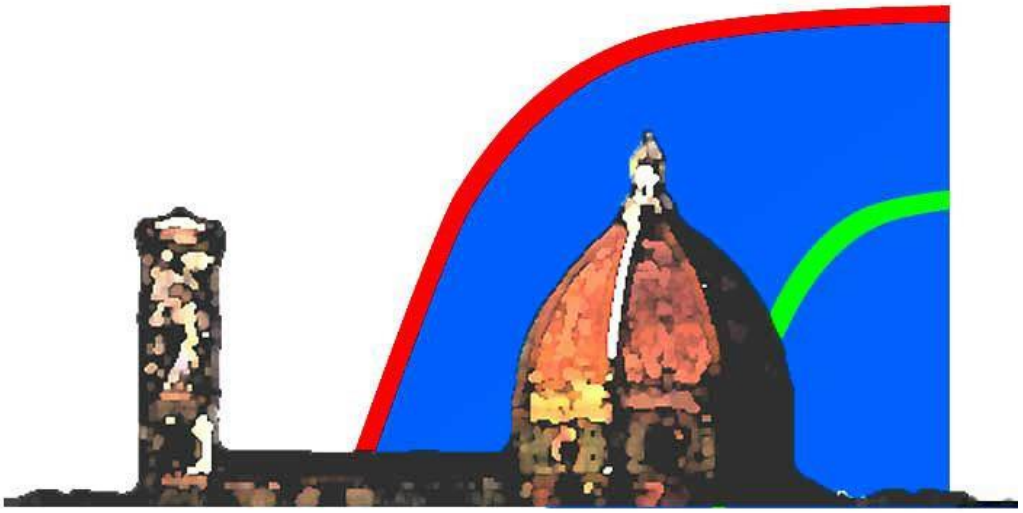
3rd Florence Conference on Phenotype MicroArray Analysis of Cells



Firenze-Italy, September 10-12, 2015

Programme and Abstracts

UNIVERSITÀ DEGLI STUDI FIRENZE

3rd Florence Conference on Phenotype MicroArray Analysis of Cells

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Programme and Abstracts

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

Contents

Welcome to the 3rd Florence Conference on Phenotype MicroArray Analysis of Cells

On behalf of the Organizing Committee, we would like to extend a warm welcome to everyone participating in the 3rd Florence Conference that will take place from Thursday afternoon until Saturday lunchtime (September 10-12, 2015).

The title of this year's event "Florence Conference on Phenotype MicroArray Analysis of Cells" reflects our aspiration to provide an opportunity for researchers working in different fields of biology to share, compare and evaluate multiple types of Phenotype MicroArray data, and to show the numerous applications of this high-throughput phenomic technology and its potential in the cell phenotyping area. For the first time, the Florence Conference programme includes a significant number of talks on the application of Biolog Phenotype MicroArray to eukaryotic cell analysis, including mammalian cells and multi-organism interactions.

We believe that this third edition of the Florence Conference will provide a forum for discussing the state of the art of Phenotype MicroArray technology in relation to other omics data as well as presenting new ideas for future research. We hope that this forum will serve as a powerful tool for deepening the knowledge on microbial and mammalian cell biology and for developing better communication among scientists throughout the world.

We are grateful to the University of Florence for hosting the third edition of the Conference, and we would like to take this opportunity to thank all the Partners and Sponsors of the Conference.

We hope you have a rewarding Conference and enjoy exploring and discovering the beautiful city of Florence.

Carlo Viti

Chair of the Conference

Luciana Giovannetti

Organizing Committee

Organizing and Scientific Committee

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Charles E. Schwartz, Greenwood Genetic Center, Greenwood, SC, USA
Joana Falcão Salles, Microbial Ecology, Centre for Ecological and Evolutionary Studies, University of Groningen, the Netherlands

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Partners, Sponsors and Patrons

Programme

Thursday 10th

University of Florence, Via Gino Capponi 9 - Room 8

Pre-conference workshop on analysing PM data

9.30-12.30	**Markus Göker** **Lea Vaas** **Maria del Carmen Montero-Calasanz** **Benjamin Hofner**	Pre-conference workshop on analysing PM data with the opm package

Opening of Conference

15.00 15.45	**Registration**	
15.45-16.00	The Authorities **Carlo Viti** and **Luciana Giovannetti,** University of Florence, Italy	Welcome to Florence University Opening of the Third Florence Conference on Phenotype MicroArrays
	Opening lectures	
16.00-16.40	**Barry B. Bochner,** Biolog Hayward, CA, USA	Phenotype MicroArray technology: recent advances using microbial and mammalian cells
16.40-17.20	**José L. Martinez,** Centro Nacional de Biotecnología Madrid, Spain	Deciphering antibiotic resistance and associated fitness costs
17.20-18.00	**Marco Fondi,** University of Florence, Italy	Phenotype Microarray and metabolic modelling
18.00-18.40	**Charles E. Schwartz**, Greenwood Genetic Center, USA	Phenotype Microarray analysis may provide insight for potential therapeutic approaches in human disorders

18.40-20.30 **Welcome cocktail**

Friday 11th - morning

University of Florence, Palazzo Marucelli, Via San Gallo, 10 - Aula Magna

8.00-8.30 Registration

Section: Multi organism interactions

Chair: Renato Fani, University of Florence, Italy

Lectures

8.30-8.55	**Joana Falcão Salles**, University of Groningen, the Netherlands	A mechanistic view of microbial invasions
8.55-9.20	**Giulia Spini,** University of Florence, Italy	Effect of the plant flavonoid luteolin on *Ensifer meliloti* 3001
9.20-9.45	**Anja Rösel**, Twincore, Germany	Application of the Biolog Phenotype Microarray™ to study changes in host cell metabolism during influenza a virus infection
9.45-10.10	**George C. diCenzo**, McMaster University, Canada	Metabolic profiling provides insight into the function and evolution of the multipartite bacterial genome

10.10- 10.40 Coffee break and poster exhibition

Section: Interesting environmental microorganisms

Chair: José L. Martinez, Centro Nacional de Biotecnología - Madrid, Spain

Lectures

10.40-11.05	**Terry C. Hazen**, University of Tennessee, USA	Phenotypic and genomic heterogeneity among *Colwellia psychrerythraea* strains from distant deep-sea basins
11.05-11.30	**Stefano Mocali**, CRA, Italy	Exploring the metabolic properties of Antarctic *Pseudoalteromonas* TAC125 and TB41 strains at different temperatures through phenotype microarray analysis
11.30-11.55	**Dimitris Petroutsos**, Research Institute of Life Sciences and Technologies, CNRS, France	Color-and metabolism-dependent regulation of photoprotection in green algae
11.55-12.20	**Kourosh Salehi-Ashtiani**, New York University-Abu Dhabi, United Arab Emirates	An integrated workflow for genome-scale reconstruction of algal metabolic networks
12.20-12.45	**Dayi Zhang**, Lancaster University, United Kingdom	Mine and cultivate the uncultivable microorganisms via magnetic isolation and phenotype microarray
12.45-12.55	**Ramona Marasco**, King Abdullah University of Science and Technology, Kingdom of Saudi Arabia	Bacterial diversity and functional services within the rhizosheath of a desert plant

12.55-14.45 Lunch and poster exhibition

Friday 11th - afternoon
University of Florence, Palazzo Marucelli, Via San Gallo, 10 - Aula Magna

Section: Genotype/Phenotype 1, metabolism and taxonomy

Chair: Terry C. Hazen, University of Tennessee, USA

Lectures

14.45-15.10	**Pilar Bosch Roig,** Universitat Politècnica de València	Phenotypic microarray for cultural heritage: innovative tools for bio-cleaning approach
15.10-15.35	**Severino Zara,** University of Sassari, Italy	Inhibitory effects of L-histidine on biofilm formation and *FLO11*-associated phenotypes in *Saccharomyces cerevisiae* flor yeasts
15.35-16.00	**Renato Fani**, University of Florence, Italy	From genome to phenome and back: understanding the high metabolic versatility of *Burkholderia cepacia* complex
16.00-16.25	**Anna de Breij**, University Medical Center, Leiden, the Netherlands	A proteomic and phenomic approach to study the effect of the antimicrobial peptide OP-145 on *Staphylococcus aureus*

16.25-17.10 Coffee break and poster exhibition

Section: Genotype/Phenotype 2, metabolism and taxonomy

Chair: Irina Druzhinina, University of Technology, Vienna, Austria

Lectures

17.10-17.35	**María C. Montero-Calasanz,** Newcastle University, United Kingdom	Application of phenotype microarrays in microbial systematics: targeting difficult groups
17.35-18.00	**Jean Guard,** U.S.-Department of Agricultural, USA	Bimodality of metabolic heterogeneity across serotypes of *Salmonella enterica*
18.00-18.25	**Martina Cappelletti,** University of Bologna, Italy	Genome and phenotype microarray analyses of two *Rhodococcus* strains with environmental and industrial relevance
18.25-18.50	**Marco R. Oggioni,** University of Leicester, United Kingdom	Epigenetic control of metabolic traits in bacteria

20.00 Conference Dinner

Saturday 12th

University of Florence, Via Laura, 48 - Aula Magna

Section: Bioinformatics and modelling

Chair: María C. Montero-Calasanz, Newcastle University, United Kingdom

Lectures

8.45-9.10	**Benjamin Hofner**, Institut für Medizininformatik, Biometrie und Epidemiologie, Germany	Application of state-of-the-art machine learning techniques to the PM Data on autism-spectrum disorders-boosting with false discovery control
9.10-9.35	**Marco Galardini**, EMBL-EB, United Kingdom	Joint genomic-phenotypic analysis of bacterial species: the need for a PM data repository
9.35-10.00	**Dov Stekel**, School of Biosciences University of Nottingham, United Kingdom	HiPerFit: Software for high throughput model fitting, parameter estimation and model choice for Biolog Phenotype Microarrays
10.00-10.25	**Markus Göker**, Leibniz-Institut DSMZ, Germany	The minimum information on a phenotype microarray study (MIPS) standard

10.25-10.55 Coffee break

Section: Eukaryotic cell analysis

Chair: Barry B. Bochner, Biolog Hayward, CA, USA

Lectures

10.55-11.20	**Polona Zigon**, University Medical Centre Ljubljana, Ljubljana, Slovenia	Metabolic fingerprints of human primary endothelial and fibroblast cells
11.20-11.45	**Luigi Boccuto**, Greenwood Genetic Center, USA	Biolog phenotype metabolic microarrays: finding the link between genotype and phenotype
11.45-12.10	**Flavia Pinzari**, CRA-RPS, Italy	Phenotype MicroArrays as a tool to study niche overlap and catabolic versatility of saprotrophic fungi
12.10-12.35	**Irina Druzhinina**, University of Technology, Vienna, Austria	Phenotype MicroArrays for genome-wide analisys of carbohydrate active enzymes in eight species of the mycotrophic filamentous fungus *Trichoderma*

12.35 Closing of Conference

Poster session

Conference information

Logistic Information

Please see page 4 the directions to the Conference Venues.

Change in Location

For technical reasons, the Sant'Apollonia Auditorium is not available to us. Therefore, we have decided to move the conference to three historic buildings very close to Sant'Apollonia Auditorium. We have chosen three locations that we hope will more than make up for the change from the Sant'Apollonia Auditorium.

Thursday, 10th Sept. - University of Florence, Via Gino Capponi 9 - Room 8.

This building housed the laboratories of a number of famous scientists, the most famous of these being Hugo Schiff (1834–1915). "Hugo Schiff studied with Friedrich Wöhler in Germany, but because of his liberal views spent almost his entire career in Italy, where he prepared and characterized Schiff bases (imines) and made many other chemical investigations. His scientific career spanned more than 60 years, and he lived to see the utilization of Schiff bases in [2+2] cycloadditions with ketenes to form β-lactams" Angew. Chem Int. Ed. 47: 1016–1020.

Friday, 11th Sept. - University of Florence, Palazzo Marucelli-Fenzi, Via San Gallo, 10 - Aula Magna.

Palazzo Marucelli-Fenzi was built in the 16th century by Gherardo Silvani for the Castelli family and was later enlarged by the Marucelli family. In 1829 it was bought by Emanuele Fenzi to house his bank and his family. It is currently home to the Department of History, Archaeology, Geography, Arts and Performance (SAGAS) of the University of Florence.

Saturday, 12th Sept. - University of Florence, Monastery of Crocetta, Via Laura, 48 - Aula Magna

The monastery of Crocetta was founded by the venerable Sister Domenica del Paradiso in 1511. It owes its name to the little red cross that the nuns embroidered on their clothes. It was built in 1519 and placed under the Dominican rule. The nuns received "donations" from the "illustrious Duke Cosimo de 'Medici" from 1562. Today, after complex rebuilding, the Monastery of Crocetta houses the Department of Education and Psychology of the University of Florence.

Directions to Conference Venues

Conference Venues can be easily reached by ATAF buses that go to Piazza San Marco. All the conference rooms and the Botanic Gardens are within close walking distance (3-5 min) (page 5).

Information for Authors

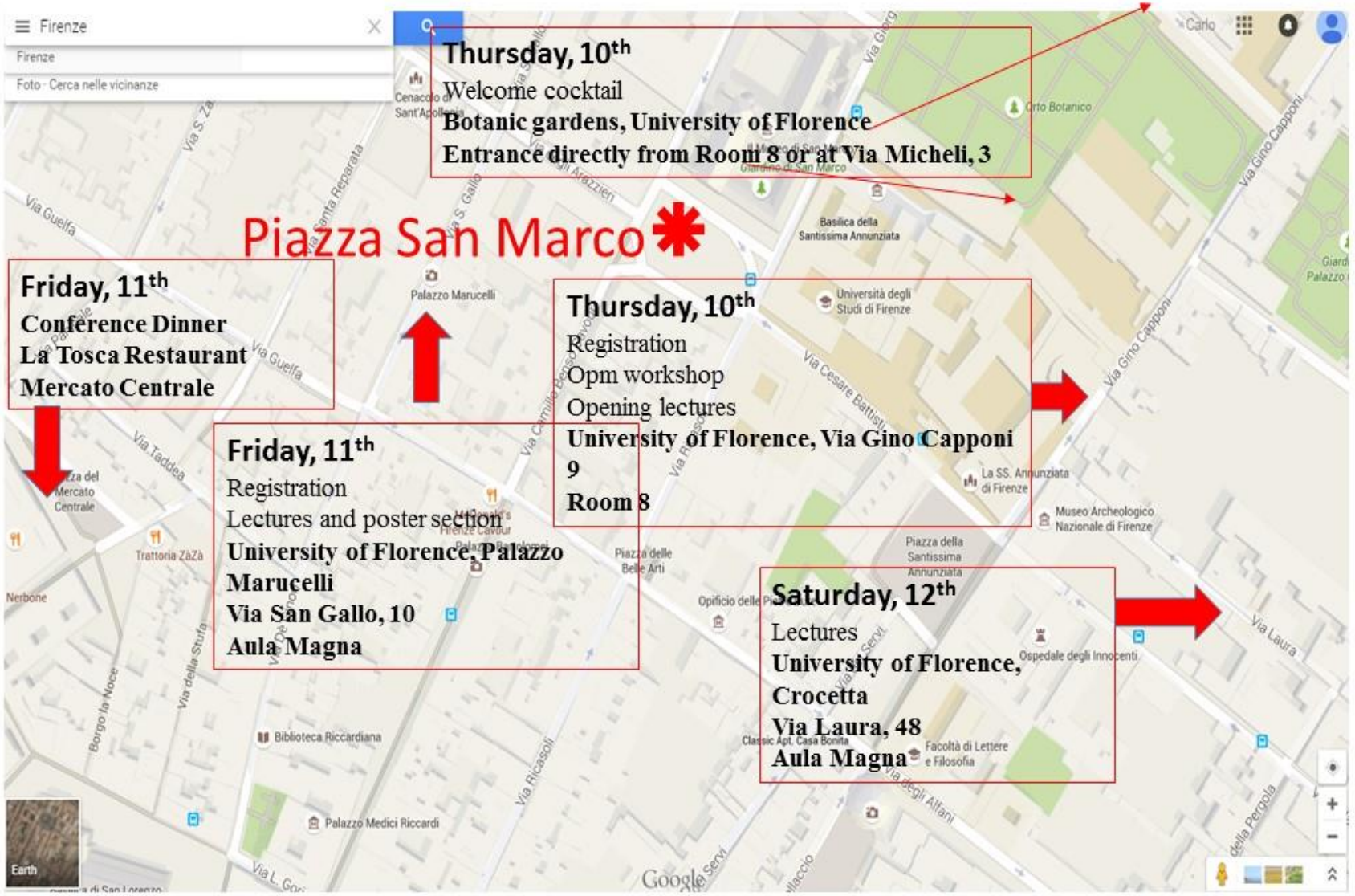
For **oral presentations**, we recommend speakers to check the day and slot in which their presentation has been scheduled. Although no substantial changes are expected at this stage, speakers will be contacted by the Organizing Committee if some minor changes are necessary.

Available time for opening lectures will be 35 minutes plus 5 minutes for audience questions, and for the other lectures will be 20 minutes plus 5 minutes.

For **poster exhibition**, presenters are reminded that posters will be exhibited on Friday 11th September, from 9:00 AM to 5:00 PM. They can be removed at the end of the session. Presenters are requested to stay close to their posters during the exhibitions.

Please remember that poster dimensions must be 80 cm wide and 120 cm high.

Special issue - A special issue of *Research in Microbiology* will be published in 2016. Authors of all the oral and poster presentations can submit manuscripts based on their presentation for consideration for the special issue. Submission of a paper to the special issue implies that it represents an original work not previously published and not being considered for publication elsewhere. Papers submitted for this special issue will undergo the normal journal reviewing process.


Thursday, 10th
Welcome cocktail
Botanic gardens, University of Florence
Entrance directly from Room 8 or at Via Micheli, 3
Piazza San Marco
Friday, 11th
Conference Dinner
La Tosca Restaurant
Mercato Centrale
Thursday, 10th
Registration
Opm workshop
Opening lectures
University of Florence, Via Gino Capponi 9
Room 8
Friday, 11th
Registration
Lectures and poster section
University of Florence, Palazzo Marucelli
Via San Gallo, 10
Aula Magna
Saturday, 12th
Lectures
University of Florence, Crocetta
Via Laura, 48
Aula Magna
Palazzo Marucelli
Via Guelfa
Via Taddea
Via della Stufa
Via Ricasoli
Via Cesare Battisti
Via Gino Capponi
Via Laura
Via degli Alfani
Via della Pergola
Piazza delle Belle Arti
Piazza della Santissima Annunziata
Basilica della Santissima Annunziata
Università degli Studi di Firenze
La SS. Annunziata di Firenze
Museo Archeologico Nazionale di Firenze
Ospedale degli Innocenti
Orto Botanico
Biblioteca Riccardiana
Palazzo Medici Riccardi
Trattoria ZaZà
Nerbone
Facoltà di Lettere e Filosofia
Google

ATAF routes

Abstracts

Part I

Lectures

PHENOTYPE MICROARRAY TECHNOLOGY: RECENT ADVANCES USING MICROBIAL AND MAMMALIAN CELLS

B.R. BOCHNER
CEO & CSO, Biolog, Inc., Hayward, CA

Phenotype MicroArray (PM) technology allows a biologist to test thousands of phenotypes of a cell line in a single experiment, to gain a comprehensive overview of the metabolism, physiology, and pathway fluxes. It provides phenomic and metabolomic information that is complementary to genomic or proteomic analysis and often more easy to interpret and more useful. The PM technology platform is applicable to a wide range of cells including bacterial, fungal, or animal and enables metabolic analysis in the context of genotype-phenotype studies. For example, it can be used for (1) analyzing cells with mutations to determine the metabolic and physiologic effects of genetic differences, (2) studying and defining cell metabolism and metabolic regulation, (3) understanding the interplay of environment and hormonal signals on cell metabolism and physiology, (4) optimizing cell culture conditions in bioprocess development and optimization, and (5) looking at the effects of drugs and other chemicals on cellular pathways. Recently applications are emerging to help analyze microbiomes and human cell-microbial cell interactions. Specific examples and discoveries will be presented to illustrate the many uses of this cell phenotyping technology.

DECIPHERING ANTIBIOTIC RESISTANCE AND ASSOCIATED FITNESS COSTS

J.L. MARTINEZ
Centro Nacional de Biotecnología, CSIC, Darwin 3. 28049-Madrid, Spain

Resistance to antimicrobial agents, including antibiotics and biocides may produce changes on bacterial physiology with relevance for the outcome of the infectious process. These changes are usually considered as *fitness cost* under the assumption that the acquisition of resistance produced a non-specific metabolic burden. This hypothesis is based on the supposed constrains that the genetic basis of resistance might impose to the bacterial cell. Resistance can be acquired as the consequence of mutations or acquisition of resistance genes. For the first, *fitness cost* should be the consequence of a certain de-adaptation of the proteins coded by the mutant alleles, that would be less proficient than the wild-type ones. For the second, *fitness costs* should be due to the use of the resources required for the replication, transcription and translation of the novel, acquired resistance genes. Whilst these hypothesis seem reasonable and have demonstrated to be true in occasions, in other cases *fitness costs* are specific for the mutation involved or for the acquired gene. In these cases, the acquisition of resistance may produce specific changes in the bacterial physiology, including those dealing with basic metabolic processes. The use of BIOLOG technology allows a precise analysis of some of these changes and serve to predict the behaviour of the resistant microorganisms in different ecosystems presenting differential nutrient resources. Along the presentation, some examples of this type of integrative analysis will be presented with a particular focus on the integration of resistance in bacterial metabolic networks.

In addition to this effect on bacterial metabolism, acquisition of resistance might alter (increase or decrease) the susceptibility to other antimicrobials besides the ones for which mutants have been selected. In these circumstances, the use of the BIOLOG technology allows a precise and wide analysis of the changes in the susceptibility to the most common antimicrobials that bacterial populations can face in natural ecosystems and in clinical settings. During the presentation, examples will be discussed on the effect that acquisition of resistance to commonly used biocides may have for the development of antibiotic resistance by human bacterial pathogens.

Curiao, T., Marchi, E., Viti, C., Oggioni, M.R., Baquero, F., Martinez, J.L., and Coque, T.M. (2015) Polymorphic variation in susceptibility and metabolism of triclosan-resistant mutants of *Escherichia coli* and *Klebsiella pneumoniae* clinical strains obtained after exposure to biocides and antibiotics. *Antimicrob Agents Chemother* 59: 3413-3423.

Olivares, J., Alvarez-Ortega, C., and Martinez, J.L. (2014) Metabolic compensation of fitness costs associated with overexpression of the multidrug efflux pump MexEF-OprN in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 58: 3904-3913.

Olivares, J., Alvarez-Ortega, C., Linares, J.F., Rojo, F., Kohler, T., and Martinez, J.L. (2012) Overproduction of the multidrug efflux pump MexEF-OprN does not impair *Pseudomonas aeruginosa* fitness in competition tests, but produces specific changes in bacterial regulatory networks. *Environ Microbiol* 14: 1968-1981.

Sanchez, M.B., and Martinez, J.L. (2012) Differential epigenetic compatibility of *qnr* antibiotic resistance determinants with the chromosome of *Escherichia coli*. *PLoS ONE* 7: e35149.

Shcherbakov, D., Akbergenov, R., Matt, T., Sander, P., Andersson, D.I., and Bottger, E.C. (2010) Directed mutagenesis of *Mycobacterium smegmatis* 16S rRNA to reconstruct the in-vivo evolution of aminoglycoside resistance in *Mycobacterium tuberculosis*. *Mol Microbiol* 77: 830-840.

PHENOTYPE MICROARRAY AND METABOLIC MODELING

M. FONDI, E. BOSI, L. PRESTA, R. FANI
ComBo, (Florence Computational Biology Group), Dep. of Biology, University of Florence, Via Madonna del Piano 6, 50019, Sesto F.no, Florence

Living organisms continuously need to adapt to fluctuating levels of nutrients, exogenous toxic compounds and environmental stresses (e.g. temperature, pH etc.). In most cases, this adaptation process involves systemic changes at different cellular levels, including gene expression, protein synthesis regulation and metabolic reactions. Understanding the mechanisms underlying such phenotypic switches is one of the most intriguing and challenging topics in modern day biology and one of the key aims of systems biology.
The ease at which genomes are currently sequenced has assigned to genomics one of the first steps in microbial systems biology. On the other hand, genome sequence only represents a snapshot of the real phenotypic capabilities of an organism, providing very few indications on other crucial aspects of the underlying life cycle such as response to environmental and genetic perturbations, fluctuations in time, gene essentiality and so on. To gain a systemic and exhaustive description of living entities, static information deriving from genome sequence is not enough and other levels of knowledge together with adequate modeling frameworks should be exploited.
Metabolic modeling, in particular, refers to a large plethora of *in silico* approaches that can be adopted to quantitatively simulate chemical reactions fluxes within the cell. In recent years the application of such computational techniques to in depth investigate microbial metabolism has spread tremendously in microbiological research; genome scale models have revealed powerful tools to study a vast array of biological systems and applications in industrial and medical biotechnology, including biofuel generation, food production, and drug development. Well-designed metabolic models can help predict the system-wide effect of genetic and environmental perturbations on an organism, and hence drive metabolic engineering experiments. An even more realistic picture of the metabolic traits of a given organism can be obtained by exploiting high-throughput data from innovative technologies such as transcriptomics, fluxomics, proteomics. Such diverse data types can also be mapped over metabolic models and, in this way, specific functional states derived. By exploiting phenotype microarray data, for example, genome scale metabolic networks can be refined and eventually debugged to provide a more detailed picture of the actual metabolic state of a given cell.
Here, we have used constraint-based metabolic modeling and multi-omics integration for gaining a system-level understanding of the metabolic landscape of the Antarctic bacterium *Pseudoaltermonas haloplanktis* TAC125 (PhTAC125), following two main external perturbations (i.e. temperature shock and nutrients switching in a complex medium). PhTAC125 has been isolated from seawater sampled along the Antarctic ice-shell, a permanently cold environment; it is capable of growing in a wide temperature range (4 to 25 C◦) thus revealing a certain (still undisclosed) plasticity at many possible cellular levels. Furthermore, PhTAC125 has recently attracted the interest of biotechnologists, as it has been suggested as an alternative host for the soluble overproduction of heterologous proteins. Nevertheless the volumetric product yields are still poor due to low cell densities and the establishment of a fed-batch culture system relying on complex media has revealed the presence of several metabolic switches during which PhTAC125 selectively uses one (or few) carbon source(s) to sustain growth. The understanding of the actual reprogramming of the whole metabolic network in these two conditions is currently poor, despite it may have important biological/biotechnological drawbacks.

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PHENOTYPE MICROARRAY ANALYSIS MAY PROVIDE INSIGHT FOR POTENTIAL THERAPEUTIC APPROACHES IN HUMAN DISORDERS

C.E. SCHWARTZ
Greenwood Genetic Center, Greenwood, SC

My group has been utilizing the Biolog Phenotype MicroArray for the past half decade to explore its usefulness in distinguishing human disorders based on their metabolic profile. In this regard, we have been relatively successful and some of our findings are presented elsewhere at this meeting (Boccuto et al.)

Just as important, our Biolog analyses have allowed us to gain valuable insight into potential therapeutic approaches for some human disorders. We have successfully applied the Phenotype MicroArray platform, plates PM M1-M8, to establish: 1) Both fibroblasts and lymphoblasts from patients with the MECP2 dup syndrome exhibit abnormal utilization of critical cytokines mediating the immune response. This had led us to propose a treatment involving a combined treatment of anakinra and subcutaneous administration of IgG to lessen the number of respiratory infections which are quite deadly in these young males; 2) The abnormal response to growth factors by fibroblasts from patients with segmental overgrowth syndromes was decreased when treated with Rapamycin. We are exploring the potential of treating the overgrowth lesion by locally administering the drug; 3) Patients with Snyder-Robinson syndrome, a defect in spermine synthase, actually exhibit a metabolic profile indicating a problem in the citrate cycle, which may reflect a mitochondrial dysfunction. This dysfunction could arise from oxidative stress or an impact on the electron transport system. This has led us to consider treating the affected males, who exhibit seizures, with drugs that function by attacking the electron transport system in cells or act as antioxidants.

Taken together, these three examples demonstrate the value of the PM methodology in providing insight, sometimes not clearly evident from the phenotype, for potential therapy of human disorders.

A MECHANISTIC VIEW OF MICROBIAL INVASIONS

J.F. SALLES[1], J.D. VAN ELSAS[1], F. POLY[2], X. LE ROUX[2], C.A. MALLON[1]
[1]Groningen Institute for Evolutionary Life Sciences, University of Groningen, the Netherlands [2]Université de Lyon, INRA, CNRS, Laboratory of Microbial Ecology (UMR 5557 CNRS, USC 1364 INRA), Villeurbanne, France, j.falcao.salles@rug.nl

Biological invasions were until recently synonymous only with macroorganisms due to the long held belief that anything smaller than one millimeter was homogenously distributed on Earth and lacked an indigenous territory. However, the advent of high-throughput DNA sequencing technology and subsequent detailed characterization of microbes from environmental samples revealed that microorganisms could indeed exhibit biogeographical patterns, opening the door to a new perspective of life at the small-scale: microbial invasions. Here we make use of an invasion framework to postulate that microbial invasions are a process of (i) introduction, (ii) establishment, (iii) spread, and (iv) impact. Using a model system where we simulate the invasion of a non-pathogenic derivative of the bacterium *Escherichia coli* O157:H7 into soil communities of different diversity levels, we provide a detailed examination of the patterns and mechanisms of a microbial invasion.
Firstly, microbiostasis, used to describe the inherent invasion resistance of microbial communities, is found to act as a barrier between the establishment and spread phases. By using next generation sequencing as well as BIOLOG plates, we revealed that the strength of this the biotic resistance mechanism is dependent upon the diversity of the microbial community and amount of available resources. Across both of these gradients, *E. coli* survival is indicative of the oft-cited "diversity-invasion effect," whereby invader survival is higher in lower diverse communities and vice versa. Furthermore, the observed patterns are well predicted by the novel metrics: "community niche" and "remaining niche available to the invader." These quantifications are a unique way to measure resource use based on the competitive abilities of all individuals in the community (in the case of community niche) and the level of competition between the invader and residents (in the case of the remaining niche available to the invader). As community niche increases, invasion decreases. At the same time, the remaining niche available to the invader will also decrease, indicating that competition for resources is a major mechanism promoting invasion resistance.
Secondly, we examined whether-and, if so, how-an invasion impacts the resident microbial community. Using similar approaches, these questions are answered by measuring
four bacterial community associated parameters before and after invasion: (i) diversity, (ii) taxonomic structure, (iii) niche breadth, and (iv) niche structure. Despite the fact that *E. coli*'s invasion is never successful, we observed that impacts still occur. Invasion slightly increases diversity and significantly shifts taxonomic structure. In a similar fashion, the niche breadth and niche structure within communities also shift. Interestingly, these alterations of taxonomic and niche structure are linked, and the emergence of previously rare or subordinate
taxa that appear upon invasion account for the emergence of newly utilized niches. This behavior underscores the dynamic and unique nature of microbial invasions, which future experiments may also show modifies the invasion resistance mechanism and community functioning.
Overall, besides helping to reveal the patterns, mechanisms, and general principles of a phenomenon that has long existed but only recently discovered, these discoveries have the potential to aid many practical applications where the manipulations of microbial invasions are key. Such scenarios include the treatment of human pathogens, the effectiveness of probiotics, and the survival of biocontrol and biofertilizing agents.

EFFECT OF THE PLANT FLAVONOID LUTEOLIN ON *ENSIFER MELILOTI* 3001

G. SPINI[1], F. DECOROSI[1], M. CERBONESCHI [2], S. TEGLI [2], A. MENGONI [3], C. VITI[1], L. GIOVANNETTI[1]
[1]Department of Agrifood Production and Environmental Sciences, and Genexpress Laboratory, University of Florence, Florence, Italy
[2]Department of Agrifood Production and Environmental Science, Plant Pathology Section, University of Florence, Florence, Italy
[3]Department of Biology, University of Florence, Florence, Italy

Ensifer meliloti (formerly *Sinorhizobium meliloti*), a Gram-negative nitrogen-fixing proteobacterium, is considered a model bacterium for legume-rhizobium symbiosis. The establishment of a successful symbiosis between *Ensifer meliloti* and compatible host legumes (*Medicago* spp.) depends on a complex molecular signal exchange. The early stage of the molecular dialogue involves the release from plant roots of the flavonoid luteolin, which in turn induces the expression of rhizobial nodulation (*nod*) genes required for root infection and nodule development.
To date, several studies have contributed to characterize in detail the bacterial response to the luteolin perception as far as gene expression is concerned. Nevertheless, despite this molecular information, a global view on *E. meliloti* phenotypes affected by the plant signal luteolin is still lacking. Therefore, an extensive phenotypic investigation of luteolin effect on the nitrogen fixing *E. meliloti* 3001 has been performed.
Results revealed that the plant signal luteolin affects a wide spectrum of *E. meliloti* 3001 phenotypes. Phenotype MicroArray (PM) application pointed out an enhanced resistance phenotype of *E. meliloti* 3001 in the presence of luteolin toward a broad set of chemicals including several antibiotics, toxic ions, respiration inhibitors, membrane damagers, DNA intercalants and other potential antimicrobial agents. Moreover, the presence of luteolin significantly reduced overall N-Acyl homoserine lactones production, as well as the lag phase in relation to the inoculum cellular density, the motility and biofilm formation under nutrient-limited growth conditions. The indole-3-acetic acid (IAA) production was also found to be affected *in vitro* as response to luteolin.
Overall, our findings suggest that the plant signal luteolin triggers a pleiotropic response in *E. meliloti* 3001 related to the nutritional conditions, that is possibly unlinked to the nodulation factor biosynthesis and controls several aspects of bacterial physiology unexplored with molecular analysis conducted so far.

APPLICATION OF THE BIOLOG PHENOTYPE MICROARRAY™ TO STUDY CHANGES IN HOST CELL METABOLISM DURING INFLUENZA A VIRUS INFECTION

A. RÖSEL[1], L.A.I. VAAS[2], N. BURGDORF[1], A. MOSTAFA[3], S. PLESCHKA[3], F. PESSLER[1, 4]
[1]TWINCORE Centre for Clinical and Experimental Infection Research, Group Biomarker for Infectious Diseases, Hannover
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Background. Every year, influenza viruses infect up to 20% of the German general population. Mortality and morbidity are highest among infants and elderly individuals [1]. Data from mouse models of influenza A virus (IAV) infection suggest that infection with more virulent strains can result in a massive metabolic disorder in infected tissues in the context of a "cytokine storm". Especially changes in mitochondrial ATP- and O_2-/ROS-production via pyruvate dehydrogenase (PDH) seem to play an important role [2]. However, a global functional analysis of metabolic changes in infected human host cells has not been performed, and it is not known which other pathways may be affected.
Aims. We therefore aimed to adapt the Phenotype MicroArray™ for mammalian cells (PM-M) to investigate global changes in substrate utilization and energy metabolism in human cell lines infected with IAV.
Methods and Results. We established a protocol for IAV infection of the human lung epithelial cell line A549 and the macrophage cell line THP-1 in Biolog PM-M plates. We compared a wild-type IAV isolate (A/Giessen/6/09, H1N1) from the 2009 pandemic (WT-IAV) [3] with a reassortant, which is identical except that it contains the NS segment from the more virulent PR8 (A/Puerto Rico/8/34, H1N1) strain (RA-IAV) and has proved to be more virulent in mice and cell lines (A.M. and S.P., unpublished results). Infections were initially established on TOX1 plates using different multiplicities of infection (MOIs). Host cell respiration was analyzed with the R-package *opm* [4] at 4, 8, 12 and 24 h post infection.
Both viral strains led to changes in respiration in the A549 and THP-1 cells, albeit with different kinetics. Furthermore, a clear dose response was observed in infection with either strain.
Conclusions. These initial results demonstrate a change in the metabolic phenotype of host cells during IAV infection and suggest that the Biolog PM-M technique may be a useful tool for global profiling of host cell energy metabolism during infection with IAV and, likely, other viruses.
Outlook. We are now starting a detailed investigation employing PM-M plates 1-4 for screening metabolic activity of the infected cells on 367 different energy substrates. These experiments are geared to search for distinct pathways and metabolic hubs which are differentially changed upon infection with IAV with different virulence.

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METABOLIC PROFILING PROVIDES INSIGHT INTO THE FUNCTION AND EVOLUTION OF THE MULTIPARTITE BACTERIAL GENOME

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Historically, the bacterial genome was viewed as consisting of a single large chromosome. However, it has become evident in recent years that many species do not conform to this simplistic view and indeed have a multipartite genome, which consists of multiple large DNA fragments, or replicons. This genome architecture is particularly prevalent among plant symbionts (eg. *Sinorhizobium* and *Rhizobium*), as well as plant and animal pathogens (eg. *Agrobacterium*, *Brucella*, *Vibrio*, and *Burkholderia*). Yet despite a number of societally relevant organisms containing a multipartite genome, the functional significance and the evolutionary driving forces of this genome architecture are not fully elucidated. The genome of *Sinorhizobium meliloti*, a soil-dwelling α-proteobacterium that enters into N_2-fixing symbiosis with legumes, consists of three replicons: a 3.65 Mb chromosome, a 1.68 Mb chromid (pSymB), and a 1.35 Mb megaplasmid (pSymA). To tackle the questions surrounding multipartite genome evolution and function, we constructed *S. meliloti* strains in which pSymA (ΔpSymA), pSymB (ΔpSymB), or both replicons (ΔpSymAB) have been removed from the cell, representing a genome reduction of 20%, 25%, and 45%, respectively. We have characterized the consequences of this synthetic genome reduction through a variety of techniques, including metabolic profiling using both phenotype microarray (Biolog) technologies and HILIC-TOF-MS metabolite analyses. These two complementary approaches clearly indicated that the metabolism and metabolic capacity of *S. meliloti* is significantly disrupted following the removal of the evolutionarily older pSymB replicon, while the loss of the younger and more variable pSymA replicon had little impact. Carbon metabolism was the most severely impacted, with a nearly 70% reduction in utilizable carbon sources following the removal of pSymA and pSymB; yet, only a few of these metabolic traits appeared relevant in a sterile, bulk soil environment. Previously, we reported the construction of a *S. meliloti* deletion mutant library collection, in which each strain carries a large (~ 50 – 350 kilobase), defined deletion of either pSymA or pSymB. In ongoing work, we are screening the pSymB deletion mutants for carbon metabolic defects using the Omnilog system, with the intent of localizing the genetic basis for the phenotypes observed in the ΔpSymAB strain. These results are to be integrated with a comprehensive *in silico* metabolic model that we are currently developing. This model will be used to examine the differential contribution of each replicon to the core and accessory metabolic pathways of *S. meliloti*, and to explore the extent to which the genetic determinants of individual metabolic pathways are spread across more than one replicon. Overall, the characterizations described here provide evidence that secondary replicons are primarily niche specialized entities, and that co-evolution with the existing genome results in a gradual metabolic integration. This niche specialization implies that the secondary replicons of symbionts and pathogens are enriched in genes involved in host interaction, providing a target for genetic analysis of these processes.

PHENOTYPIC AND GENOMIC HETEROGENEITY AMONG *COLWELLIA PSYCHRERYTHRAEA* STRAINS FROM DISTANT DEEP-SEA BASINS

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16S rRNA gene sequencing is routinely used to identify the taxonomy of prokaryotes. Recent evidence suggests that microbes with nearly identical 16S rRNA genes can have substantial genotypic heterogeneity. To better understand the diversity within a single microbial species, we set out to characterize the phenotypic and genomic diversity of three strains that would be classified as *Colwellia psychrerythraea* based on 16S rRNA taxonomy. *Colwellia* are psychrophilic heterotrophic marine bacteria found in many cold ecosystems. Additionally, *Colwellia* species have been shown to respond to marine oil spills and were important members of the microbial community in the Gulf of Mexico during the Deepwater Horizon oil spill. In this study we compare the carbon source utilization profiles and genomic diversity for three *Colwellia psychrerythraea* strains isolated from geographically distant deep-sea basins.

We have recently isolated two strains of *C. psychrerythraea;* strain ND2E from the Eastern Mediterranean and strain, GAB14E from the Great Australian Bight. These two recently isolated strains were compared with the type strain *C. psychrerythraea* 34H, which was isolated from arctic sediments. To understand the phenotypic diversity of these strains, we employed Biolog phenotype microarrays to test the carbon source utilization profiles of these isolates. To investigate the genomic heterogeneity of these three strains we sequenced the genomes of the two recently isolated strains and compared them with the genome of the type strain. These three isolates share greater than 98.2% 16S rRNA identity. However, the carbon source utilization profiles were distinct for each of the strains with less than half of the carbon sources being shared between all three strains. There were also dramatic differences in the genetic makeup of these three strains. The two most closely related strains, 34H and GAB14 (99.3% 16S rRNA identity), are very divergent on the genomic level (79.8% average nucleotide identity). These differences in genomic content are in part due to large insertions and deletions, which, in some cases, correspond to predicted genomic islands. These findings combine to suggest that there can be substantial phenotypic and genomic heterogeneity among a single microbial species in different geographical locations.

EXPLORING THE METABOLIC PROPERTIES OF THE ANTARCTIC BACTERIUM *PSEUDOALTEROMONAS HALOPLANCTIS* TAC125 AT DIFFERENT TEMPERATURES THROUGH GENOMIC AND PHENOMIC ANALYSES

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About 90% of the ocean's volume is colder than 5°C and is comprised of bacteria suitably adapted to cold environments (psychrophiles).Their ability to proliferate in the cold requires a vast array of genetic and metabolic adaptations to maintain the metabolic rates and sustained growth compatible with life in such severe environmental conditions. However, the underlying molecular mechanisms and the metabolic pathways involved in the cold adaptation mechanisms are not fully understood. Thus, here we assessed the metabolic features of the marine phychrophilic strain *Pseudoalteromonas haloplanktis* TAC125, a model organisms for cold-adaptation, at 4°C and 15°C by means of a phenomic approach. Furthermore, the genomic and phenomic features of TAC125 were also compared to the contrasting cold-adapted *Pseudoalteromonas atlantica* TB41,
Although the genome size of TB41 is considerably larger than TAC125 (about 800.00bp), the higher number of genes in most of COG categories of TB41 did not reflect any higher metabolic versatility at 4°C as compared to TAC125 which, in contrast, showed a remarkable high adaptation to cold conditions. In fact, the metabolic downshift occurred between 15°C and 4°C was 8,7% and 56,1% for TAC125 and TB41, respectively. Most of the compounds catabolized exclusively at 4°C by TAC125 were biocidal or antimicrobials, other than some cold adapted molecules such as plumbagin and folate antagonist compounds. Linking genome and phenome features of the two strains through the Ductape tool highlighted the role of several substrates which are better catabolized by TAC125 and are related to several cold-adaptation mechanisms, such as glutathione metabolism and Arginine and Proline metabolism (i.e. Spermine) or vitamine B6 metabolism (i.e. Pyridoxine). Furthermore the ability of TAC125 to grow on Caprylic Acid (related to fatty acid biosynthesis) supports the link between fatty acid metabolism and adaptation to cold temperature in bacteria.

COLOR- AND METABOLISM-DEPENDENT REGULATION OF PHOTOPROTECTION IN GREEN ALGAE

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Light carries fundamental information for living organisms perceived by photoreceptor proteins, which convert it to biological signals. In photosynthetic organisms light is also the energy source to convert CO_2 into organic metabolites. When the absorbed light exceeds the photosynthetic capacity of the cells, a photo-protective mechanism, called Non-Photochemical Quenching (NPQ), is activated, that dissipates excess energy as heat. In green algae NPQ requires the high-light inducible protein LHCSR3, a nuclear-encoded chloroplast localized protein LHCSR3.

The signal transduction process that controls the expression of LHCSR3 involves calcium-signaling events and requires an active photosynthetic electron flow but the exact mechanisms and molecular actors are largely unknown.

Here we will present evidence that LHCSR3 in *Chlamydomonas reinhardtii* is under control of the blue light photoreceptor PHOTOTROPIN (PHOT) and that the PHOT-dependent LHCSR3 regulation is transduced via the kinase domain of PHOT. Besides the color-dependent regulation of LHCSR3 via PHOT, a metabolism-dependent regulation also exists, evidenced by the fact that acetate transcriptionally inhibits LHCSR3. Using a mutant strain that expresses the reporter gene luciferase under control of the promoter of LHCSR3 we applied the Biolog® Phenotype Microarray technology and found that besides acetate other central carbon metabolites transcriptionally inhibit LHCSR3.

Overall our data suggest the existence of a tightly regulated interconnection between photo-perception (through the blue light photoreceptor PHOT), photo-utilization (metabolism) and photo-protection (LHCSR3 and NPQ) in the green algae *Chlamydomonas reinhardtii*.

AN INTEGRATED WORKFLOW FOR GENOME-SCALE RECONSTRUCTION OF ALGAL METABOLIC NETWORKS

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Barriers to genotype-phenotype characterization are often encountered when newly isolated algal species are studied. Importantly, the challenge of obtaining a comprehensive metabolic description significantly impedes reconstruction of the species metabolic network. Here, we describe an integrated analysis pipeline for metabolic characterization of new algal isolates. The workflow involves *de novo* assembly and annotation of the genome, comprehensive phenotyping of metabolism through the use of *Phenotype MicroArray* (PM) technology, and reconciliation of genomic evidence with metabolic data for reconstruction of the network. We present validation of our pipeline through studies carried out on the model alga *Chlamydomonas reinhardtii*, then use a new species of Chloroidium that we isolated from the UAE, herein referred to as *Chloroidium sp. DN1*, as an example of the breadth of information that can be obtained with our approach. Our assembly of *Chloroidium sp. DN1* yielded a genome of 52.5 Mb and 10,605 predicted ORFs. Analysis of the draft genome and ORFeome revealed potential metabolic strategies to explain its adaptation to the desert climate. The isolated Chloroidium species was found to have a rapid growth rate with a broad salt tolerance, and to accumulate lipid bodies in undisturbed cultures. Our PM analyses indicated that *Chloroidium sp. DN1* is able to grow heterotrophically on 10 different carbon compounds including 2,3-Butanedione, Dihydroxy-Acetone, D-Arabitol, D-Mannitol, D-Sorbitol, D-Mannose, D-glucose, Trehalose, α-Keto-Butyric acid, D-Halose, and D-Galactose. Additionally, 38 peptides/dipeptides were found to serve as nitrogen sources promoting heterotrophic growth. We created a draft genome-scale metabolic network for *Chloroidium sp. DN1* that comprises 1,445 genes in 194 pathways. Several genes were predicted in genomic analyses that are likely to play roles in *Chloroidium sp. DN1's* broad carbon substrate uptake capabilities and adaptation to variable salt conditions. Our results validated our workflow for comprehensive characterization of new algal isolates, in turn enabling rapid identification of algae that may possess metabolic properties suitable for commercial applications.

MINE AND CULTIVATE THE UNCULTIVABLE MICROORGANISMS VIA MAGNETIC ISOLATION AND PHENOTYPE MICROARRAY

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Phenotype microarray has been widely applied for the physiological and ecological functions of pure cultivated bacteria or microbial community. Given the fact that over 99% of indigenous bacteria in natural environment are still uncultivable, they have important roles in ecological system but their phenotypes hide within the complex microbial community. For the first time, we developed a magnetic isolation system to separate the living and functional microorganisms from microbiota and achieve their cultivation by phenotype microarray. This magnetic-nanoparticle mediated isolation is a cutting-edge technique by labelling all the living bacteria in the microbial community via magnetic nanoparticles. The magnetic microbiota is cultivated in original environment and the functional bacteria lose their magnetism through division, consequently isolated from the other inert bacteria by magnetic manipulation. The isolated bacteria are still alive with their unique ecological functions and the phenotype microarray is then applied to evaluate their carbon/nitrogen utilization and growth dynamics. From the case study in a coke plant wastewater treatment in the UK, we have successfully isolated the *in situ* functional phenol degrader, *Burkholderiales*, instead of cultivable *Pseudomonas*, consistent with the results of stable isotope probing (SIP). Four carbon (D-alanine, a-D-glucose, tyramine and L-glutamine) and five nitrogen (L-arginine, L-histidine, L-citrulline, L-pyroglutamic acid and δ-Amino-N-valeric acid) sources can promote *Burkholderiales* growth and enhance the phenol degradation capacity. With the modified cultivation medium, we achieved the cultivation of the previously uncultivable *Burkholderiales* and identified their inhibition by hydroxylamine during wastewater treatment process. Without substrate labelling and complex operation, this novel technique combines magnetic-nanoparticle mediated isolation and phenotype microarray, opening a door as a cost-effective tool to reveal the *in situ* physiological behaviour and ecological functions of uncultivable bacteria in complex microbial community.

BACTERIAL DIVERSITY AND FUNCTIONAL SERVICES WITHIN THE RHIZOSHEATH OF A DESERT PLANT

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Desert plants develop specific physiological and morphological adaptations to cope with water limitation. The xeric plants of the genus *Panicum*, growing in the sand dunes of the Grand Erg Oriental of the Tunisian Sahara, is an ideal example for effective adaptations to arid conditions. It has a unique root morphology where the central root is surrounded by a cylindrical protective sand grain rhizosheath. This structure is contributed by the root 'metaorganism' (the assemblage of root hairs and tissues and the associated microorganisms), its secretions, and sand particles. All these components are presumably involved in increasing moisture and limiting desiccation, protecting the root central core. To evaluate the microbiome contribution to such a root development, we analysed the bacterial diversity associated with the root system compartments together with the metabolisms and physiological profiles of these communities. Microscopy analysis of the rhizosheath revealed a complex matrix of root hairs, sand grains and bacteria. Culture-independent analysis and bacteria isolation showed a different spatial distribution of bacteria according to the specific root system compartments, indicating that different selective pressures shape the root bacterial communities in a spatial range of a few millimeters. *Gammaproteobacteria* dominated the internal root tissues, conversely *Actinobacteria* of the *Micrococcaceae* and *Streptomycetaceae* families were enriched in the rhizosheath. In addition, the bacterial functionality of both root system fraction and bacterial strains was estimated using carbon and nitrogen substrate utilization (OmniLog™ Phenotype Microarray). The collected data indicate that the *Panicum* rhizosheath selects a unique and specific bacterial community adapted to drought conditions and provide an excellent example to study water stress resistance in the harsh conditions of the Sahara sands.

PHENOTYPIC MICROARRAY FOR CULTURAL HERITAGE: INNOVATIVE TOOLS FOR BIO-CLEANING APPROACH

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Research on biotechnology application to the field of Cultural Heritage (CH) restoration have shown how the selected microorganisms are efficient to clean particularly complex and embedded substances, process called: bio-cleaning. Bacteria are able to synthesize a group of specific enzymes for the degradation of complex materials deposited on frescos, sculptures and monuments. Several studies have used sulfate-reducing bacteria and nitrate-reducing bacteria to remove undesirable complex compounds. The bio-cleaning processes have shown to be respectful to the artwork, environmental friendly and safe for restorers because not toxic or pollutant substances are used. This technique have prove to be effective removing black crusts, salt efflorescence, and animal glue residues, in a less invasive and more specific way than the traditional cleaning techniques. However, in order to increase the diffusion of these biological techniques using living organisms for CH restoration, the use of products non-toxic, efficient and respectful with the artwork, more attention must be paid on the bacterial metabolic mechanisms occurring during bio-cleaning processes. Consequently, it is useful and important to apply to the bio-restoration field the phenotypic techniques available for a fast and profound study of the bacteria applied for bio-cleaning strategies. We have used, for the first time, the Phenotypic Microarray (PM) technique for characterizing the bacteria utilized for bio-cleaning of CHs, in order to identify the benefits and drawbacks of these biorestoration treatments.
In our experimental conditions, the potential use of Phenotypic Microarray for screening and characterization of *Pseudomonas stutzeri*, a bacterium adopted for bio-cleaning, was tested.
Several cultural conditions and parameters on PM tests were recorded including: i) best contact time between artworks and cell suspension; ii) list of un-desirable substances that are able to eliminate; iii) reduction of eventual inhibitors and interferences during bacterial activities. In particular the PM assays have demonstrated that *P. stutzeri* 5190 is able to utilize the 21.5% of the studied C-compounds (including amino acids; carboxylic acids, fatty acids, carbohydrate, esters, polymers and alcohols) and the 54.7% of nitrogen sources tested (including organic and inorganic N sources). Other PM assays have shown that these bacteria are osmotolerant until 6.0% NaCl gradient and able to grow from pH 5 to pH 10. Chemical sensibility assays show that the bacterium is able to resist medium - high dose of the majority of the tested chemical components (including a variety of inhibitors, toxic agents and antibiotics).
All these data acquired will therefore allow increasing the optimization in bioremoval efficiency and reducing artwork risks during bio-cleaning strategies.

INHIBITORY EFFECTS OF L-HISTIDINE ON BIOFILM FORMATION AND *FLO11*-ASSOCIATED PHENOTYPES IN *SACCHAROMYCES CEREVISIAE* FLOR YEASTS

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Flor yeasts of *Saccharomyces cerevisiae* are wine yeasts characterized by a distinctive biodiversity due to *FLO11* gene that codes for a hydrophobic cell-wall mannoprotein with a role in biofilm formation. Here, by Phenotype Microarray™ analysis 380 nitrogen compounds were administered to three *S. cerevisiae* flor strains handling *FLO11* alleles characterized by different expressions. Flor yeasts were able to metabolized amino acids and dipeptides as the sole nitrogen source, with the exception of L-histidine. L-histidine totally inhibited growth and its effect on cell viability was inversely related to *FLO11* expression. Hence, L-histidine did not affect the viability of the *Δflo11* and S288c strains. Moreover, L-histidine considerably decreased air-liquid biofilm formation and adhesion to polystyrene of the flor yeasts, and, interestingly, no effect on the expression level of *FLO11* gene was observed. Finally, L-histidine changed the chitin and glycan content on flor yeasts cell wall.

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FROM GENOME TO PHENOME AND BACK: UNDERSTANDING THE HIGH METABOLIC VERSATILITY OF *BURKHOLDERIA CEPACIA* COMPLEX

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Strains of the *Burkholderia cepacia* complex (Bcc) are able to colonize many different environments; they can have a free-living lifestyle, but they may also colonize multicellular eukaryotes intracellularly and, although they are considered highly beneficial in the environment, they can also cause life-threatening infections in immuno-compromised and Cystic Fibrosis (CF) patients. This heterogeneous lifestyle and the consequent high metabolic versatility is accompanied by unusually large genomes, suggesting that particular genome structures and genetic content may support and explain in evolutionary terms such high metabolic diversity.
Then, the purpose of this work was to provide a model of the relationships between genomes and phenotypic diversity in the 18 Bcc type strains, through a multi-level, systems biology approach.
The genome sequences of these 18 strains were obtained and their assembly revealed that sizes vary between 6,23 and 9,72 Mb. A Pulse Field Gel Electrophoresis analysis confirmed the presence of multiple replicons in each strain. Further analysis on the sequences obtained allowed the identification of peculiar patterns as concerning, on one hand, genes involved in pathogenesis, virulence and antibiotics resistance and on the other hand genes involved in plants growth promotion, nitrogen fixation and degradation of toxic agents.
Large scale phenotypic characterization was also performed on these strains, adopting the Phenotype MicroArray (PM) technique. The ability of these strains to grow using different carbon/nitrogen sources and to grow in the presence of different pH, osmolytes and toxic compounds was tested. In addition the M.I.C. of different classes of antibiotics were determined.
All those data were then used to perform an analysis of relationships between genome data and phenome results with the software suite DuctApe, to provide a first model of genome-metabolic description and differentiation of Bcc strains.

A PROTEOMIC AND PHENOMIC APPROACH TO STUDY THE EFFECT OF THE ANTIMICROBIAL PEPTIDE OP-145 ON *STAPHYLOCOCCUS AUREUS*

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Emergence of multidrug-resistant (MDR) bacteria is becoming a major health concern worldwide as patients infected with such strains can no longer be successfully treated. A promising alternative treatment strategy is the use of antimicrobial peptides (AMPs). Within the EU-funded 'Biofilm Alliance' (BALI) consortium, we aim to incorporate immune orchestrating synthetic antimicrobial and anti-biofilm peptides into a novel controlled release drug delivery formulation to control biomaterial-associated infections. The basis for the development of the AMPs was OP-145, previously named P60.4Ac, a 24 amino acid peptide derived from the human cathelicidin LL-37. OP-145 killed *Staphylococcus aureus in vitro* and eradicated this species from three-dimensional wounded human skin models. Moreover, OP-145 inhibited the formation of biofilms by *S. aureus* on abiotic and biotic surfaces. To get more insight into the mechanism of antimicrobial and anti-biofilm activity of this peptide, we performed proteomic and phenotype microarray analyses on *S. aureus* exposed to sub-inhibitory concentrations of OP-145. The knowledge obtained using this approach is vital to guide the design of novel and improved antimicrobial and anti-biofilm peptides.

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APPLICATION OF PHENOTYPE MICROARRAYS IN MICROBIAL SYSTEMATICS: TARGETING DIFFICULT GROUPS

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The genomics revolution is motivating the modernisation of prokaryotic systematics into a comprehensive characterisation based on the integration of "traditional" physiological and chemotaxonomic methods and genomic approaches, the so-called taxo-genomics. Nevertheless, to react to the large quantities of data produced by high-throughput sequencing technologies, it is still required that "traditional" approaches adopt reliable and standardised methodologies and increase data portability to allow an efficient correlation of the results between distinct taxonomic studies and an acceleration in microbial diversity knowledge.
Here, we illustrate the application of phenotype microarrays (PM) measurements to the characterisation of novel species in the order *Geodermatophilales*, particularly slow-growing chemoorganotrophic bacteria inhabiting dry biotopes, which are challenging to analyse. Optimised laboratory protocols provided reproducible phenotypic profiles, however, which could be explored with specifically adapted bioinformatic tools implemented in the freely available package opm for the R statistical environment. This allowed for establishing meaningful and stable markers within the order, for addressing the functionality encoded in the genomes and for reconstructing PM character evolution to supported taxonomic reclassifications in the order.

BIMODALITY OF METABOLIC HETEROGENEITY ACROSS SEROTYPES OF *SALMONELLA ENTERICA*

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Salmonella enterica subspecies I is a major cause of foodborne illness in people, and whole genome analysis has revealed that genetic heterogeneity between and within serotypes is common. What is not evident is how much phenotypic heterogeneity is present and how it correlates to genotype. To explore this issue, phenotype microarray (PM) plates 1 – 10 were used to assay respiratory activity of 3 different groupings of isolates on 950 different metabolic conditions and compounds (Biolog, Hayward, CA). Group 1 included 4 independent replications with *Salmonell*a *enterica* serovar Enteritidis strain 22079, which was previously sequenced and characterized by several analyses. Respiration on fourteen (1.48%) compounds varied significantly between different colonies of strain 22079 after accounting for technical variation; 11 were carbon sources, 2 were nitrogen sources and 1 was a phosphorous source. Analysis of a 2nd group of 8 independent field isolates of serovar Enteritidis showed greater variability, and 81 metabolites (8.5%) differed. Classes of compounds included 15 carbon, 9 nitrogen, 9 phosphorous, 3 sulfur and 45 nutritional supplement sources. Assay of a 3rd group of 4 serotypes not related to serovar Enteritidis found that 113 metabolites (11.9%) varied, and these included 13 carbon, 4 nitrogen, 4 phosphorous and 92 nutritional supplement sources. Thus, clonal selection of 22079 greatly reduced metabolic variation within serotype, and this result was expected. However, the degree to which independently acquired isolates of serovar Enteritidis varied within the same serotype was not expected, and results were that variance was closer to that observed to occur between serotypes. Given that serovar Enteritidis is considered an exceptionally clonal serovar within *Salmonella enterica*, we suggest that genomic and phenotypic analyses should be coupled to establish the origin of genomic heterogeneity occurring across *Salmonella enterica.*

GENOME AND PHENOTYPE MICROARRAY ANALYSES OF TWO *RHODOCOCCUS* STRAINS WITH ENVIRONMENTAL AND INDUSTRIAL RELEVANCE

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The *Rhodococcus* genus comprises of Gram-positive, non-motile, nonsporulating, aerobic bacteria, with a high G+C content and a mycolic acid-containing cell wall. Members of *Rhodoccocus* genus are widely distributed in soil, water and marine sediments; moreover, some of them are pathogens for humans and animals (*R. equi*) while others cause diseases of plants (*R. fascians*). Due to their metabolic flexibility and their tolerance to various stresses, they play an important role in nutrient cycling and have potential applications in bioremediation, biotransformations, and biocatalysis. Consistent with the wide catabolic diversity, they possess large and complex genomes, which contain a multiplicity of catabolic genes, a high genetic redundancy of biosynthetic pathways and a sophisticated regulatory network. Compared to the increasing number of *Rhodococcus* spp. genomes that have been sequenced, very limited studies are available on their wide metabolic abilities and on their potentials for biodegradation activities related to genomic features.
The present work focuses on the analysis of metabolic capabilities and genomic features of *Rhodococcus* sp. BCP1 and *Rhodococcus opacus* R7 that were isolated from an aerobic butane-utilizing consortium and from an aromatic hydrocarbon contaminated soil, respectively. Phylogenetic analyses and genomic comparison with correlated species were performed to characterize the unique regions present in the genome of R7 and BCP1 strains. Phenotype Microarray analysis was also conducted using the Biolog redox technology with commercially available Biolog microtiter plates (pre-loaded substrates) and with microtiter plates that were manually prepared with additional organic/ xenobiotic compounds to be tested. Compared to BCP1, R7 strain generally showed higher metabolic activities compared on the tested carbon sources and broader range of nitrogen and sulfur sources. BCP1 specifically utilized some carboxylic acids and amino fatty acids as carbon and nitrogen sources, respectively. The two strains were able to resist to various chemicals, pH values and osmolytic substances. Phenotype microarray results also highlighted new biodegradation capacities of BCP1 and R7 strains towards aliphatic and aromatic hydrocarbons as well as emergent contaminants like naphthenic acids. These metabolic data were combined with the genomic study to genetically interpret the metabolic features of these bacterial strains with potential application for bioremediation and industrial processes.

EPIGENETIC CONTROL OF METABOLIC TRAITS IN BACTERIA

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Phase variable type I restriction modification (R-M) systems have been identified and we have shown that they provide a novel bacterial epigenetic control mechanism that affects both gene expression and important bacterial phenotypes; i.e. in pathogens, these systems were linked to the capacity for generating disease (Manso et al., Nature Communications 2014). The phase variable genetic modules are based on repeated inverted copies of the specificity gene (hsdS) of the R-M system that allows for high frequency recombination conferring the R-M system with multiple different methylation target specificities. These double-hsdS modules are widespread among firmicutes. In order to identify metabolic traits associated to differential methylation of the genome, we have isolated in the species *Streptococcus pneumoniae* and *Listeria monocytogenes* variants, which express stably only one single variant of the methylation specificity determinant. Phenotype Microarray technology is currently being used to identify differences in carbon and nitrogen metabolism and variations in pH and osmolytes. The characterization of these high frequency phase variable systems is of importance, as they represent a widespread genetic module for rapid generations of subpopulations with different fitness.

APPLICATION OF STATE-OF-THE-ART MACHINE LEARNING TECHNIQUES TO THE PM DATA ON AUTISM-SPECTRUM DISORDERS – BOOSTING WITH FALSE DISCOVERY CONTROL

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Modern biotechnologies, such as phenotype microarrays (PM), often result in high-dimensional data sets with much more variables than observations (n « p). These data sets pose new challenges to statistical analysis: Variable selection becomes one of the most important tasks in this setting.
We compared 17 autism spectrum disorder (ASD) patients to 18 healthy controls in order to find biomarkers for ASD (Hofner et al., 2015). To reduce the dimensionality and to gain more biological insights, we mapped the PM data to amino acid pathways. In a second step, we used machine learning techniques to determine which of the amino acid pathways were differentially expressed in ASD patients. To be more precise, we considered the combination of model-based boosting and stability selection.
Boosting (Bühlmann and Yu, 2003) allows to estimate complex models with built-in variable selection. Stability selection was recently proposed by Meinshausen and Bühlmann (2010) as flexible framework for variable selection, and was later refined by Shah and Samworth (2013). By the use of resampling procedures, stability selection adds a finite sample error control to high dimensional variable selection procedures such as boosting.
We present results from a detailed simulation study that presents insights on the usefulness of the combi- nation of boosting and stability selection. Limitations will be discussed and guidance on the specification and tuning of stability selection will be given. The results will then be used for the detection of metabolic biomarkers for autism.
Boosting methods are implemented in the R package mboost (Hofner et al., 2014; Hothorn et al., 2010, 2013). Stability selection for boosting and other machine learning approaches is implemented in the R package stabs (Hofner and Hothorn, 2015). Data storage, manipulation and annotation for PM data are implemented in the R package opm (Go¨ ker, 2015).

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JOINT GENOMIC-PHENOTYPIC ANALYSIS OF BACTERIAL SPECIES: THE NEED FOR A PM DATA REPOSITORY

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Last decade's advances in genomics have proven that hundreds (if not thousand) bacterial genomes can be obtained with limited effort. Even more, genomic databases are filled with genomes belonging to virtually every branch of the bacterial tree of life. On the other end, the "phenotypic landscape" looks depressingly empty, hampering the development of methods and studies aimed at deciphering the translation of genetic differences to phenotypes and their evolution. Phenotype Microarrays would represent an ideal platform to construct such a "phenotypic landscape", mainly for its standardised set of conditions which allows to almost directly compare different species and strains. Despite this potential, virtually no PM raw data are available. Even though the PM raw format is a very simple and light csv or json file, almost no published study adds those files as supplementary material, but only a categorical classification of growth is reported; given the different thresholds used to define growth this is clearly a sub-optimal solution.
In this talk I will propose a few solutions to this problem, from publishing PM raw data as supplementary material to build a centralised database (a bare minimum example of such database will probably be shown). I will also show what kind of analysis could be carried out if such database existed, hoping to foster a shared initiative.

HIPERFIT: SOFTWARE FOR HIGH THROUGHPUT MODEL FITTING, PARAMETER ESTIMATION AND MODEL CHOICE FOR BIOLOG PHENOTYPE MICROARRAYS

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We have developed software to enable high throughput fitting of parametric models to Biolog data. The aim is to allow Biolog users to obtain considerably more details from their data than area under curve or end point at a fixed time. The software finds the best fit model to the data in each well, and estimates crucial parameter values, such as length of lag phase, maximal "growth" rate and yield. It can also identify and report cases of diauxie, along with associated parameters. The software has a back-end that performs the analysis in high throughput on a graphics processing card, and an easy to use front end that uses a web browser to visualize and query the data and model fits. Users can take a "plate-centric" approach, drilling down to individual wells, models and parameters, or a "query-centric" approach, to reveal, for example, those wells showing the shortest lag phase, or to perform comparisons between different conditions. We demonstrate the software, showing examples from *E. coli* and *S. cerevisiae* experiments.

THE MINIMUM INFORMATION ON A PHENOTYPE MICROARRAY STUDY (MIPS) STANDARD

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Phenotype microarray (PM) systems simultaneously capture kinetic data on nearly 2,000 phenotypes of microbes or eukaryotic cells by recording stimulation or inhibition of energy production over time with distinct substrates. A minimum standard for recording and distributing PM data, which aid, e.g., in improving genome annotation and testing physiological hypotheses, does not yet exist, however. In contrast, standards for sequence data such as the Minimum Information about a Genome Sequence (MIGS) have been established some years ago and are increasingly adopted by major sequencing centres and sequence repositories (see http://gensc.org/projects/mixs-gsc-project/).
The Minimum Information on a Phenotype Microarray Study (MIPS) project was started by the Genomic Standards Consortium to develop a standard for PM applications (see http://gensc.org/projects/mips/). PM users interested in MIPS are asked to subscribe at https://lists.sourceforge.net/lists/listinfo/genscmips-info to participate in the work on the forthcoming MIPS white paper. Contributions by researchers who apply PM to environmental samples, or organisms that are challenging to cultivate and measure, is particularly welcome.
The talk will provide an overview on MIPS version 4 to further stimulate interest in the project, to promote discussions on standards in the PM community and to attract potential participants in finishing the MIPS white paper.

METABOLIC FINGERPRINTS OF HUMAN PRIMARY ENDOTHELIAL AND FIBROBLAST CELLS

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Background: Human primary cells originating in different locations within the body could differ greatly in their unique metabolic profile, influencing how they act during pathophysiological processes. Cell metabolism could affect how susceptible or resistant they are to various disease risk factors. One way to monitor cellular metabolism is through cell growth and proliferation. Tetrazolium reduction is a ubiquitous property of cells enabling colorimetric cellular assays to be multiplexed into phenotype microarrays.
Aim: Systemic autoimmune diseases, such as rheumatoid arthritis and scleroderma show an increased risk for early development of coronary artery disease and myocardial infarction, as a consequence of endothelial dysfunction. Scleroderma is also characterized by fibrosis, resulting in skin thickening and lung damage. In order to better understand these pathophysiological processes, our specific goals were to measure metabolic activity of primary human endothelial cells and fibroblasts, as well as determine their specific metabolic fingerprints.
Methods: Human primary endothelial cells from the coronary artery (HCAEC) and from the umbilical vein (HUVEC), and fibroblasts from normal, healthy lung (NHLF) and dermal (NHDF) tissues (all from Lonza, Walkersville, MD, USA) were initially resuspended at a density of 200.000 cells/ml in Biolog IF-M1 medium with Pen/Strep, L-Glutamine and dialyzed FBS. Cells were then seeded into Biolog Phenotype MicroArray plates (PMM1-M4) at 10.000 cells/50 µl/well and incubated at 37°C, under 5% CO_2 for 18h. Following the incubation, 10 µl Redox Dye Mix MB/well was added and the plates covered with sealing tape to prevent CO_2 loss, with resumed incubation. Kinetically determined tetrazolium reduction (measured as intensity of color change) was monitored every 15 min in the OmniLog incubator/reader (Biolog Inc., Hayward, CA, USA) for 24h. During this time 367 biochemical substrates were tested for utilization.
Results: Unique patterns were observed for HCAEC and HUVEC, with HCAEC metabolizing tricarballylic acid and mannan to the highest extent, followed by pectin, gelatin, uridine, Tyr-Glu and His-Trp, Met-Met, D-Glucuronic acid, Gln-Glu and Gln-Gln. On the other hand, HUVEC metabolized D-Glucuronic acid, followed by Glu-Gln, Gln-Gln and other substrates. Fibroblasts exhibited a different pattern of utilized substrate metabolites, specifically NHLF primarily utilized dextrin, D-maltose and D-mannose, followed by maltotriose, D,L-Lactic acid, Ile-Gln, Inosine, Glycogen and Gln-Glu, Asp-Gln, Thr-Gln, Pro-Gln, Phe-Glu and Phe-Asp. NHDF showed highest tetrazolium reduction with Gln-Glu, Phe-Glu, Phe-Asp, among others.
Conclusion: We show that substrate utilization with Phenotype MicroArrays PMM1-M4 can provide metabolic characterization and a unique profile in specific primary human cell types.

BIOLOG PHENOTYPE METABOLIC MICROARRAYS: FINDING THE LINK BETWEEN GENOTYPE AND PHENOTYPE.

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Next-generation sequencing has provided researchers with a powerful tool to investigate the human genome, but has also raised the challenge of correlating the vast number of genomic variants to the individual's phenotype. The Biolog Phenotype Microarrays (PM) platform represents a novel, unbiased approach to explore multiple pathways simultaneously and to evaluate the effects of genetic variants on cell metabolism. We present three example in which the PM plates allowed us to characterize the role of different mutations in the same gene or the same pathway that were associated with diverse clinical presentations.

Example 1. FG and Lujan syndromes are both associated with mutations in the *MED12* gene but their clinical features are quite different. FG is associated with macrocephaly, short stature, intellectual disability (ID), hypotonia, and affable behavior. Lujan has mild ID, marphanoid habitus, hyperextensible joints, and behavioral issues. Metabolic profiles from lymphoblastoid cell lines showed increased metabolism of most carbohydrates, ketone bodies, and Krebs cycle intermediates in two patients with FG (p.R961W mutation) and decreased metabolism of the same compounds in one Lujan patient (p.N1007S mutation), thus perhaps providing metabolic evidence for the phenotype heterogeneity associated with the two *MED12* mutations.

Example 2. Fragile X syndrome (FRAXA) is the most common monogenic cause of ID in males and is caused by abnormal methylation and suppression of the *FMR1* gene transcription. Other than moderate to severe developmental delay, this condition is characterized by several behavioral issues, including autism spectrum disorder (ASD), anxiety, and ADD/ADHD. Investigation of lymphoblasts from 16 FRAXA patients and 20 controls suggested specific associations of behavioral features and certain metabolic profiles. Three FRAXA patients with ASD showed decreased tryptophan utilization, 4 cases with ADD/ADHD showed increased utilization of Krebs cycle intermediates and ketone bodies, and 9 cases with the diagnosis of anxiety and/or obsessive-compulsive disorder (OCD) revealed increased metabolism of glucose-related sugars, Krebs cycle intermediates and carboxylic acids.

Example 3. Abnormal activation of the Pi3K-AKT pathway has been associated with a spectrum of segmental overgrowth conditions. Metabolic analysis of fibroblasts from affected and unaffected tissues of 16 individuals with different segmental overgrowth syndromes (CLOVE, Klippel-Trenaunay-Weber, Fibro-Adipose Overgrowth) indicated that mutations affecting the Pi3K complex exhibited a reduced response to growth factors activating the pathway (insulin, IGF-1, FGF-1, GH, PDGF), while *AKT1* mutations associated with Proteus syndrome caused increased response to the same growth factors.

Taken together, these three examples demonstrate the valuable contribution of the PM plates in investigating genotype/phenotype correlations and identifying metabolic targets for future investigations and potential treatments.

PHENOTYPE MICROARRAYS AS A TOOL TO STUDY NICHE OVERLAP AND CATABOLIC VERSATILITY OF SAPROTROPHIC FUNGI

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Fungi have a range of important ecological functions associated with nutrients and carbon cycling processes in leaf litter and soil. They contribute to the total respiration of soil microorganisms for more of 90% [1]. Functional diversity investigates the role played by different species in relation to the maintenance of ecosystem functionality. In this context researches about the relations existing between fungal functional diversity and decomposition rates are important because availability of nutrients in soil is essentially due to the dynamics of the degradation of organic matter.
Fungal functional diversity studies has mainly concentrated on evaluating the ability of selected fungi to utilize simple sugars, cellulose, or lignin [1, 2]. Whilst obtaining such information permits the assignment of fungi into broad physiological groupings, this approach does not provide the tools necessary for the evaluation of large-scale patterns in fungal functional diversity, since all natural systems contain fungi capable of decomposing the three aforementioned major classes of plant compounds [3]. This shortcoming can be overcome by using the Phenotype MicroArray^{TM} system (PMs) [4] which allows for the testing of microorganisms against many different carbon sources.
The carbon source PMs were used successfully to detect the metabolic profiles of single fungal isolates representing a valuable tool for uncovering the width of fungal trophic niche [5, 6]. It must be said that PMs profiles obtained for the pure cultures can reflect only functional potential rather than *in situ* functional ability, nevertheless some phenotypic traits can result outstanding respect to others, and can ideally point some species as key stone elements of the decomposer community.
The PM system originally designed for unicellular prokaryotes was optimised for filamentous fungi by both selecting carbon sources, to obtain micro-plates particularly capable of discriminating between fungal phenotypes [4], and by suspending spores or cells within a dense inoculating fluid, resulting in more uniform growth and relying on colour formation reflecting active metabolism [4]. However, colour formation did not always coincide with growth for all the 95 carbon sources, and for some filamentous fungi biomass formation can occur without colour development, or vice versa, the colour developed in the wells corresponds to a very low biomass value. Moreover the use of PMs with slow growing, not sporulating or xerophilic fungi requires some precautions and the adoption of specific countermeasures in the preparation of the inoculum.
In this contribution will be given an overview on the use of PMs as a tool to study niche overlap and catabolic versatility of saprotrophic fungi and their further application to cultural heritage, post-harvest and bio-control case studies.

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PHENOTYPE MICROARRAYS FOR GENOME-WIDE ANALYSIS OF CARBOHYDRATE ACTIVE ENZYMES IN EIGHT SPECIES OF THE MYCOTROPHIC FILAMENTOUS FUNGUS *TRICHODERMA*

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Trichoderma reesei QM 6a is the progenitor of a plethora of high cellulases producing mutant strains now used as the major industrial source of cellulase production. Interestingly the genome of this fungus contains only a small repertoire of genes encoding cellulase- and hemicellulose-encoding genes when compared to several other fungi including other *Trichoderma* species.
The genus *Trichoderma* (Hypocreales, Sordariomycetes, Ascomycota, Dikaria) contains fungi with diverse nutritional strategies. The absolute majority of these fungi are capable to grow as saprotrophs *in vitro* but *in situ* they are most frequently found as biotrophs on other fungi (mycoparasites) and in association with plants (endophytes and epiphtes). A small group of species known for their ability to grow in soil and on the broad variety of other substrates as saprotrophs became powerful environmental opportunists that are also capable to cause mycoses in immunocompromised humans. Despite the later property, the ability to antagonise, parasitize and even kill fungi is widely applied in agriculture for biological control of plant pathogenic fungi.
The cumulative importance of *Trichoderma* fungi led to an increasing number of species for which genomic sequences are now available. To exploit this information and to learn about the genomic properties of cellulase producing and/or opportunistic and highly mycoparasitic *Trichoderma* species we investigated carbohydrate active enzymes (CAZymes) in eight species: four from the *Trichoderma* Section *Longibrachiatum* (*T. reesei*, *T. parareesei*, *T. citrinoviride* and *T. longibrachiatum*), *T. harzianum*, *T. virens* and the two from the Section *Trichoderma (T. atroviride* and *T. asperellum)*. To tightly link genotypes to phenotypes we used carbon source utilization Phenotype MicroArrays (PM) and compared the utilization of carbohydrates (poly-, oligo- and monosaccharides), the corresponding polyols as well as amino sugars and sugar acids. Highest growth rates were found on N-acetylglucosamine, D-glucuronic and D-galacturonic acid, several D-glucose- and D-galactose containing oligosaccharides as well as on D-mannose, D-fructose, D-galactose and most polyols. *T. atroviride* and *T. virens* exhibited the highest growth rate of all eight species on many of above listed carbon sources. There were, however, also species- and section-specific differences: e.g. all species from Section *Longibrachiatum* only poorly assimilated sucrose, whereas the others grew on it well. The genomic features underlying these phenotypes will be presented. Major emphasis will thereby be given to the genomic inventory of CAZymes and their evolution and differentiation during speciation in *Trichoderma*.

Abstracts

Part II

Posters

NON-PARAMETRIC ANALYSIS OF PHENOTYPE MICROARRAY™ FUNGAL RESPIRATION AND GROWTH KINETICS BY ADDITIVE MIXED MODELING (GAMM)

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The carbon source Phenotype Microarray™ (PM™) technique provides an effective approach for studying the metabolic profiles of different micro-organisms, and provides a starting point in uncovering the potential ecological niche of bacteria and fungi. Indeed, profiles can be compared across closely related strains or single isolates, and analysed in response to varying chemical and physical experimental treatments -e.g. incubation temperatures, and absence/presence of light during growth [1 , 2, 3].
Metabolic activity and growth of micro-organisms inoculated in PM™ microtitre plates is measured at different wavelengths and time intervals. For fungi, readings are made at 490 nm (mitochondrial activity) and 750 nm (mycelial growth), following 0, 24, 48, 72, 96, 168, 192 or more hours of incubation [2, 3]. Several replicate plates are usually used, and readings at each incubation interval are repeated to account for error.
As a consequence, PM™ data is highly multivariate and characterised by spatial and temporal auto-correlation. Distinct statistical packages and modelling techniques have been used to handle and analyse this type of data [4, 5]. However, most implementations have focused on bacterial studies, and have largely failed to deal with data autocorrelation and grouping structures. Importantly, fungal respiration and biomass kinetics can differ substantially from that of prokaryotes, and may therefore require distinct analytical strategies.

Within the statistical language environment provided by the R Open source software and associated packages, we explore the use of (Generalised) Additive Mixed Modelling (GAMMs) [6] in the analysis of PM™ respiration and growth curves from fungi.
This smoothing splines-based modelling technique can handle non-independent data resulting from temporal (and spatial) autocorrelation in PM™ data; while providing a non-parametric technique that can flexibly adapt to any curve shape. Further, cross-validation and Information-Theoretic (I-T) approaches can ensure identification of optimal curve parameters as for the bias-variance trade-off, strengthening the quality of inference. Here we provide a general discussion on the implementation of this modelling strategy for PM™ data, followed by a case-study on respiration and growth data from two different fungal strains and their co-inoculums. As part of the analysis we describe additional techniques that can complement a GAMM-based analysis (e.g. data-mining algorithms), or provide alternative modelling strategies (e.g Monte Carlo Markov Chain methods).

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INVESTIGATION OF BIOCHEMICAL AND MOLECULAR INHIBITOR OF ALCOHOL PLANT EXTRACT ON PATHOGENIC BACTERIA

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The finding of verities new sources of antimicrobial potential agents are needed to resist the new generations of drug-resistant microorganisms. This study is designed to compeer the antimicrobial activity of the extract of the marine macroalgae *Ulva lactuca* and the medicinal plant *Nigella sativa* against the Gram positive cocci *Staphylococcus aureus* and Gram negative bacilli *Pesudmonas aeroginosa*. The results showed high antibacterial activity, with inhibition of *S. aureus* growth up to 30 mm and 20 mm and *P. aeruginosa* growth inhibition was up to 12 mm and 15 mm, after the treated with 100 µl *U. lactuca* and *N. sativa* extracts, respectively. The MICs and MBCs were reflected with the growth inhibitor with values of 2 µl, 8 µl and 4 µl, 8 µl for *S. aureus* and *P. aeruginosa* after treated with *N. sativa* respectively. The antimicrobial activity of *U. lactuca* and *N. sativa* extracts reflect on the dry weight and the biochemical and molecular analysis. Further, the analysis of SEM showed an efficiency of the extracts on the cell wall.

EFFECTS OF B-HEXACHLOROCYCLOHEXANE AND TOLUENE ON THE CATABOLISM OF THE SAPROTROPHIC SOIL FUNGUS *PENICILLIUM GRISEOFULVUM*

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β-exachlorocyclohexane (β-HCH) is a toxic, carcinogenic halogenated organic compound, widely used as insecticide, and can cause damage to reproductive and nervous systems in mammals. Three isomers of hexachlorocyclohexane, α-HCH, β-HCH and γ-HCH, were included as persistent organic pollutants in the Stockholm Convention in 2008, and their worldwide spread and persistence represent a severe environmental problem. Fungal tolerance and biotransformation of toxic substances hold considerable promise in environmental remediation technologies as many fungi can tolerate extreme environmental conditions and possess efficient extracellular degradative enzymes with relatively non-specific activities. In this study we have studied the metabolic effects of a saprotrophic soil fungus, *Penicillium griseofulvum* Dierckx, isolated from polluted soils affected by a high concentration of isomers of hexachlorocyclohexane, in the presence of β-HCH, the most recalcitrant isomer to biodegradation. The Phenotype MicroArray™ system was used to gather information on fungal metabolism. A combined inoculum of the fungus with the same concentration of β-HCH in toluene, and one with only toluene were performed in FF MicroPlate™ arrays to evaluate co-metabolic effects of the xenobiotic and the solvent on the use of different carbon sources by the fungus. Data obtained from the Phenotype MicroArray™ assays were used in comparisons between the three growth conditions aimed at i) evaluating the overall differences in metabolism of the fungus at the presence of the xenobiotics (toluene and HCH); ii) determining the carbon sources differently catabolized by the fungus in the presence of β-HCH and iii) identifying potential inducer(s) that may increase the breakdown of toxic compounds. The overall percentage overlap in the catabolism of 95 carbon sources by the fungus exposed to β-HCH and toluene with respect to the control was 89.9% and 89.6%, respectively, at the beginning of the lag phase (48h), appearing higher (>95%) when approaching the plateau. This suggested that an initial response by the fungus to the presence of the β-HCH and also of the solvent (toluene) resulted in appreciable differences in the pattern of substrate use with respect to the control. The presence of toluene alone in the FF plates lowered the metabolic efficiency of the fungus towards some compounds, while the presence of both β-HCH and toluene had not such a strong effect with respect to the control. D-xylose, D-ribose and glycerol at the presence of toluene and/or toluene and HCH actually promoted fungal growth better than in the control. These effects could be related to possible responses of the fungus to oxidative stress induced by the presence of the xenobiotics.

BIOSYNTHESIS AND LYSINE-CONJUGATION OF INDOLEACETIC ACID IN *PSEUDOMONAS SAVASTANOI PV. NERII* INDUCES AN IMPAIRED BASAL METABOLISM AND AN ALTERATION OF ITS ABILITY TO CAUSE DISEASE.

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Besides plants, many organisms and even microorganisms are able to synthesise the major plant auxin indole-3-acetic acid (IAA), included fungi, algae and bacteria. For this phytohormone, several IAA biosynthetic pathways have been identified in plant-associated bacteria, with more than one pathway existing in certain species. In some symbiotic and nitrogen-fixing bacteria, their ability to synthesise IAA was shown to affect essential steps of their interaction with their host plants, such as the onset of symbiosis and their plant growth-promoting activity. The plant pathogenic bacterium *Pseudomonas savastanoi*, causal agent of Olive and Oleander knot disease, uses the so called "indole-3-acetamide pathway", for the conversion of tryptophan (Trp) to IAA by a two step pathway and the two enzymes Trp monooxygenase and IAM hydrolase (encoded by the *iaaM* and *iaaH* genes, respectively). Moreover, the pathovar *nerii* of *P. savastanoi* (*Psn*) is also able to conjugate IAA to lysine, giving a less biogically active compound, by the enzyme IAA-lysine synthase coded by the *iaaL* gene. Besides its role in supporting the hyperplastic growth of the typical symptoms induced by *P. savastanoi*, evidences are accumulating about the role of bacterial IAA in the plant-host pathogen interaction as well (Macconi, unpublished data).
In this work the wild type strain *Psn23* and the two mutants *Psn23 ΔiaaM* and *Psn23 ΔiaaL* were investigated by using a Phenotype Microarray approach (PM 9-20). Bacteria were grown at 26°C for 96 h, on KB medium amended with L-Trp at initial concentration of 0.01 OD_{600}. The preliminary results obtained so far showed the strain *Psn23* and the mutants *Psn23 ΔiaaM* and *Psn23 ΔiaaL* to grow similarly on all toxic compounds of panels PM 9-20 and have the same chemical sensitivity. The only exception found was 8-hydroxyquinoline, where the two mutants were severely impaired in their growth in comparison with the wild type *Psn23*. It is important to undelined that 8-hydroxyquinoline is among those phytotoxic compounds known to be present sometimes in plant exudates. Therefore, more investigation are in progress to understand how the ability of *Psn* to synthesise IAA and its specific resistance to a plant toxic substance such as 8-hydroxyquinoline are metabolically interconnected, by performing a wider phenotype microarray analysis.

ACC DEAMINASE GENES COULD BE INVOLVED IN UNUSUAL NITROGEN SOURCES UTILIZATION IN *SINORHIZOBIUM MELILOTI*

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The gene encoding the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase (*acdS*) is present in the dispensable genome of the plant symbiotic rhizobium *Sinorhizobium meliloti.* AcdS is supposed to be involved in the sequestering and cleaving of plant-produced ACC, the precursor of the plant stress hormone ethylene. However, the function of *acdS* in symbiotic bacteria has not been fully clarified and no definitive conclusion on the role of such gene on the efficiency of the symbiotic interaction have been drawn.

To clarify this issue, comparative genomic analyses of *acdS* orthologs were performed in *S. meliloti* and functional studies were carried out by expressing *acdS* from natural strains in the model strain *S. meliloti* Rm1021, which lacks *acdS* gene. Symbiotic and endophytic phenotypes of recombinant vs parental strain were evaluated with respect to competition for root nodule occupancy, plant colonization and modulation of ethylene production by the host plant. Additionally, phenotype microarray experiments were performed to investigate the metabolic function carried out by AcdS. Data showed that the *acdS* spread in *S. meliloti* through horizontal gene transfer. No increase in fitness for nodule occupancy was found overexpressing *acdS*, as well as faint effects on the modulation of plant ethylene levels were observed. Surprisingly, AcdS was shown to confer the ability to utilize formamide as sole nitrogen source. We conclude that *acdS* in *S. meliloti* could be more related to the exploitation of unusual nitrogen sources, in connection with rhizospheric colonization or endophytic life-style than to the symbiotic interaction.

PLASMID PROFILE AND ANTIBIOTIC SUSCEPTIBILITY OF *VIBRIO VULNIFICUS* ISOLATED FROM COCKLES (*ANADARA GRANOSA*) IN SELANGOR MALAYSIA

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Background: Vibrio vulnificus infections are the worldwide public health problems associated with illnesses resulting from consumption of raw or partially cooked seafood and exposure with the contaminated sea water. The aim of this study is to isolate and identify *V. vulnificus* from cockles in Malaysia and detect antibiotic susceptibility and plasmid profiles.
Methods: A total of 78 cockles, 40 from wet and 38 from supermarket were examined for the presence of *V. vulnificus* using TCBS and Chromagar Vibrio. The antibiotic susceptibility tests were performed for 12 antibiotics by the disc diffusion method. For molecular identification of the isolates, the 16S ribosomal RNA gene fragment was amplified by polymerase chain reaction and the nucleotide data were subjected to BLAST analysis.
Results: The morphological and biochemical test detected *V. vulnificus* in 32% of the cockle samples all of which showed resistance to two to eight antibiotics. Plasmids were found in 92% of the isolates with 10 different profiles having two to four plasmids each. Based on 16S rRNA gene sequence homology15 isolates were identified as *V. vulnificus*, five as other Vibrios and five as nonvibrios.
Conclusion: We have observed a marked difference between morphological methods and molecular methods in identification of *V. vulnificus* indicating the inadequacy of the morphological technique in discriminating the *Vibrio* species. The occurrence of *V. vulnificus* in the cockle samples is quite high so consumption of uncooked and semicooked cockles should be avoided in order to prevent food-borne infection by this pathogenic bacterium.

ELUCIDATION OF METABOLIC NETWORK OF *HELICOBACTER PYLORI* CLINICAL STRAINS IN MALAYSIA

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Helicobacter pylori colonizes almost half of the human population worldwide. *H. pylori* strains are genetically diverse and specific genotypes are associated with various clinical manifestations including gastric adenocarcinoma, peptic ulcer disease (PUD) and non-ulcer dyspepsia (NUD). To understand the metabolic differences among *H. pylori* strains, we investigated four Malaysian *H. pylori* clinical strains that we had sequenced and a standard strain, *H. pylori* J99, at the phenotypic level using the Biolog Phenotypic Microarray system to corroborate genomic data. We initiated the analyses by predicting carbon and nitrogen metabolic pathways from the *H. pylori* genomic data. Biolog PM aided to validate the prediction. We have identified a core set of nutrient source that was utilized by all strains tested and another set that was differentially utilized by only the local strains. Our data serves as an insight for future challenges in correlating inter-strain metabolic differences in *H. pylori*.

STUDY OF THE MICROBIOTA OF LACTIC ACID BACTERIA AND BIFIDOBACTERIA OF BOVINE COLOSTRUM

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Colostrum is the first milk produced by the mammary gland during the early days of postpartum period. The production, composition and physical characters of bovine colostrum vary greatly with individual breeds, feeding, length of dry period of cows and time postparturition. In addition to normal nutrients such as proteins, carbohydrates, fats, vitamins and minerals, colostrum contains many other biologically active constituents such as growth factors, antimicrobial compounds and immune-enhancing components. The role of colostrum is not only to provide nutrition, but also to provide protection against infection. The microbiota of colostrum could also be involved in this protective effect through gut colonization and stimulation of the immune system as demonstrated in humans. Several studies on the microbiota of human breast milk showed the dominance of lactic acid bacteria (LAB), bifidobacteria (BFB), staphylococci and streptococci. Conversely, the microbiota of bovine colostrum, particularly regarding LAB and BFB populations, is still poorly studied.
In the present study, the microbiota of six samples of bovine colostrum has been investigated. To overcome the limitations of culture-dependent methods, the complexity of the microbial communities was characterized by a physiological analysis using the Biolog EcoPlates method, and by a molecular analysis using the LH (Length Heterogeneity)-PCR method. The Biolog EcoPlates system allowed to obtain a metabolic profile of the whole microbial community based on the different use of 31 carbon sources. The LH-PCR allowed the identification of the most representative bacterial genera or species based on the length of specific DNA fragments, by comparison with an internal database of LAB and BFB fragments. The changes in colour intensity in Biolog EcoPlates wells were measured until 120 h and the average well color development (AWCD) for each colostrum sample was calculated. The data were statistically analysed and the physiological diversity of communities was determined by using diversity indices (Shannon-Weaver index – H; Evenness – E; Richness - R). The LH-PCR profiles evidenced the differences of LAB and BFB communities in each sample. These bacterial groups were present in all samples, but the species attributed on the basis of the peaks detected in each electropherogram were different. The differences of LAB and BFB species present in colostrum samples were also confirmed by culture-dependent methods. Strains isolated from agar media and identified by sequencing of 16S rRNA gene, belonged to *Lactobacillus* (4 species), *Enterococcus* (4), *Streptococcus* (2), *Lactococcus* (3), *Leuconostoc* (2), and *Bifidobacterium* (3). In conclusion, the combined use of classical culture-dependent method and metabolic or molecular culture-independent techniques allowed a deep characterization of the LAB and BFB populations. Culture-independent methods provided a reliable picture of the diversity of bacterial ecosystems increasing the knowledge on function and structure of bovine colostrum microbiota.

APPLICATION OF PHENOTYPIC MICROARRAYS TO CHARACTERIZE THE MULTI-ANTIBIOTIC RESISTANCE BACTERIA ISOLATED FROM ONSITE WASTEWATER TREATMENT TECHNOLOGIES

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Wastewater treatment approaches vary from the conventional centralized systems to the entirely onsite decentralized and cluster systems. The centralized systems which are usually publicly owned collect and treat large volumes of wastewater for entire large communities. On the other hand, decentralized onsite systems treat wastewater of individual homes and buildings. The wastewater treatment plants are sources of resistance genes in the natural environment, and that they play an important role in the ecology of antibiotic resistance both intrinsic and acquired. They are interfaces between different environments and provide an opportunity for resistance to mix between pathogens, opportunistic pathogens, and environmental bacteria. Antibiotic resistance is a highly selectable phenotype, and can be detected using growth inhibition assays performed in broth or by agar disc diffusion.
The scope of the study was to apply Phenotype Biolog MicroArray (PM) technology to test the sensitivity of the bacterial strains isolated from onsite wastewater treatment facilities to numerous antibiotics. The following isolates were chosen for the study: *Serratia marcescens ss marcescens, Pseudomonas fluorescens, Stenotrophomonas maltophilia, Stenotrophomonas rhizophila, Microbacterium flavescens, Alcaligenes faecalis ss faecalis, Flavobacterium hydatis (26 C), Variovorax paradoxus, Acinetobacter johnsonii, Aeromonas bestiarum.* The raw influents, effluents, liquid and solid samples from the three different technologies were investigated: technology A - fixed bed reactor, technology B - trickling filter/Biofilter system with a filter consisting of rock-wool pieces on which the microorganisms grow, and technology C - aerated filter system with a fluidized bed reactor with the microorganisms growing on the surface of plastic media and suspended microorganisms. The strains evaluated were chosen from the solid medium (TSA) with the addition of the following antibiotics: tetracycline, kanamycin, streptomycin, and penicillin. PM11 and PM12 microplates, Biolog Inc. (Hayward, CA) were applied in the study. PM analysis showed a gain of phenotype (resistance or positive growth) of the strains to 34 antibiotics. The strains were classified as multi-antibiotic resistance bacteria. PM technology allows phenotypic testing to become a simple analysis on gene expression and allows to directly observe the consequence of a genetic change.

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COMPARATIVE GENOMICS ANALYSIS OF *DICKEYA SOLANI* STRAINS

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Dickeya solani can cause the blackleg and soft rot disease on potato plants and tubers. In the past 15 years this species has spread in the European potato plantations that resulted in large income losses mainly due to the extensive rotting of tubers in storage and no rising of potato plants in the field. Genomes of four *D. solani* strains indicating the differences in the virulence were compared to six genomes of this species available in the GenBank. The purpose of the study was to find differences/similarities in genes coding for *D. solani* virulence factors and their regulators.
The *D. solani* strains that differed in virulence: IFB0099 and IFB0158 showing high virulence on potato, IFB0221 manifesting medium virulence on potato and IFB0223 with low virulence on potato were sequenced by 454 and PacBio next generation sequencing methods. So far six other *D. solani* strains isolated in different European countries have been sequenced, scaffolded and preliminary annotated. We used these genomes and the genome of fully annotated closely related *D. dadantii* 3937 strain for comparative analysis of genes encoding virulence factors and their regulators. General features such as genome size, number of ortholog groups, number of open reading frames were similar. The *D. solani* pangenome and accessory genome was determined.
The 41947 total proteins belonging to the 10 strains have been grouped into 5045 orthologous groups, 3809 belonging to the core genome and 413 belonging to the accessory genome and 823 belonging to the unique genome. The presence of the virulence factors genes (such as those coding for pectate lyases, cellulase, regulators of pectinolysis) in the 10 genomes has been verified by performing a Blast-BBH search, using the genes of *D. dadantii* 3937 as queries. All studies genes appeared to have an ortholog inside the genome of each *D. solani* strain. Most of the tested virulence related genes are highly conserved.
Comparative analysis of the 10 *D. solani* genomes confirmed the low variability between the strains shown previously in MLST, rep-PCR and PFGE analysis. The study suggested that the differences in the virulence of *D. solani* strains are rather due to the regulation of virulence factors expression than to the presence of the specific virulence factor(s).

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MICROBIAL DEGRADATION OF CYANOBACTERIAL-PRODUCED EXOPOLYSACCHARIDES IN *MICROCISTIS* BLOOM FORMATIONS IN AN ISRAELI EUTROPHIC LAKE

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Microcystis spp. is a cyanobacterial species that commonly forms blooms in euthrophic lakes and grows in colonial forms. There are environmental, ecological and health concerns regarding the expansion of these bio-formations, since they determine water quality deterioration, generate anoxia and alter existing food webs. In addition, toxin secondary metabolites pose serious hazards for humans and livestock [1]. Cell aggregation is possible thanks to microbial-produced exopolysaccharides (EPS), produced mainly by *Microcystis* and other bloom-associated cyanobacteria that embed *Microcystis* cells within the bloom colony. During extensive blooming, EPS may be produced in large amounts, that in one case were reckoned to occupy 0.0001-0.007 % of an eutrophic lake water volume in the epilimnion [2].
Beside their structural role, EPS represent a huge carbon input available to bacterial heterotrophic population during water-blooming. By these means, the study of EPS degradation is very important to describe C cycle within the community. At the same time, the individuation of those bacterial species in the community that are more able to degrade EPS could lead to optimize new biotechnological approaches to control water-bloom spread.
In this work, *Microcystis* biomass was harvested from the surface layer of lake Kinneret (Sea of Galilee, Israel) and isolation procedures were performed to obtain axenic cultures of *Microcystis*-associated heterotrophic bacteria. By using Biolog carbon substrate utilization approach, the growth of 4 different bacterial strains was tested on cyanobacterial EPS alone, and combined to 8 different carbon sources (namely pyruvate, gluconate, ribose, glucose, galactose, xylose, glutamic acid and yeast extract).
Results showed that some of the used carbon sources stimulated the growth on cyanobacterial EPS, the most relevant being glutamic acid, which produced a relevant growth of one of the strains.

Acknowledgments
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THE BIOLOG PHENOTYPE MICROARRAY™ ELUCIDATES METABOLIC DIFFERENCES AMONG GENETICALLY CONSERVED MORPHOLOGICAL VARIANTS OF *BURKHOLDERIA PSEUDOMALLEI*, STRAIN MSHR5848

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Burkholderia pseudomallei (*Bp*) is the causative agent of melioidosis with infections ranging from acute and rapidly fatal to prolonged and chronic. *Bp* is a potential biothreat agent due to its high aerosol infectivity, ability to cause severe disease with nonspecific symptoms, and its resistance to multiple antibiotics. Additionally, no vaccine is available. Unlike many *Bp* strains, which are known to exhibit random variability in traits such as colony morphology, the Department of Defense's Unified Culture Collection stock of BURK178 (derived from *B. pseudomallei* strain MSHR5848) exhibits two distinct and relatively stable colony morphologies: a smooth, glossy, pale yellow colony (type 1) and a flat dry white colony (type 2). These MSHR5848 derivatives were extensively characterized to identify differences associated with type 1 and type 2 colony morphologies. Here, we present whole genome sequence comparisons, multiple locus sequence type analysis and restriction fragment length polymorphisms to demonstrate that the morphological variants are genetically conserved. In contrast, the Biolog GEN III OmniLog ® and Phenotype Microarray™ (PM) profiles reveal distinct metabolic differences between the variants. Furthermore, growth curves show that the type 1 variant grows more rapidly than the type 2 variant. Finally, there is a difference in virulence noted between the two variants with type 2 being more virulent in a mouse model while type 1 is less virulent for mice but more cytotoxic in a macrophage model. Thus, despite their very similar genetic composition, these two variants have distinct phenotypic and *in vivo* differences. Statistical analysis of the phenotype microarray data has narrowed the 1,920 assayed substrates to a manageable subset which differentiates the two morphological variants. These can be utilized for future studies in the pathogenesis of *Bp* as well as the development of vaccines and therapeutics.

CHARACTERIZATION OF *SMC03167* GENE ENCODING FOR A MAJOR FACILITATOR SUPERFAMILY EFFLUX PUMP IN *ENSIFER MELILOTI*

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The efflux pump systems are one of the major mechanisms conferring multidrug resistance phenotypes in bacteria. Efflux pumps extrude a wide variety of chemically unrelated compounds assisting bacteria to cope with various stresses and antimicrobial substances.
In *Ensifer meliloti*, which is a nitrogen-fixing symbiont of legume plants *(Medicago spp.)*, the contribution of efflux pumps to host nodulation ability is actively investigated. The *SMc03167* gene of *E. meliloti*, which encodes a QacA/EmrB efflux protein of the major facilitator superfamily (MFS), was found to be up-regulated in presence of the flavonoid luteolin, known as an inducer of nodulation process. Therefore, to deepen our knowledge about the SMc03167 gene, we compared the chemical sensitivity profile of the *E. meliloti SMc03167*-defective mutant with its the wild-type (strain 1021). Hundreds of different toxic compounds were tested in presence of the inducer luteolin using the Phenotype MicroArray (PM) high-throughput technology. Then the symbiotic phenotype of the two strains was evaluated by plant nodulation assays.
PM analysis revealed that *SMc03167* gene inactivation increased susceptibility to a range of compounds belong to classes already known to be efflux targets of the MSF transporters, such as QACs (quaternary ammonium compounds), bisguanides, bis-phenols and dyes. In addition, the *SMc03167* mutant compared to the wild-type showed an enhanced sensitivity also for compounds previously not associated with MFS transporters. Such compounds include chelators, inhibitors and antibiotics.
The symbiosis assays revealed a lower ability to promote plant growth and a reduced nodulation efficiency of *E. meliloti SMc03167*-defective mutant than its wild-type strain.
Overall, these findings suggest that the SMc03167 efflux pump of *E. meliloti* contributes to mediate resistance toward plant antimicrobials and rhizospheric toxic compounds, possibly in relation with an efficient plant root nodulation and then symbiotic interaction.

SHIGELLA SPECIES CAN BE DIFFERENTIATED BY THEIR ABILITY TO USE DIFFERENT CARBON SUBSTRATES

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The prevalent method to differentiate the four subgroups of *Shigella* is serotyping. Metabolic phenotypes have also been used but in a limited way. For example, *S. dysenteriae* can be differentiated from the other three subgroups by its D-mannitol negative phenotype. In this study, we have probed further the use of metabolic phenotypes to distinguish the four *Shigella* species based on utilization of carbon sources using the Biolog phenotypic microarray (PM) platform. A total of 145 *Shigella* isolates comprised of *S. dysenteriae* (n=29), *S. flexneri* (n=49), *S. boydii* (n=24), and *S. sonnei* (n=43) were screened by PM. From the analysis of utilization of 190 carbon substrates, we identified 10 compounds, namely, D-ribose, D-mannitol, glycyl-L-proline, D-glucuronic acid, D-galacturonic acid, L-rhamnose, L-galactonic acid-γ-lactone, maltose, maltotriose, and dextrin, whose pattern of utilization by the *Shigella* isolates could potentially be used to differentiate the four subgroups. The ability and/or inability of these isolates to utilize a combination of these carbon sources could form the basis of a characteristic signature metabolic phenotype for each subgroup. In order to assess the genetic basis of these specific phenotypes whole genome sequencing of 72 *Shigella* strains was carried out using the MiSeq Illumina technology. In this study we focused specifically on the gene sequences of the galacturonate and glucoronate pathways since more than 90% of *S. flexneri* tested utilized these hexuronates, unlike the other three subgroups which cannot. Therefore, this phenotype can be used to distinguish *S. flexneri* from the other three species. Comparative sequence analysis of the *Shigella* genes, encoding catabolic enzymes and the transporter, involved in galacturonate and glucoronate metabolism, was performed using *E. coli* K-12 as the reference strain. Sequence analysis revealed that all the *S. flexneri* that were galacturonate and glucoronate positive had the complete set of genes required for their utilization. The negative phenotype for these two carbon sources in the other three *Shigella* species could be accounted for by the presence of premature stop codons, truncation and gene absence. Additionally, we have identified novel missense mutations that may contribute to the galacturonate and glucoronate negative phenotype. We are currently analyzing the gene sequences involved in the metabolism of the other eight carbon sources that could potentially allow for the differentiation of *S. boydii, S. sonnei,* and *S. dysenteriae.* Taking together these results could lead us to the development of molecular assays and microbiological media for the detection and identification of *Shigella.*

PHENOTYPIC CHARACTERIZATION OF *BRETTANOMYCES BRUXELLENSIS* STRAINS FOR THE TOLERENCE TO STRESSES ENCOUNTERED DURING SECOND GENERATION BIOETHANOL PRODUCTION

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Brettanomyces (teleomorph *Dekkera*) *bruxellensis* has been generally considered a spoilage yeast in fuel ethanol production plants. However, due to its peculiar carbon- and nitrogen metabolism, the yeast is also believed to hold great potential for bioethanol production in continuous reactors. Nevertheless, before actually being useful in the production of second generation bioethanol there are still some challenges to overcome. For example, the use of lignocellulosic biomass results in the need for an extensive pretreatment process during which often several inhibiting compounds are released, leading to less efficient or stuck fermentations. The objective of this study was to phenotypically characterize *B. bruxellensis* strains for tolerance to stresses typically encountered during second generation bioethanol fermentation and to develop a screening plate for the evaluation of yeast strains against stresses relevant for the second generation bioethanol production. To this end, a plate was developed containing a dose range of different inhibitors (i.e. vanillin, catechol, levulinic acid, formic acid, furfural, ethanol, low pH, and high osmotic pressure). Further the plate included a negative and positive control well. Subsequently, several *B. bruxellensis* strains from different ecological niches were screened using the developed plate. Plates were incubated for eight days at 25 °C and analyzed by the OmniLog incubator/reader (Biolog, Hayward, CA, USA). All analysis were performed in duplicate. The different *B. bruxellensis* strains were ranked by calculating the average well colour development (AWCD). Additionally, each strain was scored for its tolerance to the tested inhibitory conditions. This resulted in a huge variation among strains, demonstrating the need for screening a large collection of strains to identify superior yeast strains. Highly ranked *B. bruxellensis* strains can then be further tested for tolerance against a mixture of inhibitors and real hydrolyzed biomass fermentation broths. Additionally, features such as ethanol yield and performance in pilot plants should be evaluated to truly see its potential for industrial second generation bioethanol production.

STATISTICAL TOOLS FOR ANALYZING BIOLOG PHENOTYPIC MICROARRAY DATA

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Grouping the bacterial responses into active and non-active, normalization by removing systematic errors from the experimental data, and detection of samples with differing metabolic responses are standard procedures when analyzing phenotype microarrays. Here, we introduce a three-step pipeline with novel procedures for grouping, normalization and sample detection.

To perform the grouping into the two categories, active and non-active, we utilize a model based EM-algorithm instead of the earlier used *ad hoc* methods where the user defines a cutoff to separate the two groups from each other.

In the normalization method, we expand the existing solutions by introducing an important assumption that the active and non-active bacteria manifest completely different metabolism and, thus, should be treated separately. First, an average for both of the groups is evaluated and then the metabolic responses of the two groups are divided by the corresponding group averages.

Sample detection, in turn, provides new insights into detecting differing respiration patterns between experimental conditions, *e.g.* between different combinations of strains and temperatures, as not only the main effects but also their interactions can be evaluated. In the sample detection, the multilevel data are effectively processed by a hierarchical model in the Bayesian framework.

Throughout our analysis pipeline, including the grouping, normalization and sample detection, a logistic model is considered to be suitable for describing the metabolic intensity curves produced by the Biolog experiments.

Our pipeline is implemented in R language on the top of opm R package and is freely available for research purposes.

COMMUNITY LEVEL PHYSIOLOGICAL PROFILING OF MICROBIAL POPULATION SETTLED DENITRYFICATION FIELD BIOREACTORS.

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The BIOLOG EcoPlates (BIOLOG Inc.) were used to estimate functional diversity and determinate community-level physiological profiling of heterotrophic bacterial populations, settled in field denitrifications bioreactors filled with different organic substrates. Used substrates were inoculated with additional culturable and/or unculturable microorganisms in comparison with no inoculated control bioreactors.
Obtained results show that straw as a substrate features the highest microbial activity estimated as maximum value of Average Well Colour Developmnet (AWCD). The microbial activity of straw significantly increased after inoculation by addition of both culturable and mix culturable/unculturable microorganism. Simultaneously, metabolic microbial activity of slowly decompositable brown coal is initially lower than in other bioreactors but is characterised by upward tendency after longer time than in other fillings.
Finally, our metabolic analyses of microbial populations settled bioreactors filled with mix of straw and brown coal confirmed that this filling provides the best conditions for high and stable metabolic microbial activity, regardless of used bacterial activators.

Key words: BIOLOG system, community-level physiological profiling, AWCD, microbial communities, denitrification microorganisms

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DOES CO-CULTIVATION WITH A PATHOGEN CHANGE THE NUTRIENT-UTILIZATION PATTERNS OF ENDOPHYTIC FUNGI?

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Endophytic fungi are of attracting increasing interest as biocontrol organisms against pests and diseases. Endophytes appear to interact with other, co-occurring fungi through diverse mechanisms. For instance, the results of our recent studies indicate that a single endophyte isolate may antagonize a co-occurring pathogen through chemical antagonism as well as through effective competition for nutrients. Information about the fungal nutrient utilization capacities is needed in order to better understand how the *in planta* interactions between co-occurring fungi potentiate effective biocontrol. Investigating the fungal nutrient utilization patterns after the microorganisms have been triggered to cope with the challenging situation by growing in antagonism assays *in vitro* it was aimed for mimicking the *in planta*-like situation. Here, we hypothesized that the nutrient utilization patterns of a fungal isolate differ depending on whether the fungal cells are collected from a single or dual culture. The study objects were endophyte isolates originating from elm (*Ulmus* spp.) trees. The endophytes were selected based on their differential correlation with the susceptibility of the host elms to Dutch elm disease, and because of their different *in vitro* interactions with the causal agent of this disease, *Ophiostoma novo-ulmi*. Using phenotype microarrays, we compared the carbon and nitrogen utilization patterns in cells of three isolates that were grown in dual cultures with three isolates of the pathogen or in single cultures. Our results provide insights into the dynamics of antagonism between plant pathogens and endophytes with biocontrol potential

THE ND4BB INFORMATION CENTRE & THE SPECIAL CASE OF PHENOTYPE MICROARRAY DATA

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The New Drugs 4 Bad Bugs (ND4BB) initiative is a series of programs designed to directly address some of the scientific challenges associated with antibacterial drug discovery and development. The over-arching concept of ND4BB is to create an innovative public-private collaborative partnership that will positively impact all aspects of Antimicrobial resistance research with benefit on the future discovery and development of novel agents for the treatment, prevention and management of patients with bacterial infections.
One important objective of ND4BB is to develop a data repository providing easy and integrable providing a key information base for research projects focused on antibiotic resistance. All consortia participating in studies conducted under the ND4BB programme will be expected to contribute data to the ND4BB data hub and collaborate to share data and experience as widely as possible amongst all programme members and the antibiotic research community as a whole.
Here we present the technical setup of the ND4BB Information Centre and describe the specific challenges of the distinct data type of Phenotype Microarray data. The necessary data preprocessing done using the dedicated R-package opm [1] builds the basis and resulting specific needs for data storage and integration concepts together with accessibility demands are explained.

[1] L.A.I. Vaas, J Sikorski, B Hofner, A Fiebig, N Buddruhs, HP Klenk, M Göker. Bioinformatics 29 (14), 1823-1824.

CORRELATION OF GENOME SEQUENCE DATA AND OMNILOG PHENOTYPIC MICROARRAY DATA AMONG SHIGA-TOXIN PRODUCING *ESCHERICHIA COLI*

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Background: Shiga-toxin producing *E. coli* (STEC) are asymptomatically carried in the gastrointestinal tracts of ruminants and are associated with food- and water-borne human disease outbreaks and sporadic infections. Symptoms can range from diarrhea to the life-threatening hemolytic uremic syndrome. Among STEC certain serotypes and lineages within serotypes are more frequently associated with human disease than others. Further, they may differ in their ability to colonize animals and their survival in the environment. Despite the importance of this group of pathogenic bacteria, the link between phenotype and genotype is still largely unknown. In this study we examined the whole-genome sequence (WGS) and phenotypic profile of 143 STEC of a variety of serotypes isolated over a period of approximately 20 years, from bovine, human, and environmental sources.
Materials and Methods: Genomes were sequenced using Illumina MiSeq at Genome Quebec or the National Microbiology Laboratory in Winnipeg, Canada. Sequences were assembled using Spades. Comparative genomics were performed using Panseq. Phylogenetic trees were created using FastTree2. Phenotypic microarray analyses were performed using the Biolog Omnilog system, and the opm package for R. Statistical analyses were performed using R.
Results: The phylogeny based on single nucleotide polymorphisms (SNPs) among the 143 genomes from various STEC serotypes showed that most clades were serotype specific; with occasional outliers clustering apart from other strains of the same serotype, as was found for the O121 and O103 serogroups. Similarly, clustering of the same strains based on the utilization of carbon sources, osmolytes and pH generated a tree with a similar topology to that based on WGS data. Additionally, the three genetic lineages of serotype O157:H7 were apparent in the clustering of phenotypic microarray data. Lastly, phenotypic utilization profiles capable of identifying sub-groups of bacteria, both within and between serotypes, were determined.
Discussion: Phylogenies based on phenotypic markers recapitulate the groupings of bacterial strains obtained through SNP whole-genome phylogeny. Intriguingly, known genetic lineages such as those within serotype O157:H7 were distinguished based on phenotypic profile and sets of markers specific to these subgroups were identified. Potential implication of this work includes the development of selective media for particular serotypes, clades and lineages or biochemical tests to identify specific bacterial sub-groups most frequently associated with human disease.

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