary metabolite that proved to be highly toxic and has been classified by the IARC as potentially carcinogenic to humans (Group 2B).
With regard to this, although differentiation between *Penicillium* and *Aspergillus* is reliably attainable by the morphological method, such method cannot be used to differentiate *P. verrucosum* from *P. nordicum*, as they are morphologically undistinguishable: only further cultural tests on selective media and the latest PCR techniques can at present do this. Moreover, as only little experimentations have been carried out on these strains by using additional techniques such as the measurement of consumption of carbon-based sources i automated systems (i.e. Biolog Microstation™) and the measurement of OTA produced by RP-HPLC, we applied a polyphasic approach to differentiate and to characterize some *Penicillium verrucosum* and *Penicillium nordicum* strains. In particular, we use:
(i) a cultural technique with two substrates (DYSG and YES) selective for these species;
(ii) a molecular diagnostic PCR recently set up;
(iii) an automated system based on fungal carbon source utilisation (Biolog Microstation™); (iv) and an RP-HPLC analysis to quantify OTA production.
Thirty strains from the SSICA collection, isolated from meat products and formerly identified as *Penicillium verrucosum* by the morphological method, were re-examined by both cultural tests and a PCR test: they all resulted to belong to the species *Penicillium nordicum*.
Their biochemical and chemical characterization supported the results obtained by cultural and molecular techniques applied and showed a different ability in *P. verrucosum* and *P. nordicum* to metabolize carbon-based sources and to produce OTA at different concentrations, respectively.
In particular, by means of Biolog Microstation™ we succeed to carry out a metabolite profiling of *P. nordicum*, in order to create a reliable "*P. nordicum* User Database" suitable for future identifications of isolates belonging to this species. The metabolic profiles of all the strains were then compared by using the RetroSpect™ Trending & Tracking Software and we found out that ß-Hydroxy-butyric acid and D-Cellobiose proved to be diagnostic carbon-based metabolized sources for *P. verrucosum* and *P. nordicum*, respectively.

RHIZOSPHERE BACTERIA CONTROLLING PLANT GENE REGULATION – FIRST RESULTS IN *ARABIDOPSIS* ECOTYPES

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Numerous investigations indicate the ability of rhizosphere bacteria to enhance plant growth Merbach *et al.* (1999, 2009). They showed increased amounts of exudates in plants which were colonized by rhizobacteria resulting in a higher phosphate-solubility. The rhizobacterium *Enterobacter radicincitans* DSM 16656 colonizes roots and shoots of cereals (Remus *et al*., 2000) and *Brassica* species (Schreiner *et al*. 2009). Besides strong growth- and yield increase in *E. radicincitans* -colonized pepper, also kohlrabi and radish showed an enhanced growth of tuber, shoot and leaves after DSM 16656 -inoculation.
According to these observations the effect of growth promoting bacteria like the well characterized strain DSM 16656 was investigated on the model plant *Arabidopsis thaliana*. Possibly the colonization of DSM 16656 depends on the plant glucosinolate composition (Schreiner *et al*. 2009). Therefore different *Arabidopsis* ecotypes with different glucosinolate patterns were used. Interestingly, DSM 16656 has a growth promoting effect on only one out of six tested ecotypes whereas growth of other ecotypes was similar to the control or even suppressed after DSM 16656 treatment. Hence, a microarray of leave and root tissue was initiated to find genes expressed differently in DSM 16656 treated plants compared to non-inoculated control treatments. Further investigations will be focused on the question how growth promoting bacteria regulate phosphate and nitrogen pathways in *Arabidopsis*.

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FREQUENCY DISTRIBUTION AND CHANGING PATTERN IN HEPATITIS C VIRUS GENOTYPES: A 10 YEARS DATA ON 20552 CHRONIC HCV CARRIERS.

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Information regarding the changing pattern in hepatitis C virus (HCV) genotypes/subtypes circulating in Pakistan and resulting disease outcome is not well known. The specific objective of this study was to find out the frequency distribution of HCV genotypes and changing pattern of various HCV genotypes over time in well-characterized Pakistani HCV isolates in different age and gander groups.

The genotype distribution of HCV from all the four provinces of Pakistan was tracked for a period of 10 years (2000-2009) on total 20552 consecutive patients, with 6216 (53.4%) males & 5436 (46.65%) females using type-specific genotyping assay.

Of these total, 16891 (82.2%) HCV patient's samples were successfully genotyped. Of the successfully typed genotypes 12537 (74.2 %) were with 3a, 1834 (10.9%) with 3b, 50 (0.24%) with 3c, 678 (3.3%) with 1a, 170 (0.83%) with 1b, 49 (0.24%) with 1c, 431 (2.1%) with 2a, 48 (0.23%) with 2b, 3 (0.01%) with 2c, 13 (0.06%) with 5a, 12 (0.06%) with 6a, 101 (0.49%) with 4, and 965 (4.7%) were with mixed genotype infection. A changing pattern was seen for all the prevalent genotypes of this country. Three genotypes such as 2a, 2b and 4 were gradually decreased and were completed eradicated from Pakistan. Genotype 3b was gradually decreased and is near to eradication. The proportion of genotype 3a increased gradually throughout this 10 years period. Two genotypes 1a and 1b suddenly increased in years 2003-2005 and then decreased significantly.

A changing pattern of HCV genotypes prevalence was observed in Pakistan overtime, with an increase in the relative proportion of genotype 3a & mixed genotypes and a decrease of genotypes 3b, 2b, 4, 5a and 2a. This changed HCV genotype pattern might have dual impacts, first it may require new therapeutic strategies, and secondly it may have potential clinical implications on HCV disease outcome.

SYSTEMATIC ANALYSIS OF IgG ANTIBODY IMMUNE RESPONSE AGAINST VARICELLA ZOSTER VIRUS (VZV) USING A SELF-ASSEMBLING PROTEIN MICROARRAY

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Varicella zoster virus (VZV) is a human herpesvirus encoding at least 69 distinct viral proteins which causes chickenpox after primary infection and shingles during reactivation and which is particularly important in pregnancy and immunocompromised patients. Current serodiagnostic tests are either based on whole cell lysates or glycoprotein preparations. In order to investigate the humoral immune response to VZV infection or vaccination in more detail, and to improve the currently available diagnostic assays, we developed a nucleic acid programmable protein array (NAPPA) containing all 69 VZV proteins and performed a detailed analysis of 68 sera from individuals with either no, a previous or an acute VZV infection. In addition to the known reactive glycoprotein antigens (ORF 5, ORF 14, ORF 31, ORF 37, ORF 68), we discovered IgG antibodies against a variety of other membrane (ORF 2, ORF 24), capsid (ORF 20, ORF 23, ORF 43) and tegument (ORF 53, ORF 9, ORF 11) proteins, as well as other proteins involved in virus replication and assembly (ORF 18, ORF 25, ORF 26) and the transactivator proteins ORF 12, ORF 62 and ORF 63. All of these antigens were only reactive in a subset of VZV-positive individuals. A subset of the newly identified VZV antigens was validated by western blot analysis. Using these seroreactive new VZV antigens, more sensitive assays and tests distinguishing between different clinical entities may be developed.

BACTERIAL COMMUNITIES ASSOCIATED TO OUTDOOR CULTURES OF THE MARINE MICROALGA *TETRASELMIS SUECICA*.

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Microalgae in culture collections are usually not axenic, *i.e.* they are associated with bacteria that were co-enriched and isolated together with the algal cells and then selected during algal maintenance and culture. Moreover outdoor cultures of microalgae are carried out in non-sterile conditions and are thus susceptible to contaminations. Several studies have reported that bacteria may both stimulate or inhibit algal growth (1); they are able to produce algicidal compounds, regulate algal production of toxins (2) and alter algal physiology (3). Therefore they can play an important role on the stability and productivity of microalgal cultures. Although many microalgal cultivation technologies have been developed and culture conditions are optimized to improve biomass productivity, little is known about bacterial communities associated to microalgal mass cultures. The objective of the present study was to analyze the bacterial community associated with an outdoor culture of *Tetraselmis suecica* strain OR and to determine the influence of this community on the algal growth. *Tetraselmis suecica* is a marine flagellate green alga of the class Prasinophyceae widely used in aquaculture, which has shown a high probiotic potential and an inhibitory activity against *Vibrio* species (4).
T-RFLP was applied to follow the dynamics of the bacterial community associated to the culture in different periods of the year. Culturable bacteria isolated from the laboratory culture (used as inoculum for the outdoor culture) and from the outdoor culture collected eight months after inoculation were clustered into operational taxonomic units by ARDRA analysis and then identified through 16S rDNA sequencing. Batch cultures inoculated with outdoor, laboratory and axenic cultures were carried out in 0.5-L bubbled tubes under laboratory conditions to evaluate the influence of bacterial communities on algal growth.
The outdoor algal culture showed a lower diversity in the associated bacterial communities, compared to the laboratory one, and a seasonal variation of the bacterial population, with only few ribotypes constantly present in the algal culture. Strains belonging to the *Roseobacter* clade and to the *Flavobacteriales* and *Rhizobiales* groups appeared to be closely associated to the culture. The presence of an associated bacterial community demonstrated a positive influence on the algal growth under laboratory conditions (higher productivity than the axenic culture).
From these results it appears that the bacterial flora in *Tetraselmis suecica* cultures is only in part resilient and that it has a positive effects on culture productivity. Further studies will be carried out to better understand the role of the closely associated bacteria on algal growth and bioactivity under laboratory conditions.

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ANALYSIS OF METABOLIC PROFILE OF A FLOR YEAST MUTANT OF *SACCHAROMYCES CEREVISIAE* BY USING PHENOTYPE MICROARRAY™ TECHNOLOGY

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Sardinian wine strain of *Saccharomyces cerevisiae* used to make sherry-like wines form a biofilm at the air-liquid interface at the end of the ethanolic fermentation, when grape sugar is depleted and further growth becomes dependent on the access of oxygen. We previously showed that *FLO11*, which encodes for a hydrophobic cell wall glycoprotein, is required for the air-liquid interfacial biofilm and that the biofilm cells have a buoyant density greater that the suspending medium. We also performed a transcriptome microarray experiment, comparing the cells in the bottom of the vessel versus cells in the biofilm, but the obtained results were not exhaustive enough. The aim of this study has been the comparison of wild type flor yeast with its isogenic derivative containing a knockout of *FLO11* gene by using Phenotype Microarray™ analysis, for simultaneously testing cellular phenotypes especially related to biofilm formation. We analysed wild-type and mutant for metabolic, osmolyte, pH and chemical sensitivity panels. Results obtained after an incubation of 96 hours showed differences between wild-type and Δ*flo11* behaviour. Particularly, mutant showed, compared with the wild-type, inability to metabolize peptides mostly formed by lysine and/or histidine and other amino acids and, on the contrary, ability to growth in presence of different osmolytes at different concentrations and different ranges of pHs.

BIOCOMPETITION AS A TOOL TO COPE WITH THE PROBLEM OF AFLATOXIN CONTAMINATION OF FOOD AND FEED COMMODITIES: MAY PHENOTYPE MICROARRAY TECHNOLOGY CONTRIBUTE TO THE INDIVIDUATION OF "GOOD" COMPETITORS?

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Aspergillus flavus is a well known plant pathogen responsible of economic losses and food safety concern due to the ability of mycotoxin production. Aflatoxin contamination of crops is a significant problem worldwide, and many Countries have set more or less strict limits for mycotoxin presence in feed and food commodities. An interesting strategy to cope with aflatoxin contamination in susceptible crops involves the use of intraspecific competition to interfere with mycotoxin production by the relevant *Aspergillus flavus* strains. Atoxigenic *A. flavus* strains ($afla^-$), unable to produce the relevant mycotoxin, have been already used as bio-competitors to decrease aflatoxin accumulation on cotton, maize and peanuts fields. Selecting a strain performing as a strong bio-competitor is not a straightforward task since it depends on previous assessment of various interacting factors conditioning the relative fitness of the strains in a given ecological niche. Reconstruction experiment have been generally performed in laboratory conditions to uncover the biological mechanisms underlying the efficacy of atoxigenic strains in preventing aflatoxin production and/or to give a preliminary indication of strain performance when released in the field.
Our present goal is 1) characterization of *A. flavus* population colonizing the corn field of the Po valley and 2) setting a strategy for the identification of "good" competitors among $afla^-$ strains isolated from the above mentioned *A. flavus* population. For this purpose we are performing competition experiments in laboratory conditions and designing "high throughput" procedures to test as many as possible environmental conditions that may affect competition efficacy. Moreover, $afla^-$ strains are generally considered to be derived from aflatoxin producer strains ($afla^+$) that have lost the capacity to produce the relevant mycotoxin as a result of mutation(s) affecting gene(s) belonging to the Aflatoxin biosynthetic cluster. $afla^-$ strains are obviously expected to be altered in one or more steps of secondary metabolism. However the story might be more complex than expected, since regulatory interactions between pathways of primary and secondary metabolism have already been described. In this respect, Phenotype MicroArray technology may represent an additional value for our goal if the specific metabolic features of different $afla^-$ strains could be correlated to their competitive ability. In the present study we have performed, as a preliminary approach, experiments to verify if $afla^+$ and $afla^-$ strains may be differentiated by the use of the Biolog FF physiological identification kit.

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FUNCTIONAL DIVERSITY OF FUNGAL ASSEMBLAGES DECOMPOSING THE LEAF LITTER OF A NATURAL MEDITERRANEAN ENVIRONMENT

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Saprotrophic fungi have a wide range of important ecological functions associated with nutrients and carbon cycling processes in leaf litter and soil. Functional diversity of some strains of filamentous fungi associated to leaf litter decay in the Mediterranean maquis was analysed and compared by means of Biolog™ FF microplates . The aim of the study is the evaluation of the relationship between the potential activity showed by pure fungal isolates (*in vitro*) and their actual ecological role in the field. Fungal frequencies and occurrence during the trophic succession on the natural resource were studied and used as key for understanding the metabolic profiles obtained *in vitro*. This approach can clarify the role of some key fungal species in the decomposition process within natural ecosystems.
The study was carried out in the Natural Reserve of Castel Volturno (Southern Italy) where litter bags of pure and mixed leaves of low maquis plant species were incubated in one-year field experiment. Fungal species were isolated from decaying leaves and identified. Many species were not easy to be isolated in fact their reproductive structures have a close physical relationship with the natural substrate. Thirteen of these fungal isolates (including *Beltrania querna* Harkn, *B. rhombica* Penz. and *Circinotrichum maculiforme* Nees.) were selected being representative of the relevant fungal community. Three categories of occurrence frequencies were used to group the fungal species. Moreover, three different sampling times were considered. Assemblages of fungal species based on their natural occurrence in the field were compared with their potential metabolic abilities by means of multivariate statistical analysis (Discriminant Analysis and ANOVA). Results showed that fungi, during the trophic successions, occurred on the natural resource with a frequency that was strongly related to their metabolic profile as expressed *in vitro* with Biolog FF plates. Fungi replaced one another on the natural substrate in space and time, and the relative abundances of each species changed together with the potential ability of utilising particular clusters of substrates.
The possibility of utilizing different spatial and temporal trophic niches driven by succession give a likely explanation of the positive effects that an apparent redundance of fungal species can have on leaf litter decomposition.

MICROBIOLOGICAL CHARACTERIZATION OF RHIZOBIA FROM *PHASEOLUS VULGARIS* CROPPED IN SEMI-ARID SOILS MANURE MANAGED

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Beans (*Phaseolus vulgaris* L.) are widely consumed around the word and their adaptability depends on the climatic condition in the cropped area. It as been reported that under semi-arid condition nodule formation and rhizobial nitrogen fixation are highly affected by environmental constrains: salinity, drought, high temperature.
Therefore, for the cultivation of legumes in semi-arid area under environmental friendly conditions the application of sustainable agronomic practices (i.e. manure, green manure and organic farming) is required.
The present study aimed to investigate the capabilities of sole-carbon-source utilization of rhizobia isolated from semi-arid soils, in Sud Italy, where common bean *(P. vulgaris)* is traditionally cultivated in the dry season adopting sustainable agronomic soil management.
Plant infection tests were performed in Gibson tubes (*in vitro* systems for *Leguminosae-Rhizobium* symbiosis studies) using plantlet of *P. vulgaris* as host plant trap.
Rhizobial isolates from nodules were characterised by means of plant infectiveness assays and BIOLOG bacterial identification system (GN2 microplates and microstation, BIOLOG ™, Hayward, CA, USA). Furthermore, in order to compare rhizobial catabolic ability of organic substrates the niches overlapping indices (NOI) were evaluated for each rhizobial group recovered from the different sites.
The results obtained show that the most probable enumeration (MPN) of rhizobia does not change significantly in soils with different content of soil organic matter (SO) and same management with locally available organic materials. In all soils the occurrence of dominant *Rhizobium* spp. strains were observed: putative rhizobial *Agrobacterium*-like isolates were moreover recovered from nodular habitat. Comparison of NOI highlight nutritional versatility of some isolates suggesting the high metabolic affinity of rhizobia from sites with different SO and managed with buffalo manure.

COMPARING *YERSINIA ENTEROCOLITICA* BIOTYPES USING THE PHENOTYPE MICROARRAY

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Yersinia enterocolitica is a heterogeneous species comprising five biotypes (BTs) based on biochemical differences and with varying degrees of pathogenicity. It is found in a wide range of environments and some biotypes are able to cause disease such as gastroenteritis in humans. In some countries it rivals *Salmonella* as a foodborne pathogen and because it can grow at refrigeration temperature it is an increasing concern in terms of food safety. In this study we used bacterial Phenotype MicroArrays to characterise representative strains of the five BTs at two different temperatures. We also screened tolerance to compounds in complex media. The data obtained will help confirm conventional biotyping for *Y. enterocolitica* and be used to identify new metabolic pathways that can ultimately be compared back to genomic data to establish a link between genomic and phenotypic differences.

THE EFFECT OF ROOT LEACHATES FROM SIX DIFFERENT PLANTS ON SOIL MICROBES ASSOCIATED WITH TWO PLANT SPECIES AS INDICATED BY BIOLOG ECOPLATES™

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Microbes are known to secrete, use and/or alter chemical compounds in soil. Amongst these compounds are root exudates of plants. Many root exudates contain allelopathic chemicals. The allelopathic process involves secretion of bioactive compounds or allelochemicals, by plants or microbes, which inhibit or stimulate growth of neighbouring plants. In this glass-house study, Biolog EcoPlatesTM were used to indicate the effect of root leachates from six different plants on the soil microbial populations associated with wheat and ryegrass plants respectively, grown in pots with soil from the same origin as that of the soil in the pots of the donor plants. Root leachates from donor plants were added to the acceptor plants (wheat and ryegrass) on a weekly basis until plants reached maturity. Soil samples from acceptor pots were used to inoculate Biolog EcoPlatesTM and the carbon utilisation patterns were compared to the pattern obtained for the soil microbial populations before treatment commenced. From the results it is clear that Biolog EcoPlatesTM can be used successfully to indicate allelochemical activity in the soil.

PRELIMINARY TECHNOLOGICAL CHARACTERIZATION OF *LACTOBACILLUS PLANTARUM* ISOLATED FROM CHEESES

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Lactobacillus plantarum is one of the non-starter lactic acid bacteria (NSLAB) commonly found among the adventitious microorganisms that develop in nearly all cheese varieties. NSLAB are usually present because of post-pasteurization contamination, but may also constitute part of the raw milk microflora and survive pasteurization There is increasing interest in the use of NSLAB as adjunct cultures in cheese manufacture to improve flavor and to accelerate ripening. As well as other LAB, *L. plantarum* has complex nutritional requirements and multiple amino acid auxotrophies. Its growth and survival in protein-rich substrates depends on their proteolytic and peptidolytic system. Proteinases play a crucial role in hydrolysis of proteins into oligopeptides, then peptides are transported into bacterial cell by specific transport system and degraded to free amino acids by a range of peptidases.

This work was aimed to estimate some dairy technological characteristics of 57 *L. plantarum* strains, 30 of them showing putative probiotic properties, isolated from cheeses.

Milk coagulation ability and proteolytic activity were evaluated in 10% (w/v) reconstituted skim milk, inoculated with 1% (v/v) of each strain previously grown in sterile milk, and incubated at 37°C. The ability of the strains to coagulate milk and the measures of pH were recorded at 2 and 7 days of incubation. The proteolytic activity, with *o*-phthaldialdehyde spectrophotometric assay at 340 nm (OPA test), was determined after 7 days. Aminopeptidase (AP) activities were determined on 4 β-naphtil aminoacids (Arg-βNa, Leu-βNa, Lys-βNa, Ser-βNa) and 1 β-naphtil dipeptide (Phe-Pro-βNa) in phosphate buffer solutions at 0.656 mM, pH 6.5. Washed cells from overnight MRS broth cultures were resuspended at 1.25 OD_{550nm}, and incubated 30 min at 40°C.

As expected, owing to their lower ability to metabolize lactose, the strains presented a slow milk fermentation capability. Only 17 strains were able to coagulate milk, causing a pH decrease to 4.3-4.7, often only after 7 days of incubation. For 14 of the 17 acidifying strains also the proteolytic activity resulted very low, with OD_{340nm} values ≤ 0.1.

AP activities, with some difference among strains, were always higher on Leu-βNa and Lys-βNa and lower on Arg-βNa, Ser-βNa and Phe-Pro-βNa. About 80% and 96% of the strains showed AP activities on Leu- and Lys-βNa, with values between 15-30 μg of β-naphtil released/h/ml, respectively. Another 18% of the strains showed activity on Leu-βNa higher than 30 μg β-Na/h/ml. This activity is important because it plays a key role in the hydrolysis of bitter peptides, and can impact on cheese quality by releasing of free leucine which is one of the precursor of branched chain fatty acids as aroma compound.

The main findings of this study can be summarized as follows: (i) the recovery of strains with low acidifying and proteolytic activities makes *L. plantarum* a good candidate as adjunct cultures because they could be used without deviations from cheese production standards; (ii) the presence of secondary proteolytic properties, as evidenced by a specific aminopeptidase activities, suggests the choice of selected strains to be applied to enhance cheese quality. Further phenotypic and technological strain characterizations are necessary to better define role and use according to specific applications for different cheese varieties.

PHENOTYPIC CHARACTERIZATION OF *SACCHAROMYCES CEREVISIAE* STRAINS DOMINATING SPONTANEOUS WINE FERMENTATIONS IN DIFFERENT WINERIES

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In spontaneous alcoholic fermentation of grape must, different yeast species are usually involved but the predominance of *Saccharomyces cerevisiae* on the other species because of its high ethanol tolerance has led to its recognition as the principal wine yeast. However, natural *S. cerevisiae* populations show a great intraspecific diversity so that many different strains can occur simultaneously or in succession during the whole fermentation process. The dynamics of *S. cerevisiae* strains during spontaneous wine fermentations have been extensively investigated in several oenological areas using various molecular methods such as mithocondrial DNA restriction analysis, the comparison of chromosomal DNA profiles and the amplification of different DNA regions by polymerase chain reaction. According to these surveys, in the majority of cases, each fermentation process is carried out by a small number of strains (one to three) occurring at higher percentages (more than 50% of the total biomass) associated with a variable number of secondary strains that are present at lower percentages. Nevertheless, due to serial dilutions during wine samples plating, these major and minor strains are to be considered as the *S. cerevisiae* dominanting the wine fermentations. In spite of this consideration, the higher frequency of some yeast strains could imply their better adaptation to the environmental conditions as a consequence of different types of selective pressures. In this work, to evaluate whether some technological characters of oenological interest such as the fermentation vigour, the ethanol tolerance and the presence of killer factor could be involved in determining the different frequencies of yeast strains, several *S. cerevisiae* isolates from spontaneous wine fermentations carried out in three wineries located in different and distant areas in Tuscany, were taken into consideration. All the assayed yeast strains, previously differentiated by mitochondrial DNA restriction analysis, were reported as major or minor strains depending on their frequency was more than 30 or less than 15%, respectively. The production of fermentation compounds as qualitative characters was also determined. All the oenological traits were evaluated by carrying out small-scale fermentations in synthetic grape must at 28°C and the time course of the fermentations was monitored daily by determining weight losses. At the end of the fermentation process, the concentration of the main oenological compounds (ethanol, glycerol, organic acids, higher alcohols etc.) was determined by HPLC and GC. Concerning the technological properties, no significant ($p < 0.05$) differences were found between major and minor strains. However, the principal component analysis of the fermentation products pointed out a strain differentiation according to the winery where the yeast strains were isolated, suggesting the occurrence of a strict relationship between *S. cerevisiae* strains and their isolation sources.
In conclusion, results obtained form this phenotypic characterization didn't furnish a possible rationale for the occurrence of major and minor *S. cerevisiae* strains in spontaneous wine fermentations and suggest that other cytological or biochemical or physiological properties could be involved in determining the dominance of one strain over others.

HIGH RESOLUTION MELTING ANALISIS IS USEFUL FOR RAPID AND ACCURATE DISCRIMINATION OF *P. SAVASTANOI* STRAINS AND PATHOVARS

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Pseudomonas savastanoi pv. *savastanoi* is the causal agent of olive knot disease. The strains isolated from oleander and ash belong to the pathovars *nerii* and *fraxini*, respectively. When artificially inoculated, pv. *savastanoi* causes disease also on ash, and pv. *nerii* attacks also olive and ash. Surprisingly, nothing is known yet about their distribution in nature on these hosts and if spontaneous cross-infections occur. On the other hand sanitary certification programs for olive plants, also including *P. savastanoi*, were launched in many countries. European certification programmes classify this bacterium as a quarantine microorganism and it would be necessary to set up a large number of diagnostic protocols for an easy and rapid identification of this bacterium even on/in asymptomatic propagation material.
The aim of this work was to develop an innovative PCR-based tool for the rapid and differential detection of these *P. savastanoi* pathovars. Here for the first time High Resolution Melting Analysis (HRMA), a modern and very efficient technique for SNP analysis, was applied to simultaneously discriminate and detect phytopathogenic bacteria *P. savastanoi* pv. *savastanoi*, pv. *nerii* and pv. *fraxini*.
Specific PCR primers for some *P. savasatanoi* highly-conserved genes were designed, supposed to be able to detect SNPs specifically present on these genes, as previously assessed by sequencing. The specificity of all these assays was 100%, as established by testing sixteen *P. savastanoi* strains, belonging to the three pathovars examined and having different geographical origins.
We were able to discriminate each *P. savastanoi* pathovar, with a single HRM run. Moreover, we demonstrated the possibility to use HRMA in multiplex assay, and we are also able to associate this type of analysis to the phylogeny of this species.
Compared with the other methods already available for *P. savastanoi*, the identification procedure here reported has the same reliability but is a faster and more economic tool both for epidemiological and ecological studies on these pathovars, and for diagnostic procedures monitoring the asymptomatic presence of *P. savastanoi* on olive and oleander propagation materials.

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CHARACTERIZATION AND SELECTION OF LACTIC ACID BACTERIA TO BE USED TO RESTRICT BIOGENIC AMINE ACCUMULATION IN DRY FERMENTED SAUSAGES

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Biogenic amines (BAs) are nitrogenous low molecular weight organic bases that can be found in several foods, especially in fermented foods, mostly as a consequence of the decarboxylation of their respective free precursor amino acids, through the action of substrate-specific microbial decarboxylases. These molecules, owing to their psychoactivity and vasoactivity, can cause different toxicological effects to humans, depending on the specific BA, its concentration and individual sensitivity. In addition, BAs are known to be potential precursors of carcinogenic nitrosamines. Since fermented sausages are known to sustain a potentially high accumulation of BAs, because their high protein content associated with the proteolytic activity of the microbial populations occurring during fermentation and ripening provide precursors for BA formation, most research activities in recent years are addressed to solve or to reduce this problem. The addition of negative amine-producer starter cultures to carry out a controlled fermentation could be an advisable biotechnological practice to prevent excessive BA accumulation in dry sausages, but, at least in most cases, it does not suppress survival and growth of indigenous bacterial populations able to decarboxylate BA precursors. In this connection, the current main challenge is to develop starter cultures selected from indigenous populations, well adapted to a particular product and possibly highly competitive against the contaminant microorganisms, so that both typical characteristics and safety of a traditional fermented sausage could be attained.
In this study, 47 isolates of lactic acid bacteria (LAB), obtained from two traditional fermented sausages produced using meat of two local breeds, *Cinta senese* and *Nero siciliano*, without adding commercial starter cultures, were characterized in order to select appropriate starter cultures. By PCR-ARDRA analysis, 36% of the isolated belonged to *Lactobacillus plantarum* species, 49% to *Lactobacillus sakei* and the remaining 15% to *Lactobacillus curvatus*. The isolates were characterized in respect to their technological properties, i.e. growth and growth rate at different salt or nitrate concentrations, acidifying capability, gas production from glucose, incapability to produce BAs, proteolytic and lipolytic activities. All the experimental data were statistically treated by cluster analysis. In order to identify the most appropriate LAB isolate(s), one "virtual" isolate, possessing all the desired technological properties and thus representing the "best" LAB, was included into the cluster analysis of the experimental data. Two isolates, both belonging to the *L. plantarum* species, were selected because of their smallest linkage distance from the virtual strain. Moreover, the competitiveness of the two selected isolates against a BA producing bacterial strain was assayed in SB broth, a medium widely used to simulate the technological conditions of sausage manufacturing. HPLC analysis of the spent medium, carried out after 15 days of incubation, showed that only one isolate, originally obtained from a sausage made with *Nero siciliano* meat, was able to inhibit completely any release of BAs, suggesting its use as a starter culture in the manufacture of fermented sausages of *Nero siciliano* breed.

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GENETIC ANALYSIS OF N-ACETYLGALACTOSAMINE AND GALACTOSAMINE UTILIZATION BY *E. COLI* O157:H7 AND *E. COLI* C

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The pathway for N-acetylgalactosamine (Aga) and D-galactosamine (Gam) catabolism in *Escherichia coli* was proposed by *in silico* studies of the genes of the *aga/gam* cluster of *E. coli* C. An intermediate in the pathway is Gam-6-P, which is converted to tagatose-6-P by Gam-6-P-deaminase/isomerase coded by *agaI*. *E. coli* C has an intact *agaI* but the *agaI* gene in *E. coli* O157:H7 (strain EDL933) is annotated as a split gene because a mutation in the 72nd codon leads to a premature stop codon. Both *E. coli* strains utilize Aga but only *E. coli* C can utilize Gam. If the *agaI* gene is essential for Aga catabolism in both strains then in *E. coli* O157:H7 the two split genes together must be coding for a functional enzyme. To test this, both the split genes of *agaI* in *E. coli* O157:H7 and the intact *agaI* in *E. coli* C were deleted and tested for Aga and Gam utilization by phenotypic microarray (PM) and by plating on MOPS minimal agar medium with the desired carbon sources. Furthermore, in order to test if the glucosamine-6-P deaminase/isomerase coded by *nagB* of the related N-D-acetylglucosamine catabolic pathway can substitute for *agaI* in the utilization of Aga and Gam (in *E. coli* C), Δ*nagB* deletion mutants and double knockout Δ *agaI*Δ*nagB* deletion mutants of *E. coli* C and O157:H7 were also constructed. The phenotypes of these knockout mutants were analyzed by PM analysis and by plating. The Δ*nagB* and Δ *agaI*Δ*nagB* mutants showed the expected phenotype by PM in that they were unable to utilize N-acetylglucosamine, N-acetylmannosamine, and N-acetylneuraminic acid. Importantly however, the utilization of Aga in *E. coli* O157:H7 and that of Aga and Gam in *E.* coli C were unaffected in Δ*agaI*, Δ*nagB,* and Δ *agaI*Δ*nagB* mutants of these strains. Thus, neither *agaI* nor *nagB* seem to be necessary for the utilization of Aga and Gam in *E. coli.* We are currently investigating the role of other genes of the *aga/gam* cluster in the utilization of Aga and Gam in *E. coli*.

PHENOTYPIC ANALYSIS OF *ERWINIA CHRYSANTHEMI* MUTANTS AFFECTED FOR GENES POTENTIALLY INVOLVED IN CARBOHYDRATE CATABOLISM

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Erwinia chrysanthemi (renamed *Dickeya dadantii*) is a plant pathogenic bacterium which has a large capacity to degrade oligosaccharides present in plant tissues. Sucrose (α-D-Glc*p*-(1↔2)β-D-Fru*f*) and raffinose (α-D-Gal*p*-(1→6)-α-D-Glc*p*-(1↔2)β-D-Fru*f*) are the most abundant soluble carbohydrates in plant tissues. The two genomic clusters *scrKYABR* and *rafRBA* contain genes potentially involved in their catabolism. Phenotypic analysis of *scr* and *raf* mutants revealed cross-links between the assimilation pathways of different oligosaccharides. Sucrose catabolism is mediated by the genes *scrKYAB*. The *rafRBA* cluster is sufficient for melibiose catabolism (α-D-Gal*p*-(1→6)-D-Glc*p*). However, it is incomplete for raffinose catabolism which needs two additional steps. We showed that, in *E. chrysanthemi*, these steps are provided by *scrY* and *scrB*. While *E. chrysanthemi* is unable to grow with lactose (β-D-Gal*p*-(1→4)-D-Glc*p*) as a sole carbon source, regulatory mutants with an inactivated RafR repressor can assimilate lactose using the de-repressed non-specific transport system RafB.

To test a large range of carbon sources, the *scr* and *raf* mutants were compared to the wild-type strain on Biolog Phenotype Microarray plates PM1 and PM2 for carbon catabolism. The mutant affected for the permease RafB was unable to assimilate either raffinose or melibiose. The mutant with an inactivated sucrose hydrolase ScrB was incapable of growth with either sucrose or raffinose. The mutant affected for the porine ScrY showed a reduced growth with either sucrose or raffinose. The mutant with an inactivated α-galactosidase RafA appeared unable to assimilate raffinose, melibiose and α-methyl-D-galactoside. Thus, in *E. chrysanthemi*, the α-galactosidase RafA is necessary for the cleavage of raffinose, melibiose and also of α-methyl-D-galactoside. The RafR$^-$ mutant was able to assimilate lactose but also lactulose (β-D-Gal*p*-(1→4)-D-Fru*f*), melibionic acid (α-D-Gal*p*-(1→6)-D-GlcA) and stachyose (α-D-Gal*p*-(1→6)-α-D-Gal*p*-(1→6)-α-D-Glc*p*-(1↔2)β-D-Fru*f*). It also showed and increased growth with β-methyl-D-galactoside. Thus, derepression of the *raf* genes could trigger the assimilation of various oligosaccharides related to their usual substrates, melibiose and raffinose. The *raf* cluster could facilitate either the uptake of these compounds by the permease RafB (lactose, lactulose and β-methyl-D-galactoside) or their cleavage by the α-galactosidase RafA (α-methyl-D-galactoside), or both activities (melibionic acid and stachyose).

Genome analysis was performed to identify other gene clusters potentially involved in carbohydrate catabolism, on the basis of the conservation in at least two bacterial species of adjacent genes encoding enzymes together with a transport system and a regulator. Systematic inactivation of a gene in each cluster will be followed by analysis of the mutants on Biolog plates PM1 and PM2. Preliminary data obtained on 10 mutants suggest a potential function for two of the selected clusters.

ADAPTATION OF THE BIOLOG PHENOTYPE MICROARRAY ™ TECHNOLOGY TO PROFILE THE OBLIGATE ANAEROBE GEOBACTER METALLIREDUCENS

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The Biolog OmniLog® Phenotype MicroArray (PM) plate technology was successfully adapted to generate a select phenotypic profile of the strict anaerobe *Geobacter metallireducens.* The profile generated for *G.m.* provides insight into the chemical sensitivity of the organism as well as some of its metabolic capabilities when grown with a basal medium containing acetate and Fe(III).

The PM technology was developed for aerobic organisms. The reduction of a tetrazolium dye by the test organism represents metabolic activity on the array which is detected and measured by the OmniLog® system. We have previously adapted the technology for the anaerobic sulfate reducing bacterium D*esulfovibrio vulgaris*. In this work, we have taken the technology a step further by adapting it for the iron reducing obligate anaerobe *Geobacter metallireducens*.

In an osmotic stress microarray it was determined that the organism has higher sensitivity to impermeable solutes 3-6% KCl and 2-5% NaNO3 that result in osmotic stress by osmosis to the cell than to permeable non-ionic solutes represented by 5-20% ethylene glycol and 2-3% urea.

The osmotic stress microarray also includes an array of osmoprotectants and precursor molecules that were screened to identify substrates that would provide osmotic protection to NaCl stress. None of the substrates tested conferred resistance to elevated concentrations of salt. Verification studies in which *G.m.* was grown in defined medium amended with 100mM NaCl (MIC) and the common osmoprotectants betaine, glycine and proline supported the PM findings. Further verification was done by analysis of transcriptomic profiles of *G.m.* grown under 100mM NaCl stress that revealed up-regulation of genes related to degradation rather than accumulation of the above-mentioned osmoprotectants. The phenotypic profile, supported by additional analysis indicates that the accumulation of these osmoprotectants as a response to salt stress does not occur in *G.m.* and response to stress must occur by other mechanisms.

The Phenotype MicroArray technology can be reliably used as a rapid screening tool for characterization in anaerobic microbial ecology.

EVOLUTIONARY ECOPHYSIOLOGY OF BARK BEETLE ASSOCIATED FUNGI

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Due to its great phylogenetic and ecological diversity the genus Geosmithia represents an exceptional model for the study of evolutionary ecology of fungi living in the symbiosis with the subcortical insects, and for the elucidation of the origin of ambrosia fungi. It is advantageous that Geosmithia species with shared traits have a different distribution in the phylogenetic tree and *vice versa*. This enables taxonomically unbiased testing of the hypotheses.

In this study, we used the Phenotype MicroArray analysis to investigate physiological adaptations of *Geosmithia* fungi to different ecological strategies. Carbon utilization profile was examined as possible adaptation to different host trees (broad leaved *versus* coniferous) and to the level of coevolution with insect vector (obligatory symbiosis of so called ambrosia fungi with xylemophagous insects *versus* weaker symbiosis with phloemophagous insects). We also made preliminary experiments to explore whether there is some evolutionary or ecological pattern in utilization of nitrogen, sulphur and phosphorus sources, or in dependence on some nutrient supplements.

TEMPERATURE AFFECTS SOLE CARBON UTILIZATION PATTERNS OF *CAMPYLOBACTER COLI 49941*

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Campylobacter spp. are small, asaccharolytic bacteria exhibiting unique nutritional and environmental requirements. *Campylobacter* spp. exist as commensal organisms in some animal species, yet are estimated to be the most common causative agents of foodborne illness in humans. *C. jejuni* is most often associated with poultry, while *C. coli* are more frequently associated with swine. Temperature has been suggested to trigger potential colonization or virulence factors in *C. jejuni* and recent studies have demonstrated temperature-dependent genes are important to colonization. It is possible that temperature-dependent colonization factors are in part responsible for the species specific colonization characteristics of *C. coli* also. We determined utilization of 190 different sole carbon substrates by *C. coli* ATCC 49941 at 37^{o}C and 42^{o}C using phenotype microarray (PM) technology. Temperature did affect amino acid utilization. L-asparagine and L-serine allowed significantly (p=0.05) more respiration by *C. coli* ATCC 49941 at the lower temperature of 37^{o}C as compared to 42^{o}C. Conversely, L-glutamine was utilized to a significantly greater extent (p=0.015) at the higher temperature of 42^{o}C. Other organic substrates exhibited temperature dependent utilization including succinate, D,L-malate and propionate which all supported active respiration by *C. coli* to a significantly greater extent at 42^{o}C. Further investigation is needed to determine the basis for the temperature-dependent utilization of substrates by *Campylobacter* spp. and their possible role in species specific colonization.

THE ROLE OF ARBUSCULAR MYCORRHIZAL (AM) FUNGI/PSEUDOMONAS INTERACTIONS IN THE AM-MEDIATED BIOCONTROL OF THE SOILBORNE PATHOGEN *FUSARIUM OXYSPORUM* F.SP. *RADICIS-LYCOPERSICI*

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Arbuscular mycorrhizal (AM) fungi, obliged symbiots of most land plants, have been shown to favour the host plant mineral and water nutrition and to increase resistance to abiotic and biotic stresses leading to biocontrol. Various bacteria with biocontrol abilities (especially Pseudomonas spp.) were isolated from AM structures and the mycorrhizosphere (the zone of soil influenced by the AM-root system). To test if AM-bacteria interactions are involved in the AM-mediated biocontrol, Ri T-DNA-transformed *Cichorium intybus* roots, colonized with the AM fungus *Glomus intraradices* DAOM197198, were inoculated on bi-compartmented Petri dishes, *in vitro*. After 4-5 weeks of growth, a bacterial suspension (*Pseudomonas fluorescens* WCS365, *Pseudomonas putida* PCL1760 or *Pseudomonas chlororaphis* PCL1391, with a specific mechanism of biocontrol against *Fusarium oxysporum* f.sp. *radicis-lycopersici* - forl) was inoculated on the so-called root-free compartment. Biofilm formation and attachment were observed by confocal microscopy on AM dead and alive hyphae and on spores for all Pseudomonas strains, with significantly more importance for *P. fluorescens* WCS365 and *P. putida* PCL1760 than *P. chlororaphis* PCL1391. A study of the tripartite Pseudomonas/*G. intraradices*/forl interactions in the *in vitro* root-free compartment, under investigation, will permit to relate bacterial capacities of attachment and biofilm formation on AM network to reduction in the pathogen proliferation. Moreover, in order to investigate the mechanisms involved in AM/Pseudomonas interactions, the priming effects on gene expression of *G. intraradices* and Pseudomonas spp. is being studied by comparing both partner transcriptomes in single and dual growth conditions, in the *in vitro* root-free system, by microarray analysis.

EFFECT OF CULTURE SITE ON MICROBIOLOGICAL AND BIOCHEMICAL CHARACTERISTICS, DURING REFRIGERATED STORAGE, OF OYSTERS (*CRASSOSTREA GIGAS* THUNBERG) REARED IN ORBETELLO LAGOON (CENTRAL ITALY) AND MANFREDONIA SEA (SOUTHERN ITALY)

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Shelf-life of oysters can be short and variable, due to the fact that the flesh has a high a_w, neutral pH, and composition that makes it easily perishable, also because of the presence of psychrotolerant spoilage bacteria related to environmental conditions as water quality, salinity and temperature. Aim of the work was the evaluation of the influence of culture site on quality changes of oysters during the post-harvest refrigerated storage.
Oysters of commercial size were collected from 2 different Italian sites: 1) Orbetello lagoon (Tyrrhenian sea, Italy) and 2) off the coast of Manfredonia (Adriatic sea, Italy). The oysters (n. 39 and n. 52; 155.13±32.98 and 149.56±25.36 g for weight; 117.75±15.19 and 116.16±10.00 mm for length, in site 1 and 2, respectively) were analysed for two significant bacterial groups: pseudomonads (*Pseudomonas* agar base plus the selective agent *Pseudomonas* CFC supplement) and *Micrococcaceae* (Mannitol salt agar), after 1, 3, 7 and 10 days of refrigerated storage at 4 °C. The soft parts of oysters were analysed for the energetic content (measured by *bomb calorimetry*) and for pH From oysters of each site, 45 strains of pseudomonads were isolated and purified. Total DNA was extracted from the isolates and 16S rDNA was PCR-amplified using FD1 and RD1 primers. Amplicons were subjected to ARDRA analysis using the restriction endonuclease *Cfo*I. Representative strains of ARDRA groups were identified by 16S rDNA sequencing.
About the bacterial groups, a low number of pseudomonads was registered in oysters of both sites: around 3.4 log CFU g^{-1} on the 1st day after harvesting; afterwards the pseudomonads increased, but only up to 4.5 log CFU g^{-1} on the 7th day, in oysters from site 1 and on the 10th day in oysters from site 2. It was observed a higher number of *Micrococcaceae* and a similar behaviour in oysters of both sites: around 5.0 log CFU g^{-1} on the 1st day, afterwards the number increased up to 6.5 and 6.9 log CFU g^{-1} on the 7th day and on the 10th day respectively. 16S rDNA sequencing indicated that the analysed isolates, from Orbetello and from Manfredonia sites, belong to *Pseudomonas* spp. During the storage, the pH values showed a small, even if significant, increase (from 6.10 to 6.43). Any significant difference in behaviour of both bacterial groups and of pH values on oysters from both sites was observed. The energetic content of the ash free dry matter showed significant differences between the oysters from the different sites, the oysters from Orbetello having soft parts with an energetic content of about 4453.51 cal/g against a value of about 3978.77 cal/g found in oysters from Manfredonia. The difference of the two rearing environments for the trophic conditions may have influenced the biochemical composition of soft parts, thus explaining that result.
In conclusion, the oysters farmed in sites very different for environmental parameters showed a similar behaviour during the period of refrigerated storage, maintaining appreciable quality characteristics during the ten days of refrigerated storage. However, the peculiarities of the two sites for trophic and physico-chemical parameters have significantly influenced biochemical composition of the edible part, confirming the key role that the environment exerts on this trait.

CHARACTERIZATION OF BACTERIAL COMMUNITIES ASSOCIATED WITH CULTURES OF *NANNOCHLOROPSIS* SP. F&M-M24

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Microalgal cultures are generally non-axenic, but associated with different species of bacteria. In recent years, the study of the interactions between algae and bacteria in the "phycosphere" has received increasing attention (Cole, 1982; Fukami et al., 1997; Grossart e Simon., 2007). This is due to the influence that bacteria have on microalgal physiology and on the culture performances. As the exudates produced by the algae modify the presence and activity of the distinct bacterial communities associated, the bacterial component in algae cultures may stimulate or inhibit algal growth.
In the present work, the bacterial communities associated with cultures of *Nannochloropsis* sp. F&M-M24 were studied. This microalga is widely use as aquaculture feed and has a good potential as an alternative source of oil for biodiesel, EPA, antioxidants and other high-value products (Rodolfi et al., 2009). Different growth tests have been performed in bubbled-tubes cultivating the algal strain without its associated bacteria (axenic culture), in the presence of the total bacterial community or in the presence of few bacterial phenotypes. Some trials have been also carried out in mixotrophy using glycerol as source of organic carbon.
A molecular characterization of the diversity of the bacterial communities associated with *Nannochloropsis* sp. F&M-M24 cultures was also performed. Samples of cultures maintained in different conditions were collected: two from laboratory cultures, one from an outdoor culture in a GWP photobioreactor (Rodolfi et al., 2009), one from the reference culture in presence of glycerol and one from the axenic culture. The polymorphism derived from the length of the terminal restriction fragment (T-RFLP) of the gene for the 16S ribosomal subunit (16S rDNA) of these communities has been analyzed. To identify some of the signals found in T-RFLP electropherograms, the technique has been applied to bacterial isolates from the reference community. The isolates were clustered into operational taxonomic units by ARDRA analysis and then identified through 16S rDNA sequencing.
It was observed that laboratory conditions tend to reduce the diversity of the associated microbial communities, while external factors, such as the addition of an organic carbon source or the highly variable conditions to which are exposed the cultures kept outdoors, lead to a greater diversity of the associated bacterial flora. From the study it clearly emerges that the bacteria may influence microalgae either promoting or otherwise reducing their growth. Further work with individual bacterial isolates of either stimulating or inhibiting strains will help to clarify the complex interrelationships within the "phycosphere".

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NICHE OVERLAP AND FUNCTIONAL REDUNDANCY OF FUNGAL SPECIES THAT AFFECT CELLULOSE-BASED CULTURAL HERITAGE

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A considerable number of fungi have been described as having the power to attack cellulose, and several studies on the in vitro dynamic of cellulose degradation by filamentous fungi have been published, but little is known about the functional diversity of fungal communities colonizing manmade cellulose-based objects under artificial environmental conditions like the indoor environments. Many authors demonstrated the key role played by microfungi in these processes, including biological foxing. A great number of fungal species is involved in paper biodeterioration and it is well known that environmental conditions (e.g. temperature, humidity, light) influence the process and its rate. Filamentous fungi can tolerate desiccation, high salt concentrations and heavy metal compounds that are present in inks and pigments, and are thus frequent inhabitants on paper and parchment supported objects. The various methodologies for isolating and identifying fungal agents are in general complex, even if based on biomolecular techniques. Indeed, several recent works dealing with staining of paper adopt DNA analysis in order to identify the fungi involved in staining processes. In spite of the objective of this kind of analysis, some questions arise by observing the list of fungal species detected. The fungal assemblages that develop on a book behave similarly to the communities of decomposers that, in natural environments, metabolise dead organic matter. Like in natural environments the diversity-functioning relationship is driven by the presence or absence of key species, by niche differentiation and species interaction. The term "niche" in the ecological context refers to the way in which an organism or population utilises its immediate environment. An ecological niche is defined by different biotic and abiotic variables. According to the competitive exclusion principle, no two species can occupy the same niche in the same environment for very long. Ecological niches have been described as "hypervolumes", that is to say multi-dimensional resource spaces exploited by organisms. Indoor environments are composed of artificial niches in which manmade materials are stored and assembled to form simplified "ecosystems", where only a few "ruderal" species can grow and reproduce. In these extreme circumstances scarce water and limited nutrients mean that only a few microbial and fungal species are able to dominate. In this study nutritional analysis based on the use of Biolog Phenotype MicroArray (PM) technique (Biolog Inc.,Hayward, CA) were applied to study the ecology of fungi inhabiting valuable objects of art, and to describe the spoiling mechanisms of the different substrata. Strains of ten fungal species isolated from objects of art supported on paper from different ages were identified and inoculated in Biolog™ FF microplates. The optical density at 750 nm (OD750) (mycelial growth) and OD490 (mitochondrial activity) were measured after 24, 48, 72, 96,168, 192 and 240 h using a microplate reader (Biolog Inc., Hayward, CA). The ability of Biolog ID MicroLog system towards the identification of the strains isolated from materials was evaluated. Moreover, statistical multivariate analysis and niche overlap indexes were applied to the dataset to define the common metabolic traits of the fungi that caused damage to cellulose-based cultural heritage.

WILD TYPE AND MUTANT STRAIN OF *ASPERGILLUS FLAVUS* COMPARED BY MEANS OF PHENOTYPE MICROARRAY TECHNIQUE: METABOLIC EFFECT OF INDUCED PEROXISOME HYPERPROLIFERATION

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Several factors regulate aflatoxin (AF) biosynthesis in Aspergillus sect. Flavi. Particular attention has been deserved in literature to fungal lipid metabolism and to the role of parasite-host interaction on AF biosynthesis and lipidic pathways. Experimental evidence suggested that fungi use endogenous lipid metabolism enzymes and endogenous oxylipins as a significant part of their pathogenic strategies to successfully colonize the host, reproduce, and synthesize toxins. Moreover, recent studies indicate that peroxisomes (P), cell organelles mainly involved in lipid catabolism in all eukaryotes, play a role in the distribution of energy and carbon sources needed for AF/ST biosynthesis. Under environmental stress, such as during interaction with the host or nutrient depletion, P proliferate and enhance fatty acid (FA) oxidation. To study whether peroxisome functionality, oxidative stress and oxylipins formation are related to aflatoxin biosynthesis in A. flavus a gene encoding for a virus (Cymbidium ringspot virus) protein, p33, which is able to induce peroxisome proliferation, was inserted in NRRL 3357 WT strain. The peroxisome hyperproliferation in Afp33DsRED strains was demonstrated by TEM analysis. The WT and the mutant strains were compared by means of metabolic performances against different carbon sources. The Biolog Phenotype MicroArray (PM) technique (Biolog Inc.,Hayward, CA) was used to investigate the effects of mutation on carbon assimilation patterns. The optical density at 750 nm (OD750) (mycelial growth) and OD490 (mitochondrial activity) were measured after 24, 48, 72, 96,168, 192 and 240 h using a microplate reader (Biolog Inc., Hayward, CA). The test indicated that the substrate utilisation (OD490nm) of the two strains (WT and AfP33DsRED) started to diverge in the use of a few carbon sources use after just 24h of incubation. In particular, AfP33DsRED metabolises Succinic acid mono-methyl ester (MeSuc) better than WT. A higher catabolic ability towards MeSuc is supposed to increase the oxidation of acetyl-CoA. Conversely, the mutant strain appeared less efficient in the use of L-Pro and some simple carbohydrates (fructose, sorbose) as substrate. The Phenotype MicroArray results were consistent with the hypothesis that Afp33DsRED strain differed from the WT in an up-regulation of the lipid metabolism (upregulation of the TCA cycle, FFA beta-oxidation and TG accumulation), which could be the cause for the induction of a hyperoxidant status (higher oxylipins formation) and aflatoxin biosynthesis enhancement both in vitro and in vivo (maize seeds).

SCREENING OF POTENTIAL PROBIOTIC PROPERTIES OF *LACTOBACILLUS PLANTARUM* ISOLATED FROM ITALIAN AND ARGENTINEAN DAIRY PRODUCTS

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Lactobacillus plantarum is a member of the facultatively heterofermentative group of lactobacilli. It is a heterogeneous and versatile species that is encountered in a variety of environmental niches, including dairy, meat, fish, and many vegetable or plant fermentations. *L. plantarum* strains have also been found in many cheese varieties. Moreover, strains of *L. plantarum* have proven ability to survive gastric transit and colonize the intestinal tract of humans and other mammals. These findings have led to the inclusion of this organism in currently marketed probiotic products.
This study investigated the probiotic potential of 26 *L. plantarum* strains isolated from dairy products. Selected strains showed high resistance to 100 mg/l of lysozyme under conditions simulating the *in vivo* dilution by saliva, good adaptation to simulated gastric juice at pH 2.5, and a moderate to low bile tolerance. The capacity to agglutinate yeast cells in a mannose-specific manner and the cell surface hydrophobicity were found to be variable among strains. Similarly, a strain-specific agglutinating phenotype was observed after agglutination assay with concanavalin A, thus confirming the binding capacity to yeast cells. Very high ß-galactosidase activity was shown from a considerable number of the tested strains, whereas a variable prebiotics utilization ability was observed, with notable preference for lactulose, corn fiber, and, to a lesser extent, raffinose. The only antibiotic resistance observed in this study was to tetracycline, which was observed in two highly resistant strains harboring the *tet*M gene.
Overall, phenotypic data were subjected to multivariate statistical analysis by using the Principal Component Analysis (PCA). The biplot obtained from the statistical analysis showed the formation of two main groups, which were separated on the basis of their bile resistance and tolerance to simulated gastric juice.
This study allowed to screen a number of *L. plantarum* strains possessing good potential for a probiotic application. The results revealed also considerable heterogeneity among the target strains and highlighted the possibility to design multiple cultures to cooperatively link strains showing the widest range of useful traits. More specifically, statistical analysis allowed to detect strains to be tested as adjunct starter cultures for the manufacture of probiotic fermented foods. Further studies are in progress in our laboratories to evaluate the ability of *L. plantarum* strains to multiply in milk, to withstand food technological stresses, and to resist to phage attack.

HEPATITIS C VIRUS INFECTION: A REVIEW OF THE CURRENT AND FUTURE ASPECTS AND CONCERNS IN PAKISTAN

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Hepatitis C virus (HCV) is the major etiological agent of hepatitis. It infects 200 million people worldwide and 85% of them could develop chronic hepatitis, liver function failure or hepatocellular carcinoma. Hepatitis C is rapidly emerging as a major health problem in developing countries like Pakistan with prevalence rate of 10% and genotype 3a is the most prevalent. Here, approximately 80% of infections proceed to chronic infection and infected blood is the primary route of spread. In Pakistan, about 75% of patents do not receive standard anti HCV therapy (Interferon + Ribavirin) and of the 25% that do receive such treatment, the SVR rate is 60 - 70%. This review is designed to cover the information about the status of HCV in Pakistan with major focus on its prevalence, genotypes, current diagnostic assays, available therapies and treatment outcomes. The present review further emphasizes the need to uncover exact HCV prevalence rate in the country, to develop diagnostic assays based on local genotype, to understand the interaction between HCV genotype 3a genes and cell line genes responsible HCV pathogenesis. In addition, this review discusses the need for the generation of infectious pseudo particle of HCV as a potential vaccine, to investigate DNA base vaccine, or siRNAbased anti HCV approaches for our local genotypes.

DEVELOPMENT OF AN INTEGRATED SYSTEM TO STUDY *PSEUDOMONAS MENDOCINA* BIOFILM

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Biofilms, the form commonly taken by microorganism in environment, are microbial communities of surface-attached cells embedded in a self-produced extracellular polymeric matrix. The strategy of microbial growth that leads to biofilm formation, compared with planktonic growth, results in the development of a microbial community that is characterized by a marked resistance to conventional biocides, antibiotics and to the action of environmental stresses, including those due to heavy metals.
The aim of this work is the development of an integrated system based on two high-throughput technique, the Calgary Biofilm Device (commercially avaible as the MBEC™ assay - Innovotech) and the Phenotype MicroArray (PM) – Biolog, to study phenotypes of biofilm cultures of *Pseudomonas mendocina* strain 34, a chromate-resistant bacteria.
The MBEC™ assay allows microorganisms to grow on 96 identical pegs protruding down from a plastic lid. By placing the biofilms on the pegs into the wells of a microtiter plate, an array of antimicrobial compounds with varying concentrations can be assessed. Since the PM can test up to 2000 cellular phenotypes simultaneously, we would to join the MBEC™ assay with the Phenotype MicroArray technology in order to study chemical sensitivity of *P. mendocina* biofilm.
Preliminary experiments indicated that the dyes used in Phenotype MicroArrays system are not suitable to monitor biofilm activity. Dye A is insoluble and remains inside biofilm and dyes D,E,F,G and H, which are more soluble, can be measured by Omnilog only after a long time of incubation. In order to overcome this problem we have used resazurin, a non-toxic, water-soluble dye which is reduced by electron transfer reactions associated with respiration. The reduced resazurin can easily measured by a fluorimeter like Typhoon (Amersham Biosciences).
In this way we were able to analyze the activity of *P. mendocina* biofilm after 3, 7 and 24 hours of incubation in presence of some toxic compounds. First results, using potassium chromate, benzalkonium chloride, triclosan and kanamycina, indicated that the set up protocol permits to analysed the activity of *P. mendocina* biofilm by using Calgary device approach combined with micro well plates. Further experiments are need in order to increase reproducibility of assays and to adapt the Calgary device to PM plates.

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METABOLIC FINGERPRINTING; AN EFFICIENT METHOD FOR DEPICTION OF RHIZOBIAL DIVERSITY

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Mucuna prurience, a promising medicinal and forage legume (cultivated as a cover crop) has been selected for the study of nodulating bacteria because *Mucuna prurience* is a drought resistant herb, nitrogen fixer, synthesize medicinally important secondary metabolites, and is used as a forage and cover crop. This crop has been found to be a big resource of rhizobial diversity. This is an unexplored legume regarding rhizobial diversity. *Mucuna prurience* plants collected from three different zones (restricted, buffered and disturbed) of Himalayan range. Initially total thirty one (MU1- MU31) rhizobial strains isolated from root nodules of *Mucuna prurience*, growing in different zones of Himalaya. Out of 31 isolates five isolates MU_{26}, MU_{27}, MU_{28}, MU_{29}, MU_{30} and MU_{31} were slow grower. Metabolic characterization of isolates as well as standard type strains was performed by using BILOG plates. There was considerable diversity observed on the basis of amino acid and carbohydrate utilization pattern. Most of the isolates used DL-leucine invariably. Out of 31 tested isolates, MU_{12} showed maximum growth in DL-leucine. L-valine was maximally utilized by standard strains. Casine was least preferred amino acid for the growth among rhizobia. Amino acid utilization pattern of *Mucuna* isolates could not provide much information regarding their distribution pattern in different zones. Hence other mode of characterization of rhizobia was chosen i.e carbohydrade utilization. On the basis of carbon utilization pattern, Results of carbon utilization showed that *Mucuna* isolates did not show much similarity with standard *Rhizobium* strains in their carbon utilization pattern.

Analysis of results by principal component (PCA) suggested that isolates from *M. prurience* formed a separate cluster from authentic strains of rhizobia. So, they had their specific and wide choice for carbon sources in comparison to authentic rhizobial strains. *M. prurience* and authentic reference strains occupied same position which was occupied by maltose, tween 20, tween 80, inulin and mannitol that means the behavior of all the isolates from *M. prurience* and reference strains was same towards utilization of above mentioned carbohydrates though complex carbohydrates were less utilized. We also found that *Mucuna* isolates recovered from restricted zone, preferred to utilize pentose sugars reflecting the specific nature of these isolates in carbon utilization through pentose phosphate pathway. *Mucuna* isolates isolated from buffered and restricted zones were able to utilize all the carbon sources including hexoses, pentoses, disaccarides as well as complex carbon like tween 20 and tween 80.

So, the diversity of isolates from *M. prurience* will depend more on their utilization pattern of carbohydrate rather than their utilization pattern of amino acids.

PHENOTYPIC MICROARRAY CHARACTERISATION OF *SALMONELLA TYPHI*

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Salmonella enterica serovar Typhi, a causative agent of typhoid fever, is strictly a human host pathogen. Typhoid fever affects roughly 17 million people annually causing approximately 600,000 deaths. *S.* Typhi is a multi-organ pathogen that inhabits the lymphatic tissues of the small intestines, liver, spleen, and the bloodstream of infected humans. It is common in developing countries with poor sanitary systems. Typhoid fever is endemic in Malaysia and occasionally outbreaks do occur. It is spread through the oral -fecal route and carriers are also the source the infection. We have previously characterize some outbreak and sporadic strains by pulsed field gel electrophoresis and virulotyping based on 22 virulence genes. Each outbreak strain was characterized by a particular pulsotype and PFGE could differentiate outbreak from sporadic cases of typhoid fever. Majority of the strains had the similar virulotypes. The objective of this study was to correlate the genotypic characters with the phenotypes via the BIOLOG Phenotype MicroArray system. Eight *S.*Typhi strains, including a carrier and an environmental (sewage) strain, were selected on the basis of the different pulsotypes and virulotypes for the study. The variation in carbon utilisation (PM1 and PM2 plates) and response to various osmolytes and pH (PM9 and PM10 plates) of the selected *S.* Typhi strains were compared and correlated to their origin, pulsotypes and virulotypes. All the tests were performed in duplicates. The data presented here is preliminary assessment for suitability and efficacy of Phenotype MicroArray in high-throughput phenotypic characterization of *Salmonella* Typhi for a better understanding in the epidemiology of this pathogen.

ENVIRONMENTAL DEPENDENCY ANALYSIS FOR GENE KNOCKOUTS IN *ESCHERICHIA COLI* BASED ON PHENOTYPE MICROARRAY

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Systematic studies have revealed that gene deletions often display little phenotypic effects under laboratory conditions and that in many cases gene dispensability depends on the experimental conditions. To elucidate the environmental dependency of genes, we analyzed the effects of gene deletions by Phenotype MicroArray™ (PM). To identify genes that showed significant phenotypic changes when deleted, we converted time-series data from reference 1 wild type and 300 mutants in *Escherichia coli* K-12 to vectors. We then classified observations from all PM screenings into two categories, setting significant positive or negative phenotypic changes and no significant change.

Wild-type *E. coli* K-12 BW25113 showed no respiration for 709 (36.9%) of the 1920 conditions examined and negative effects for 12 (0.6%) conditions. The wild-type strain showed respiratory activity for 1199 medium conditions. For 12 of these conditions, all 300 deletion mutants gave no significant phenotypic change. Under these 1199 conditions, 39 mutants on average showed significant phenotypic changes under each condition. On average each mutant showed differences under 156 conditions. Taken together, these results suggest that at least eight screening conditions and more than eight knockout genes are required for genotype-phenotype functional analyses.

All vector data indicated that the gene knockout effects for 10 mutants (Δ*lpd*, Δ*crp*, Δ*purH*, Δ*pyrF*, Δ*atpF*, Δ*atpH*, Δ*aceE*, Δ*sdhB*, Δ*aceF*, Δ*ycdY*, and Δ*purC*) are significant regardless of the medium conditions. These mutants show markedly lowered respiration under more than 861 medium conditions. Hence, they display low degrees of environmental dependency. Six of these 10 genes are involved in energy production and conversion according to the COGs. On the other hand, ten single-gene mutants showing high degrees of environmental dependency included 7 *y*-genes: *sprT*, *ydcK*, *ydiM*, *ymfG*, *yncG*, *yfeA*, and *ycbZ*. All 10 mutants showed no significant phenotypic changes with glucose, galactose, and glycerol conditions corresponding to the PM1-C9, PM1-A6, and PM1-B3 plates, respectively. The high environmental dependency of these single-gene mutants provides clues for identification of the respective gene functions. Our results revealed divergence of the environmental dependency of the gene among the knockout genes.

PHENOTYPIC CHARACTERIZATION OF *LACTOBACILLUS SANFRANCISCENSIS* STRAINS ISOLATED FROM TRADITIONAL SOURDOUGH PRODUCTS

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Sourdoughs are very complex biological ecosystems where the microbial composition and the interactive effects among endogenous and exogenous factors lead to the predominance of unique communities of lactic acid bacteria (LAB) and yeasts, with large genotypic and phenotypic variability within each species. Several studies have shown that sourdough lactobacilli exhibit unique technological properties related to flavor, texture, staling, and shelf-life of sourdough products (Di Cagno *et al.*, 2007). On the other hand, the knowledge of the metabolic features of the bacterial strains occurring in a sourdough process is of capital importance to understand their possible interactions with yeasts and their contribute to the organoleptic and technological attributes of the final products.

In this work, the metabolic behaviour of several strains of *Lactobacillus sanfranciscensis*, isolated from industrial sourdoughs used for the manufacture of typical sweet leavened baked products, belonging to two distinct ribogroups (DSM 20663 ribogroup and ATCC 27651 ribogroup, according to Foschino et al., 2001, and here referred to as ribogroups I and II, respectively) and characterized at strain level by RAPD analysis, was investigated.

The strains were grown in MR3 medium, an experimental culture medium simulating the chemical composition of a dough refreshment (thus containing maltose, glucose and fructose at a concentration of 30 g/l, 6 g/l and 6 g/l, respectively), at 30 °C and under anaerobic condition until the stationary phase. At this point, HPLC analysis of spent media was carried out to determine the amount of both consumed substrates and produced metabolites (lactic and acetic acids, ethanol and mannitol).

No significant difference ($p<0.05$) was observed among the strains for growth rate and growth yield in MR3. As concerns the metabolic behaviour, no significant difference resulted between the two ribogroups in either maltose consumption or lactic acid and ethanol production, whereas a significant difference ($p<0.05$) in the utilization of glucose and fructose was observed. Indeed, the ribogroup I strains used a greater amount of glucose rather than ribogroup II strains, even if a large biodiversity among the strains belonging to the same group was observed. Moreover all the strains of *Lb. sanfranciscensis* ribogroup I used almost all the available fructose to produce mannitol in a molar ratio of 1:1; on the contrary, most strains belonging to the ribogroup II demonstrated unable to reduce fructose to mannitol. As expected, the reduction of fructose to mannitol was coupled to a proportional production of acetic acid. This result is of great practical importance because the amount of acetic acid is widely known to affect the fermentation ratio (lactic acid to acetic acid molar ratio) and, hence, to affect both the aromatic profile and the resistance of the gluten structure.

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CHARACTERIZATION OF ISOLATED *CRONOBACTER* (*ENTEROBACTER*) *SAKAZAKII* CULTURES USING REAL-TIME PCR, BIOLOG GEN III AND ID 32E

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This study was to characterize suspected *Cronobacter* (*Enterobacter*) *sakazakii* isolates from previous studies in Universiti Kebangsaan Malaysia (UKM) and *Cronobacter* spp. and *Enterobacter* spp. pure cultures from University College Dublin (UCD). There were 37 isolated cultures from previous studies in UKM which were isolated from powdered infant and children formula milk, raw milk and pasteurized milk. The nine pure cultures from UCD included *C. sakazakii* (ATCC 29544), *C. muytjensii* (ATCC 51329), *C. malonaticus* (E 825), *C. turicensis* (E 866/ 23032), *C. dublinensis* (CFS 237), *C. genomospecies* I (E 797), *E. pulveris* (Z 601), *E. helveticus* (Z 513) dan *E. turicensis* (Z 5708). All the isolated cultures were enriched in *Cronobacter* Screening Broth (CSB) at 41.5°C ± 1°C for 24 ± 2 hours. Broth that changed colour from purple to yellow was streaked on a chromogenic Chromocult® *E. sakazakii* (CES) agar and incubated at 44°C ± 1°C for 24 ± 2 hours. Presumptively positive colonies were streaked on non-selective media which was Tryptone Soy Agar (TSA) and incubated at 25°C for 24 ± 2 hours. Yellow pigmented colonies were confirmed using Real-time PCR (BioRad, model iCycler iQ5), Biolog GEN III (Biolog Inc.) and ID 32E (bioMérieux). Real-time PCR method confirmed that five (83.3%) pure *Cronobacter* spp. from UCD were confirmed as *C. sakazakii* except *C. dublinensis* whereas 2 *Enterobacter* spp. were also confirmed as *C. sakazakii* except *E. turicensis*. The identity for 35 (94.6%) out of 37 isolated cultures from previous studies in UKM were confirmed as *C. sakazakii* using Real-time PCR. ID 32E confirmed that *C. sakazakii* and *C malonaticus* pure cultures together with 32 (86.5%) isolated cultures from previous studies in UKM were *E. sakazakii*.

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