ROSARIO 2016
Bolsa de Comercio

Second Latin American Metabolic Profiling Symposium

October 10-12

Abstract Book

2nd Latin American Metabolic Profiling Symposium 2016

Rosario | Argentina | October 10-12-2016

Abstract Book

Contents

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Program

Monday October 10th | NMR. Mass Spectrometry. Data Analysis. Instrumentation & Methodology

IBR- PREDIO CONICET ROSARIO ' Ocampo y Esmeralda

Tuesday October 11th | Bolsa de Comercio de Rosario | Paraguay 777

8:00 Registration

8:45-9:00 Opening session

Metabolomics in health

9:00 - 10:00 **Elaine Holmes.** *Plenary Lecture "Metabonomic Applications in Health"*

10:00-10:30 Coffee Break

10:30-11:00 **Ana Paula Valente.** *"Salivary metabolite signatures of children with and without dental caries lesions and with type 1 diabetes: an NMR study".*

11:00-11:30 **Maria Eugenia Monge.** *"High accuracy prostate cancer detection using human blood serum metabolomic profiling".*

11:30-11:45 Break

11:45-12:15 **Carlos Pirola.** *"Metabolomics of the metabolic syndrome components".*

12:15-12:45 **Isabel García Pérez.** *"Assessing the healhtiness of dietary patterns using metabolic profiling".*

13:00-14:00 LUNCH

14:00-14:30 BRUKER

14:30-15:00 **Pablo Hoijemberg.** *"Improving identification of compounds in metabolomic studies through correlation and statistics".*

15:00-15:30 **Julien Wist**. *"Dereplication and identification of new metabolites: a common database of reference compounds".*

15:30-16:00 Coffee Break

16:00-18:00 Poster Session

18:30-19:30 **Elaine Holmes. Imperial College. Keynote address open to general public.** *"Metagenome and Metabolome in nutrition"*

Wednesday October 12th

Applications of Metabolomics in Plant

8:45-9:45 **Ute Roessner.** *Plenary lecture "The potential of metabolomics in the plant sciences".*

9:45-10:15 Coffee Break

10:15-10:45 **Fernando Carrari.** *"Metabolic profiles as tools in new breeding technologies for crop species".*

10:45-11:15 **Adriano Nunes Nessi**. *"Comprehensive metabolic reprograming in freshwater Nitzschia palea strains undergoing nitrogen starvation is likely associated with its ecological orig".*

11:15-11:45 **Camila Caldana.** *"Metabolomics as a tool for elucidating plant growth Regulation".*

11:45-12:00 Break

12:00-12:30 **Guillermo Moyna.** *"Selection of hybrids assisted by nuclear magnetic Resonance and chemometric methods: aplication to mandarins (Citrus reticulata)and barley (Hordeum vulgare)".*

12:30-13:00 **Fabiana Drincovich.** *"Exploring the metabolic diversity of peach fruits from different varieties allowed the identification of quality metabolic biomarkers".*

13:00-14:00 LUNCH

14:00-14:30 **Soledad Cerutti.** *"Analytical strategies for the quantitative evaluation of naturally ocurring targeted toxins in plant-derived foods".*

14:30-15:00 **Maria Ines Zanor.** *"Metabolomics for deciphering the function of unknown proteins".*

15.00-15:30 **Julien Wist.** *"Metabolic fingerprinting for determination of coffee origin: what's next?*

15:30-16:00 **Ana Scopel**. *"Changes in metabolite profiling of flourensia campestris along an environmental gradient".*

16:00-16:30 Coffee Break

16:30-17:45 Oral presentations

17:45-18:30 Round table

Timetable

	Monday October 10th	Tuesday October 11th	Wednesday October 12th
08:00-08:15		**Registration**	
08:15-08:30			
08:30-08:45			
08:45-09:00		Opening session	Ute Roessner
09:00-09:15	Ute Roessner, Mass spectrometry	Elaine Holmes	
09:15-09:30			
09:30-09:45			
09:45-10:00			Coffee Break
10:00-10:15		Coffee Break	
10:15-10:30			Fernando Carrari
10:30-10:45	Coffe break	Ana Paula Valente	
10:45-11:00			Adriano Nunes Nessi
11:00-11:15	Julien Wist, NMR Data acquisition	María Eugenia Monge	
11:15-11:30			Camila Caldana
11:30-11:45		Break	
11:45-12:00		Carlos Pirola	Break
12:00-12:15	Lunch		Guillermo Moyna
12:15-12:30		Isabel García Perez	
12:30-12:45			Fabiana Drincovich
12:45-13:00		Julien Wist	
13:00-13:15	Ute Roessner, Mass spectrometry data analysis	Lunch Break	Lunch Break
13:15-13:30			
13:30-13:45			
13:45-14:00			
14:00-14:15		Bruker	Soledad Cerruti
14:15-14:30			
14:30-14:45	Coffe break	Pablo Hoijemberg	María Inés Zanor
14:45-15:00			
15:00-15:15	Julien Wist, NMR Data Analysis	Julien Wist	Julien Wist
15:15-15:30			
15:30-15:45		Coffee Break	Ana Scopel
15:45-16:00			
16:00-16:15	Bruker SOPs	Poster Session	Coffee Break
16:15-16:30			
16:30-16:45			Oral presentations
16:45-17:00			
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17:45-18:00			Round table
18:00-18:15			
18:15-18:30			
18:30-18:45		Elaine Holmes, Open Presentation	
18:45-19:00			
19:00-19:15			
19:15-19:30			

Conferences

METABOLOMICS AS A TOOL FOR ELUCIDATING PLANT GROWTH REGULATION.

Camila Caldana [1,2]

[1]Brazilian Bioethanol Science and Technology Laboratory, Brazilian Center for Research in Energy and Materials; [2] Max Planck Partner Group at Brazilian Bioethanol Science and Technology Laboratory

camila.caldana@bioetanol.org.br

Rising demand for food and fuels makes it crucial to develop breeding strategies for increasing crop yield/biomass. Plant biomass production is tightly associated with growth and relies on a tight regulation of a complex signalling network that integrates external and internal stimuli. The main goal of our group is to elucidate the processes underlying plant growth and production of biomass. As plant growth is closely linked to central metabolism network, we use gas chromatography coupled to mass spectrometry (GC-MS) technology for a comprehensive coverage of primary metabolism pathways (e.g. organic and amino acids, sugars, sugar alcohols, phosphorylated intermediates and lipophilic compounds). In my presentation, I will provide examples of i) the potential of metabolite profiles to predict plant performance as biomarkers; ii) how metabolites can regulate plant growth (e.g, axillary meristem outgrowth).

ANALYTICAL STRATEGIES FOR THE QUANTITATIVE EVALUATION OF NATURALLY OCURRING TARGETED TOXINS IN PLANT-DERIVED FOODS

Cerutti, S.

Instituto de Química de San Luis (CONICET-UNSL), Analytical Chemistry Division, National University of San Luis, San Luis, Argentina.

E-mail: ecerutti@gmail.com

Keywords: Mycotoxins; Targeted analysis; Agricultural samples; Mass Spectrometry.

Food safety is a matter of global importance. Due to the changes in climatic conditions and environmental pollution, new toxic agents are identified and new toxic effects recognized. Therefore, the health and trade consequences of toxic chemicals in food also have global implications.

Surveillance studies have shown that mycotoxin (poisonous, low molecular weight, secondary metabolites of molds) contamination is a world-wide problem, especially in developing countries, where suitable cultivation, processing and storage technologies are implemented with difficulty. In recent times, exposure to mycotoxins that can be found scattered in the environment has become a cause of growing concern. Soil, water, and air might be contaminated with outdoor environment from naturally occurring metabolites of fungal species that grow on a wide variety of crops or indoor environments from molds that can grow on various substrates.

In this context, ensuring food safety requires new methods for identification, monitoring and assessing of foodborne hazards during production, storage, delivery and consume. The use of metabolomics for the identification of biomarkers of the most relevant toxins that have been detected in this type of environment and their monitoring yields a more accurate risk assessment.

This presentation will summarize research and ongoing challenges on the development of analytical approaches based on mass spectrometry to successfully perform high-level research in the area of quantitative evaluation and monitoring of targeted mycotoxins relevant to the food and agricultural industry.

EXPLORING THE METABOLIC DIVERSITY OF PEACH FRUITS FROM DIFFERENT VARIETIES ALLOWED THE IDENTIFICATION OF QUALITY METABOLIC BIOMARKERS

Drincovich MF, Bustamante CA, Monti LL, Lara MV, Fernie AR

Centro de Estudios Fotosintéticos y Bioquímicos (CEFOBI), CONICET-Universidad Nacional de Rosario, Rosario, Argentina

e-mail: drincovich@cefobi-conicet.gov.ar

Keywords: chilling injury, metabolome, peach fruit, Prunus persica, quality, varieties

The application of cold after the harvest of many fruits is used to extent shelf-life and preserve quality properties. Besides, it is well known that cold causes dramatic reconfiguration of the plant metabolome as an adaptative response to avoid cold-induced damage. In the case of fruits, it is essential to analyze the changes in metabolites due to cold storage, which may have a substantial impact on organoleptic properties, human health and wellbeing. In an earlier study, using a single peach genotype, metabolomic changes induced by short cold and heat treatment, used to prevent chilling injury (CI), allowed us to identify metabolites which are involved in priming the fruit to cope with stress situations (1). Nonetheless, further metabolomic studies revealed a great diversity in the content of key metabolites among different peach varieties (2). Thus, these studies opened the question on whether it would be possible to associate a metabolic profile of a particular genotype with differential CI susceptibility in peach. Here, a metabolite profiling study after short and long cold storage of six peach varieties with differential susceptibility to CI was performed in order to identify, among the metabolic changes induced by cold, those that may be functionally related to CI resistance. Both common and distinct metabolic responses among the six varieties were detected. Common changes include a dramatic rise in galactinol and raffinose; increase in GABA, Asp and Phe; and a decrease in 2-oxo-glutarate and succinate. No cluster separation of fruits resistant to mealiness from those sensitive was found, indicating that resistance to CI can be found in different metabolic contexts. Although a direct correlation of a multigenic trait such as CI tolerance with the concentration of only one metabolite would not be expected, the degree of mealiness resistance of the different peach fruit varieties correlated to the level of raffinose following long cold storage. Thus, raffinose emerges as candidate biomarker for mealiness resistance. Interestingly, xylose is only increased by cold treatment in peach susceptible genotypes, which indicates a particular reconfiguration of the cell wall in the most susceptible varieties while being cold-stored for long period of times. When taken together, these data indicate that peach fruit differential metabolic rearrangements due to cold, rather than differential metabolic priming before cold, are better related with CI resistance. Peach fruit metabolism plasticity renders it possible to induce a diverse array of metabolites after cold, which is successful, in some genotypes, to avoid CI and preserve fruit quality.

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ACKNOWLEDGEMENTS

CONICET, ANPCYT, ALEXANDER VON HUMBOLDT FOUNDATION

ASSESSING THE HEALHTINESS OF DIETARY PATTERNS USING METABOLIC PROFILING

Isabel Garcia-Perez[1,2], Joram M Posma, Rachel Gibson, Edward S Chambers, Tue H Hansen, Henrik Vestergaard , Torben Hansen, Manfred Beckmann, Oluf Pedersen, Paul Elliott , Jeremiah Stamler, Jeremy K Nicholson, John Draper, John C Mathers, Gary Frost and Elaine Holmes.

[1] *Nutrition and Dietetic Research Group, Division of Endocrinology and Metabolism, Imperial College London, W12 0NN, U.K.*

[2] *Biomolecular Medicine, Division of Computational and Systems Medicine, Department of Surgery and Cancer, Imperial College London, SW7 2AZ, U.K.*

Keywords: metabolic profiling, dietary patterns, healthy eating policies

The burden of non-communicable diseases in populations can be reduced by implementing healthy eating policies. Accurate monitoring of changes in dietary patterns in response to policy implementation is challenging. Metabolic profiling allows simultaneous measurement of hundreds of metabolites in urine many of which are influenced by food intake. We hypothesize that metabolic profiling of urine can classify people according to dietary behaviour.

We used a randomised controlled trial in 19 volunteers to develop metabolite models of eating patterns. Volunteers were admitted to a clinical research unit for four 4-day periods. Participants were provided with all food and drink representing 25, 50, 75 and 100% of healthy eating recommendations to increase fruits, vegetables, carbohydrates, dietary fibre and to decrease total fats, sugars, and salt. Metabolic profiles were measured by ^{1}H-NMR spectroscopy. The INTERMAP U.K. study (n=225) and a healthy eating Danish cohort (n=66) were used as external validation datasets.

Models of the urinary metabolic profiles of the volunteers following the 100% and 25% healthy eating targets allowed derivation of a linear gradient reflecting the diet dietary profile within a controlled environment. Application of this model to two independent cohorts of free-living participants correlated with the healthiness of the diet, as determined by the Dietary Approaches to Stop Hypertension index based on two multi-pass 24-h dietary recalls for INTERMAP (P=5.10×10^{-6}) and 7-day food diaries for the Danish cohort (P=1.43×10^{-6}).

We demonstrate that a urinary metabolic profile developed in a highly controlled environment, independent of recorded food intake, can classify people into consumers of a healthy or unhealthy diet based on urinary metabolic patterns. This may have utility for the objective monitoring of adherence to healthful diets in a population setting.

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IMPROVING IDENTIFICATION OF COMPOUNDS IN METABOLOMIC STUDIES THROUGH CORRELATION AND STATISTICS

Pablo A. Hoijemberg,[*1,2] István Pelczer[1]

[1] *Department of Chemistry, Princeton University, Princeton, NJ 08544, USA*

[2] *Current Affiliation: Centro de Investigaciones en Bionanociencias (CIBION-CONICET), Ciudad Autónoma de Buenos Aires, C1425FQD, Argentina*

e-mail:pablo.hoijemberg@cibion.conicet.gov.ar

Keywords: Metabolomics, STOCSY, Metabolite Identification, Database Search.

In an untargeted metabolomic study the search for biomarker molecules serves to answer many questions, for which there is a need to find out the identity of these compounds of interest. Several dozen metabolites are normally detected by NMR analysis of a biofluid in measurable quantities, where the spectra can show about a few hundred peaks.

The identification of compounds is normally done with the aid of commercial software packages containing their own databases, by literature search, and/or by searches in public databases by lists of chemical shifts. The input for the database query can be improved using STOCSY, Statistical TOtal Correlation SpectroscopY [1], which is attainable given the amount of data collected for the multivariate data analysis and finds highly correlated peaks that would belong to a same molecule.

Despite its usefulness, the STOCSY analysis is tedious and cumbersome, normally obtaining a trace by selecting a driver peak to find the peaks highly correlated to it, and it is performed in a trace-by-trace fashion over all peaks of interest. Here we present a methodology developed to reduce the analysis time by increasing information recovery applying further statistical analysis on the "information redundant" STOCSY correlation matrix (all peaks included at once), yielding lists of grouped peaks for database queries that produce more reliable hits during the identification step.

The methodology adds an automated step after the correlation matrix calculation to group traces from different driver peaks based on their similarity. As the STOCSY tool suffers from overlapped peaks (that abound in some regions of the 1D ^{1}H spectrum of biofluids), good alternatives are its use on 1D ^{13}C spectra, 1D projections from ^{1}H 2D *J*-Resolved spectra, and small size data matrices like in a "spectrum-to-spreadsheet" procedure. Examples of the application of this methodology on biological samples and synthetic mixtures will be addressed.

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METAGENOME AND METABOLOME IN NUTRITION

Elaine Holmes

[1]*Computational and Systems Medicine, Department of Surgery and Cancer, Faculty of Medicine, Imperial College London, London, UK*

The contribution of the gut microbiota to human health and disease is undisputed, yet the mechanisms by which the microbiome exerts its effects remain poorly understood. Co-evolution of host and microbiome has influenced the functionality of both the microbiome and host such that metabolic complementarity exists within the microbiota and that critical biosynthetic pathways are provided for the host that significantly extend host metabolic capacity. Of the 'omics' technologies, metabonomics provides the most accessible window on investigating the impact of nutritional interventions on human health since metabolic profiles of easily obtainable biofluids such as blood plasma and urine carry information relating both to genetic and environmental influences, including contributions from the microbiome, diet and xenobiotics [1]. High-resolution spectroscopy together with multivariate mathematical modelling has been used to model the metabolic consequences of perturbing the microbiota and to profile the effects of nutritional interventions on the metabolism and the microbiome. Basic studies in germ free and antibiotic-treated animals have shown stark changes in the urinary and faecal metabolomes, which are largely restored to 'normal' following recolonization. Recent research indicates that in both germ free and antibiotic models of microbial depletion the bile acid profiles of several tissues is significantly different from conventional animals, containing a substantially higher percentage of tauro-conjugated bile acid species [2] indicating that the presence of microbiota influences the global metabolism of the host and may impact on the development of heart disease and metabolic disorders.

Landmark studies in both animal models and humans, such as those from Gordon's group, have shown that obese and lean individuals carry a different gut microbial composition. We have shown that weight reduction surgery, or Bariatric surgery induces a marked change in the composition of both the faecal Metagenome (switch towards the gammaproteobacteria) and the metabolome (altered excretion of cresols, phenols, GABA and polyamines), which show strong correlations [3]. Post surgical diet also impacts on the development and stability of the microbial-host metabolic profile.

Using the MWAS approach to characterize the impact of diet on hypertension in a large scale epidemiological, a range of metabolites were identified that differentiated populations with vastly differing blood pressure levels and lifestyles [4]. Several of the strongest candidate biomarkers of hypertension were of gut microbial or mammalian-microbial origin.

The strength of microbial influence is also illustrated by studies showing that the in utero and early postnatal environments leave a metabolic imprint, which can last decades and may help in understanding the predisposition of preterm and low birth weight individuals to metabolic syndrome [5]. Thus the pre and perinatal window provides great potential for nutritional intervention with the aim of improving long-tem health.

Metabolic profiling also has a role to play in elucidating responses to specific dietary components such as fermentable carbohydrates and their effect on metabolism and appetite regulation [6,7].

References

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METABONOMIC APPLICATIONS IN HEALTH.

Elaine Holmes.

Division of Computational and Systems Medicine, Department of Surgery and Cancer. Imperial College London, Exhibition Road, London, SW7 2AZ.

Metabonomic technologies such as NMR spectroscopy and mass spectrometry, coupled with computational modeling strategies can generate metabolic phenotypes from biofluids and tissues that reflect the health or disease status of an individual [1]. Biofluid profiles capture endogenous metabolic processes but can also inform on diet, other ingested xenobiotics and metabolites derived from processing of dietary components by the gut bacteria. Thus the biological profiles (urine, plasma, faeces, tissue biopsies, cell extracts) provide a window for viewing metabolic events that can be used in a diagnostic or even prognostic capacity and which can be used as biomarker panels to monitor response to therapeutic interventions such as drugs or surgery. Additionally the perturbed metabolites can be mapped to pathways to help understand the mechanisms underpinning disease.

Metabolic profiling has been applied to both personalized medicine and to population screening. Metabolome wide association studies (MWAS), analogous to GWAS, identify systematic changes in metabolite patterns associated with disease prevalence or risk. The first example of this approach involved the characterization of the impact of diet on hypertension in a large scale epidemiological (the INTERMAP study; International Collaborative Study of Macronutrients, Micronutrients and Blood Pressure), wherein a range of metabolites were identified that differentiated populations with vastly differing blood pressure levels and lifestyles [2]. This has since been extended to other cardiovascular disease risk factors such as obesity. Key population differences between the metabolic signatures of obesity lay predominantly in the gut microbial metabolites reflected in the urine profiles. Further examples involve exploration of the metabolic consequences of dietary interventions in obese populations establishing sub groups who demonstrate a beneficial response and in contrast sub-groups of individuals apparently resistant to dietary modulation.

One of the most effective strategies for obesity is bariatric surgery. In humans and in various rodent models of bariatric surgery, we see a marked change in both the metabolic and the metagenomic profiles and capture cross-talk between the bacteria and their host that report on the effects of the altered anatomy and hormonal changes resulting from surgery. Animal models of insulin resistance and obesity can be used to further elucidate the etiopathological mechanisms underpinning metabolic syndrome. There has been a substantial body of metabolic phenotyping work carried out on other chronic conditions including neurodegeneration, arthritis and cancers, some of which will be reviewed here. However, the technology has been equally effective in profiling acute conditions such as sepsis, parasitic infections and pneumonia.

In contrast to population screening, individuals can be sampled through time, effectively acting as their own control and the dynamic modulation of their metabolite profiles can be used to measure their response to a disease or to an intervention. We will explore a series of novel tools and technologies designed to capture multiple snapshots of biochemistry across different compartments, reviewing surgical tools linked up to mass spectrometers [3] in order to provide real time analysis during surgical procedures and frameworks for assessing individualized response to diet. In all of the application areas we will consider analytical and statistical strategies for accommodating the complexity and interactive nature of biological systems.

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HIGH ACCURACY PROSTATE CANCER DETECTION USING HUMAN BLOOD SERUM METABOLOMIC PROFILING

Monge ME,[1] Zang X,[2] Jones CM,[2] Tran LQ,[3] Zhou M,[2] Walker LDE,[4] Mezencev R,[4] Gray A,[3] McDonald JF[4,5] and Fernández FM[2,5]

[1]Centro de Investigaciones en Bionanociencias (CIBION), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Ciudad de Buenos Aires, Argentina. [2]School of Chemistry and Biochemistry, [3]College of Computing, [4]School of Biology, Integrated Cancer Research Center, and [5]Parker H. Petit Institute of Bioengineering and Biosciences, Georgia Institute of Technology, Atlanta, United States

e-mail: maria.monge@cibion.conicet.gov.ar

Keywords: prostate cancer, untargeted metabolomics, ultraperformance liquid chromatography, mass spectrometry, oncometabolomics, support vector machines

Prostate cancer (PCa) represents the second leading cause of cancer mortality in many western countries. Although the Prostate-Specific Antigen (PSA) test is widely used to screen for PCa, certain advisory groups recommend against its use because it suffers from false positive results and over-treatment. These drawbacks have led to the increased interest of using metabolite profiling to discover new differential biomarkers that could improve the specificity of PCa diagnosis.[1] In this work,[2] untargeted metabolomic profiling of age-matched serum samples from PCa patients and healthy individuals was performed using ultraperformance liquid chromatography coupled to high-resolution tandem mass spectrometry and machine learning methods. PCa was detected in serum samples with 92.1% sensitivity, 94.3% specificity, and 93.0% accuracy by means of a metabolite-based in vitro diagnostic multivariate index assay, which outperformed the prevalent PSA test. Within the panel of 40 metabolic spectral features that was found to be differential, 31 metabolites were identified by MS and MS/MS, with 10 further confirmed by standards. Numerous discriminant metabolites were mapped in the steroid hormone biosynthesis pathway. The identification of fatty acids, amino acids, lysophospholipids, and bile acids provided further insights into the metabolite alterations associated with the disease. Our current work involves the prospective analysis of a larger sample cohort (n=500) that includes samples from PCa patients before and after undergoing surgery, patients with benign prostatitis, and healthy individuals with measured PSA values, in order to evaluate the influence of ethnicity and cohort size on the robustness of the metabolic biomarker panel that we have previously found, and to discover biomarkers useful for follow-up care.

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SELECTION OF HYBRIDS ASSISTED BY NUCLEAR MAGNETIC RESONANCE AND CHEMOMETRIC METHODS: APLICATION TO MANDARINS (*Citrus reticulata*) AND BARLEY (*Hordeum vulgare*).

Lopez A[1], Migues I[2], Meneses L[3], Cardozo I[3], Hodos N[2], Lado J[4], Rodríguez C[2], Abbate S[3], Locatteli A[3], Rivas F[4], Heinzen H[2], Moyna G[1,*]

[1] *Departamento de Química del Litoral, CENUR Litoral Norte, Universidad de la República, Ruta 3 Km 363, Paysandú 60000, Uruguay.*

[2] Departamento de Química Orgánica, Facultad de Química, *Universidad de la República*, Avda. Gral. Flores 2124, Montevideo 11800, Uruguay.

[3] *Polo Agroalimentario y Agroindustrial Paysandu, CENUR Litoral Norte, Universidad de la República, Ruta 3 Km 363, Paysandú 60000, Uruguay.*

[4] *Instituto Nacional de Investigaciones Agropecuarias – Salto Grande, Camino al Terrible S/N, Salto 50000, Uruguay.*

e-mail: gmoyna@fq.edu.uy

Keywords: Citrus reticulata; Hordeum vulgare; Metabolomics; NMR.

Metabolomics is quickly becoming a standard tool for the analysis of agricultural products.[1,2] Using this methodology it is possible to differentiate crops and certify the geographical origin of foodstuffs, to identify populations that are resistant to pests or tolerant to specific environmental conditions, and to select varieties based on desirable organoleptic characteristics. Since late 2011 our group has undertaken a number of projects involving NMR-based metabolomic analysis of a variety of crops and farm products, and two examples of these efforts are presented. One involves the differentiation of mandarins varieties using ^{1}H NMR metabolic profiles, as well as the correlation of these data with organoleptic properties and acceptability among consumers. The other one concerns the selection of barley varieties resistant to aphids based on the ^{1}H NMR fingerprint of leaf extracts, as well as the identification of phytochemical markers associated with this resistance among the different genotypes. To conclude, a brief discussion of potential applications of this and related approaches to other agroindustrial production chains of local an regional relevance is presented.

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COMPREHENSIVE METABOLIC REPROGRAMING IN FRESHWATER NITZSCHIA PALEA STRAINS UNDERGOING NITROGEN STARVATION IS LIKELY ASSOCIATED WITH ITS ECOLOGICAL ORIGIN

Nunes-Nesi A[1], Machado M[1], Bromke M[2], Araújo WL[1], Szymanski J[3] and Willmitzer L[2]

[1] *Max-Planck Partner Group at the Departamento de Biologia Vegetal, Universidade Federal de Viçosa, 36570-900 Vicosa, Minas Gerais, Brazil*

e-mail: nunesnesi@ufv.br

[2] *Max-Planck-Institute of Molecular Plant Physiology, Am Muhlenberg 1, 14476 Potsdam, Golm, Germany*

[3] Tel Aviv University, Department of Computer Science, Tel Aviv 6997801, Israel

Keywords: Diatom, Lipidomics, Metabolomics, Nitrogen, Nitzschia palea

Nitrogen deficiency can increase the lipid content in certain microalgae species, including diatoms. However, the molecular and metabolic basis of such changes remains rather unclear. We analyzed strains of freshwater *Nitzschia palea* collected from an eutrophic pond and from an artificial rock. The habitats, differing in light and nutrient availability, lead to two metabolically distinct strains, BR006 and BR022. Differential accumulation of primary compounds, membrane lipid composition and fatty acid saturation were observed. Metabolic and biophysical analysis demonstrated differential sensitivity to N regimes: depleted, replete and saturated. Whereas N depletion leads to typical stress-related responses in both strains, including reduction of protein and photosynthesis, the response observed in BR006 is far more severe. Our results demonstrated that these strains developed distinct metabolic responses to N conditions. BR022 is able to maintain cellular homeostasis and slows down growth according to N availability. In contrast, BR006 maximizes growth rate even under N limitation, by triggering stress response, relocating carbon pool to lipid compounds and quickly reaching growth arrest after N exhaustion. We identified a relationship between habitat characteristics and metabolic responses, providing a metabolic perspective on ecological plasticity of *N. palea* which helps it to survive a wide range of habitats.

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METABOLOMICS OF THE METABOLIC SYNDROME COMPONENTS

Pirola C.J.

Institute of Medical Research Buenos Aires (University-National Scientific and Technical Research Council) , Ciudad Autonoma de Buenos Aires, Argentina.

e-mail:pirola.carlos@conicet.gov.ar pirola.carlos@lanari.fmed.uba.ar

Keywords: obesity, type 2 diabetes, hypertension,NAFLD,.

From the pioneer study of Cheng [1], we have hypothesized that transaminases reactions are deregulated in the Metabolic Syndrome (MetS) [2;3], a complex syndrome chracterized by at least 3 of 4 major components, central obesity, glucose intolerance or insulin resistance, hypertension and dyslipidemia, and frequently associated with cardiovascular risk. Then, in a collaborative study with the Dr Levi's group (Framingham, MA, USA), we conducted independent discovery and replication cross-sectional studies to characterize metabolomics signatures of obesity, dyslipidemia, and dysglycemia. With 5-7 years of follow up from discovery study participants, we identified single metabolites and multi-metabolite panels associated with longitudinal changes in body mass index, lipid levels, and glucose levels [4]. The following are highlights of our study:

· We used gas chromatography-mass spectrometry (GC-MS) to characterize cross-sectional metabolomics markers of metabolic risk factors in 650 Framingham Heart Study participants.

· Top results were independently replicated in 670 BioImage Study participants.

· Branched-chain amino acid markers of obesity and dysglycemia revealed a glutamic acid-enhanced signature that may represent a systemic metabolic derangement in transaminases-mediated pathways.

· Longitudinal results highlighted alterations in bioactive lipids, with lysophospholipid derivatives as markers of change in body mass index and sphingomyelins associated with change in lipids.

· Our multi-metabolite panels explained up to 15.3% of serial change in metabolic traits.

In addition, metabolomics profiling in "in vitro" models may help to understand the mechanisms behind the association of genetic variants with MetS components [5] as will be shown in the presentation.

Understanding the pathways represented by metabolic profiling may help unravel molecular derangements contributing to the MetS and its risk factors.

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THE POTENTIAL OF METABOLOMICS IN THE PLANT SCIENCES

Ute Roessner
A/Prof Ute Roessner ARC Future Fellow
President, the Metabolomics Society
Deputy Head - School of BioSciences
University of Melbourne SAGE Athena SWAN Self Assessment team Member - developing, supporting & promoting diversity & inclusion The University of Melbourne
u.roessner@unimelb.edu.au
School of BioSciences, The University of Melbourne, 3010 Victoria, Australia

A plant's survival and reproduction is entirely dependent on its ability to adapt to an unpredictable and dynamic environment. We are using metabolomics as a functional genomics approach to increase our understanding of how plants develop mechanisms to adapt to or tolerate abiotic stresses. Metabolomics is an emerging field in the suite of 'omic' approaches for Systems Biology. The goal of metabolomics is to detect the presence of all small-molecules in a biological sample. This presents a significant challenge due to their chemical diversity and large concentration ranges requiring the application of complementary analytical approaches, including mass spectrometry coupled to chromatography to increase the coverage of metabolites analysed. The array of approaches will be summarised and their application in biological systems demonstrated from our research program on salinity stress in barley (*Hordeum vulgare* L.) which is an essential food and brewing crop. We are focusing on salt stress perception and responses in roots, which involve complex changes at the physiological, metabolic, molecular, transcriptional, and genetic level. Evidence is accumulating that lipid signalling is an integral part of the complex regulatory networks in the responses of plants to salinity through modifications of membrane lipids to produce different classes of lipid and lipid-derived messengers. Initial analyses indicate that different tissue types within the root respond differently to salt stress in tolerant and sensitive cultivars. Here we study the root responses to salinity using a combination of next generation RNA-sequencing, cell wall composition analysis and targeted metabolite and lipid analyses of three key sections of barley roots. We are also using modern lipidomics technologies to compare the root plasma membrane compositions of different barley genotypes with contrasting salinity tolerance levels upon salt stress in addition to MALDI-FT-MS based imaging to monitor spatial distributions of metabolites and lipids across root sections. Transcriptomics results are now being integrated with spatial biochemical data, enhancing our understanding of system-wide and tissue-specific responses of roots to salinity stress. Given the lack of fundamental knowledge of the genes and proteins involved in signalling, cell wall and lipid metabolism under salinity stress, and the enormous potential for biotechnological application in this area, our results provide insight into novel mechanisms responsible for salt tolerance of barley.

SALIVARY METABOLITE SIGNATURES OF CHILDREN WITH AND WITHOUT DENTAL CARIES LESIONS AND WITH TYPE 1 DIABETES: AN NMR STUDY

Valente, AP

Federal University of Rio de Janeiro, CNRMN, Rio de Janeiro, Brazil.
e-mail:valente@cnrmn.bioqmed.ufrj.br
Keywords: NMR, Saliva, Caries, Type 1 diabetes, Metabolomic profile.

A metabolomic approach was used to analyze children's saliva metabolites and to correlate with specific biological states. The analysis of salivary metabolites is a growing area of investigation with potential for basic and clinical applications. Analyses of children's saliva in different dentitions and with or without caries could potentially reveal a specific profile related to oral disease risk (1,2). We also analyzed the salivary components of type I diabetic children (DM1) under six years of age, to assess oral health related to diabetes control, as well as metabolite profiling using NMR (3). Nuclear Magnetic Resonance (NMR) is well suited for mixture analysis followed by Principal Component Analysis used to identify differences in the salivary metabolites. The NMR data and PLS-DA were able to classify saliva from children in different health states and the metabolite profiles could be analyzed. Our data also showed similar salivary metabolite profiles for healthy subjects despite the differences in their oral hygiene habits, socioeconomic status and food intake.

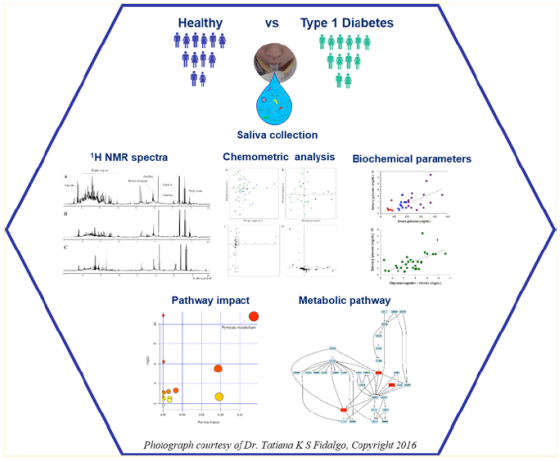

Figure 1: Schematic representation of the study approach to analyze children's saliva using NMR, from reference 3.

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FAPERJ, CNPq, INCT-INBEB

METABOLIC FINGERPRINTING TO DETERMINE THE ORIGIN OF COFFEE: WHAT'S NEXT?

Wist JW[1], Medina J[1], Arana VA, Bernal A[1].
Chemistry Department, Universidad del Valle, Cali, Colombia.
e-mail:julien.wist@correounivalle.edu.co
Keywords: metabolic profiling, origin determination, coffee, plsr, IR-ATR

The quality of Colombian coffee explains its high market value. At the same time, the steady production do not suffice to supply for both exportation and internal market and thus forces Colombia to import coffee from neighbouring countries. Both factors raise concerns of fraud by adulteration with beans from different origins. Recently our group showed that NMR could distinguish between coffees from Colombia and from 24 other origins, including neighbours. Although several degrees of variance were included in the experimental design, all samples were prepared in a very standard fashion by lightly roasting green beans prior to extraction in methanol. Clearly this doesn't represent a real life scenario where bags of roasted coffee are randomly picked from a shelf, thus without any control on the roasting process. How will our model behave if such samples are analyzed? To answer this question, 40 samples were prepared at 4 different degrees of roasting, medium, medium dark, dark and very dark and tested against our model (169 train, 42 test). As expected, the accuracy of the prediction drops dramatically from Q^2=0.87 to 0.58, even after excluding very dark samples whose profiles are too affected and thus unsuitable for analysis. This adverse situation can be reversed by excluding specific regions of the spectrum that are most affected by the roasting process and determined by PLS regression. After exclusion Q^2 values of 0.8 were achieved.

Despite these promising results, it is clear that the volume of exportations, the cost of NMR per sample and the difficulty to setup and run an NMR facility at harbour rules out this technique. A body of work exists that reports on how several other analytical methods, NIR, mIR, IRMS, perform to discriminate coffee by species or origins. However, these publications are based on on small sample size with few well determined origins from different continents. Here we present what is to the best of our knowledge the first attempt to compare several spectroscopic methods for the purpose of determination of origin of coffee. To achieve this goal, a set of 97 samples (14 origins) were analysed by NIR, mIR-ATR and NMR. Results (Figure 1) show that NIR performs worse, while mIR-ATR and NMR perform similarly. As a conclusion, mIR-ATR emerges as a powerfull candidate for large scale screening at harbours.

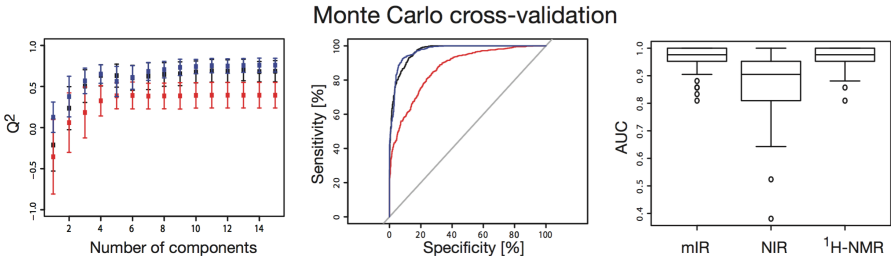

Figure 1: Q^2, ROC curves and areas under the ROC curves. Red represent NIR, blue mIR-ATR and black NMR.

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ALMACAFE, BRUKER, NESPRESSO

DEREPLICATION AND IDENTIFICATION OF METABOLITES: A COMMON DATABASE OF COMPOUNDS

Wist JW[1], Castillo A[1], Bernal A[1], Kostro D[2], Zasso M[2], Patiny L[2].
[1]*Chemistry Department, Universidad del Valle*, Cali, Colombia.
[2]*Institute of Chemical Sciences and Engineering, Ecole Polytechnique Fédérale de Lausanne, 1015 Lausanne, Switzerland*

e-mail:julien.wist@correounivalle.edu.co
Keywords: metabolic profiling, dereplication, repository

Untargeted analysis shifted the paradigma of complex mixture analysis. While the traditional approach requires prior knowledge or a "hunch" about compounds involved in a metabolic process of interest, untargeted analysis permits to discover relevant biormarkers from a large number of simulateneously observed compounds.

However, the assignment of a statistically relevant variable to a molecule is the bottleneck of untargeted studies, first because this task is still performed manually and second, and most importantly, because it requires to recur to a database of known metabolites. Several repositories exist where to deposit spectral information, such as BMRB to just mention one. But while these repositories contain a wealth of information, including raw data, its use is impractical for most user. Superimposing a reference spectrum on top of a metabolic profiles requires substantial efforts beyond the reach of most research groups. Automated queries to retrieve a list of candidate molecules are not possible.

Here we present a proof of concept of repository that allows to:

- Control the configuration, acquisition and upload of spectra of reference compounds
- Readily import new entries by simple drag and drop
- Perform the assignment of the spectra online
- Retrieve referenced compounds that match a particular signal
- Superimpose the results on top of an experimental profile

A such system is available at http://sample.cheminfo.org and is use is free. If a local version of the system is necessary it can be deployed readily. The source code can be found on github/cheminfo.

The platform consists of 3 separated layers: storage, processing and statistics, and visualization. This clear separation allows experienced user to create new tools depending on their needs by scripting an extensive library of functions that permit to manipulate NMR and MS data.

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Oral presentations

USING METABOLOMIC APPROACHES TO EXPLORE DIVERSITY WITHIN HEALTHY POPULATION FROM THE CENTRAL REGION OF ARGENTINA.

Burdisso P[1], Coscia A[1], Sciara M[2], Vazquez MP[3], Vila A[1], Rasia RM[1].

[1]*Plataforma Argentina de Biología Estructural y Metabolomica (PLABEM) IBR, Rosario, Argentina.*
[2]*Centro de Diagnostico Medico de Alta Complejidad (CIBIC) Rosario, Argentina.*
[3]*Genomics and Bioinformatics, Instituto de Agrobiotecnología de Rosario - CONICET Rosario, Argentina.*

burdisso@ibr-conicet.gov.ar
NMR, Metabolomics, metabolomic diversity.

Metabolomics is the large-scale study of small molecules, commonly known as metabolites, within cells, biofluids, tissues or organisms. The non-invasive nature of this technique allows the global study of metabolic profiles assuring the characterization of an individual metabolic phenotype.

Metabolic phenotypes are the products of interactions among a variety of factors like genetics, diet, lifestyle, environment and gut microbiome. In order to investigate metabolic phenotype diversity across and within healthy people from the central region of Argentina, we conducted an NMR-based metabolomics analysis on biofluids samples collected from healthy volunteers with complete metadata records including 18 biochemical tests, lifestyle and diet factors and gut microbiome analysis. We analysed spectra from plasma and urine specimens for each of 150 participants from the project: *"Argentine Gastrointestinal Microbiome and Metabolome"* involving 4 population samples aged 18-50 in Rosario, Paraná, Rafaela and Venado Tuerto. CPMG and NOESY spectra were pre-processed and spectral data was extracted for multivariate data analysis. PCA and OPLS-DA models were constructed. As we expected, urinary and blood metabolite patterns for each region samples are not significantly differentiated, nevertheless we could detect patterns between outliers and the associated metadata. The present work defines criteria and variability within the local population that will have to be considered in future studies.

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OPTIMISED METABOLIC PHENOTYPING WORKFLOW USED FOR CLINICAL RESEARCH AT THE CLINICAL PHENOTYPING CENTRE

Gómez-Romero M[1], Lewis M[1], Horneffer-van der Sluis V[1], Herendi L[1], Jimenez B[1], Lodge S[1], Lees H[1], Correia G[1], Pearce J[1], Holmes E[1], Nicholson J[1]

[1]*Computational and Systems Medicine, Department of Surgery and Cancer, Faculty of Medicine, Imperial College London, London, UK*

m.gomez-romero@imperial.ac.uk

Keywords: Metabolic phenotyping, clinical research, analytical workflow, nuclear magnetic resonance, mass spectrometry.

The analysis of biofluids and tissue samples taken at different stages of specific patient journeys identifies a profile of chemicals, or *metabolic phenotype*, which is the combined product of the patient's genetic profile, lifestyle habits and the environment. This phenotype can be linked to clinical outcomes and can be used to advance disease diagnostics, prognosis and treatment.

The Clinical Phenotyping Centre (CPC) is an analytical facility established in April 2012 within the Stratified Medicine theme of the Department of Surgery and Cancer (Imperial College). The CPC provides infrastructure and services to support translational research, employing a metabonomic approach to derive novel diagnostic and prognostic biomarker models.

No single analytical platform can provide comprehensive coverage of all the metabolites contained in a biological sample, therefore the CPC brings together state-of-the-art technologies and combines powerful analytical strategies with advanced multivariate statistical tools. In order to obtain meaningful results, it is critical to define the purpose of the study clearly as it will determine the design and metabonomic approach for the study. For novel clinical studies, a discovery strategy with a non-biased broad metabolite coverage is recommended. This approach, known as global metabolic profiling, allows evaluation of endogenous as well as drug derrived metabolites and the use of complementary H^1-NMR and UPLC-MS assays can maximise the metabolite coverage. For hypothesis driven studies, a targeted approach is recommended. The CPC can also develop UPLC-MS targeted assays providing quantitative information on a specific class of molecules or metabolic pathway. Both of these approaches require specific sample handling or optimisation of instruments. Detail of the two approaches will be discussed together with examples of their clinical application.

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NIHR IMPERIAL BIOMEDICAL RESEARCH CENTRE, MRC-NIHR NATIONAL PHENOME CENTRE (NPC)

ANALYSIS OF DIFERENCES IN CHEMICAL PROFILES OF THE MIXTURES FROM ^{1}HNMR COUPLED TO PRINCIPAL COMPONENT ANALYSIS (PCA)

Mario O. Salazar

Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario-CONICET. Suipacha 531 Rosario (2000), Argentina.

e-mail:msalazar@fbioyf.unr.edu.ar

Keywords: Complex mixtures, ^{1}H NMR, PCA.

Since few decades ago NMR has been developed as a tool to obtain fingerprints of complex mixtures. In order to investigate the differences in such complex chemical profiles, the wide range of data obtained from NMR spectra can be analyzed with the assistance of statistical methods such as PCA. Naturally occurring mixtures like juices present a wealth of metabolites, and therefore chemical information which is characteristic of each mixture. We have used NMR coupled to PCA to analyze compare different orange species of genus citrus observing that discrimination between spices Citrus juice (orange, tangerine, grapefruit, etc.), orange (*C. sinensis*) juice from diferent varieties, orange juice produced from different geographic region (Argentina).

Chemically Engineered Extracts (CEE) are semi-synthetic mixtures of compounds produced by diversification of natural extracts (NE) through chemical transformation of common chemical functionalities in natural products into chemical functionalities rarely found in nature.[1] The analysis of chemical profiles of the mixtures (CEE versus NE) through ^{1}HNMR coupled PCA is a useful methodology to determine the effect of the reactions appied on the total composition of the mixtures.

Through this methodology we have estimated the success of chemical reactions such us sulphonylation and bromination, on the composition of crude extracts.[1,2,3] The score plot showed discrimination between the two groups by principal components (PCs), 1 and 2 (**Figure 1**, sulphonylation of p-toluenesulfonyl chloride). CEEs showed a positive PC2 value mainly due to the positive effect on PC2 of the signals corresponding to the p-toluene moiety introduced to the natural components of the starting mixtures. This effect was confirmed by comparing integrated areas of signals corresponding to the molecular portions from the addition of reagents used.[2]

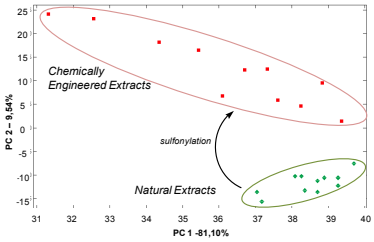

Figure 1: Score plot of PCA of ^{1}H NMR data from 11 NE (green) and 11 CEE (red) produced by reaction with p-toluenesulfonyl chloride.

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METABOLIC PROFILE ANALYSIS OF DIFFERENT *Fusarium* SPECIES– INTEGRATION OF ^{1}H-NMR-DEREPLICATION ALGORITHM AND MS/MS MOLECULAR NETWORKING FOR THE IDENTIFICATION OF SECONDARY METABOLITES

Selegato, D.M.[1], Freire, R.T.[2], Pilon, A.C.[1], Pavarini, D.P.[1], Carnevale Neto, F.[2], Castro-Gamboa, I[1].

[1] *Nucleus of Bioassays, Ecophysiology and Biosynthesis of natural products (NuBBE), IQ/Ar, Chemistry Institute – São Paulo State University São Paulo – UNESP, Araraquara, SP, Brazil. e-mail:denisemselegato@gmail.com*

Keywords: Senna spectabilis, rhizosphere, Fusarium, dereplication, 1H-NMR computacional algorithm, fusaric acid, MS/MS molecular networking, beauvericin.

Dereplication is of fundamental importance in the current bioprospecting programs. and emerges as a rapid way of identifying known compounds in mixtures, minimizing time, effort, and cost, as well as accelerating the selection of biologically promising samples and the identification of known chemotypes. The major challenges of dereplication techniques are matrix complexity and the elucidation of low abundance metabolites. To overcome this problems, new methodologies have been developed, processing metabolic profile data by multivariate analysis and other pattern recognition techniques, such as molecular networking and Principal Comonent Analysis (PCA). The main goal of this work is to analyse metabolic profiles of *F. solani* and *F. oxysporum* isolated from *Senna spectabilis'*s rhizosphere, aiming to dereplicate important and bioactive metabolites. To achieve this goal, and to overcome data complexity, we have integrated two dereplication methodologies applied to ^{1}H-NMR-based metabolomics[2] and tandem mass spectrometry pattern recognition[3]. The first uses PCA loading values of ^{1}H-NMR data to select important peaks and identify metabolites responsable for PCA separation. The second, named MS/MS Molecular Networking, organizes tandem MS data based on chemical similarity, allowing the dereplication of known molecules, and their analogues, a obstacle not overcome by other methodologies. By applying the first strategy, it was possible to identify and mathematically separate ^{1}H-NMR peaks responsible for *F.* solani and *F. oxysporum* metabolic differentiation, identifying three known molecules **(1)** fusaric acid, produced majorly by *F. oxysporum*, a mycotoxin with high comercial value, and two enniatin **(2)** HA23 and **(3)** beauvericin, produced by *F. solani* and *F. oxysporum*, respectively. Complementarily, by target analysing of all metabolites identified above using MS/MS Molecular Networking, we were able to furthwer identify eight other analogues **(A)** six enniatins, three of them known molecules but firstly described for *F. oxysporum* beauvericin F **(A1)**, beauvericin D **(A2)** and beauvericin G2 **(A3)**; and three firstly reported depsipeptides **(A4 – A6)**; and **(B)** two picolinic acid analogues 5-(3-buten-1-yl)-pyridine-2-carboxylic **(B1)** and 5-(but-3-en-1-yl)-6-oxo-1,6-dihydropyridine-2-carboxylic acid **(B2)**. Therefore, by integrating two newly developed dereplication methodologies, we have succefully dereplicated 11 abundance secondary metabolites, among known and newly describied, as well as high and low abundance compounds.

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Posters

METABOLIC PROFILING FOR THE EVALUATION OF MARINE BACTERIA AS A SOURCE OF COMPOUNDS FOR PHYTOPATHOGEN CONTROL

LA Betancur J[1,2], SJ Naranjo G[1,3], DM Vinchira[1], NC Moreno S[1], LA Maldonado M[4], ZR Suarez M[5], A Acosta[6], L Castellanos H[1], FA Ramos R[1*]

[1]*Universidad Nacional de Colombia sede Bogotá, Facultad de Ciencias, Departamento de Química, Grupo de investigación: Estudio y Aprovechamiento de Productos Naturales Marinos y Frutas de Colombia. Bogotá, Colombia.* [*]*e-mail: faramosr@unal.edu.co*

[2]*Universidad de Caldas*

[3]*Universidad Politécnica del Ejército*

[4] *Universidad Autónoma Metropolitana*

[5]*Investigación y Desarrollo. Empresa Colombiana de Productos Veterinarios VECOL S.A.*

[6]*Universidad de La Sabana*

Keywords: *Marine bacteria, Metabolic profiling, anti-phytopatogen activity, Inhibition Quorum Sensing*

Marine environments represent an understudied resource for the isolation of bacteria with the potential to produce bioactive compounds.[1] The metabolomics aproach is a comprehensive strategy which allows profiling of a complex mixture of numerous metabolites in a crude extract as well as targeting substances that can be correlated to a certain biological activity before beguin any time-consuming isolation procedure.[2] In the present study, we conduct LC-MS based metabolic fingerprint analysis in order to evaluate the chemical composition of 33 bacterial isolates obtained from sediments, algae, sponges and soft corals from Providencia and Santa Catalina coral reef. The isolates were affiliated to 8 genera: *Streptomyces*, *Micromonospora*, *Gordonia* (Phylum Actinobacteria); *Stenotrophomonas* and *Proteus* (Phylum Gammaproteobacteria); *Bacillus, Lisinibacillus and Paenibacillus* (Phylum Firmicutes). For metabolic profiling, LC-MS analysis was conducted for the ethyl acetate extracts of each strain. The preprocessing of the data was performed in MZmine software and subsequently analyzed using the Statistical Package SIMCA-P 14 (Umetrics, Sweden). The metabolomic approach was performed by HCA, and OPLS-DA, including activity data used against pathogens. Based on the taxonomical data, screening information of antibacterial and Quorum quenching activity against phytopathogenic strains, as well as the metabolic profiling of the strain organic extracts, we selected 8 out of the 33 the isolates for follow-up chemical isolation and structure identification work.

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NON-VOLATILE PUTATIVE KAIROMONES MEDIATING DIAPHORINA CITRI OVIPOSITION

Burgueño A.P.[1], Amorós M.E.[1,2] , Moyna G.[3] , Buenahora J.[2] , Rossini C.[1]
[1] *Facultad de Química, UdelaR, Montevideo, URUGUAY*
[2] *Instituto Nacional de Investigación Agropecuraria, INIA SG, Salto, URUGUAY*
[3] *Departamento de Química del Litoral, UdelaR, Paysandú, URUGUAY*

e-mail: aburgueno@fq.edu.uy
Keywords: kairomone, citrus greening disease.

Huanglongbing, currently the most destructive citrus disease worldwide, is dispersed by the Asian citrus psyllid (ACP), Diaphorina citri (Hemiptera: Psyllidae). During host plant search, ACP can integrate information from flushing shoots including visual and odor cues [1]. A preference greenhouse experiment carried out using 6 citrus species: Citron (Citrus medica), Eureka lemon (Citrus limon), Cravo lemon (Citrus limonia), Rough lemon (Citrus jambhiri), Sweet orange (Citrus sinensis) and Duncan grapefruit (Citrus paradisi) has shown an oviposition preference of D. citri for Duncan grapefruit, Rough lemon and Sweet orange. These potential host plants exhibited emissions of volatile organic compounds that grouped preferred species together primarily due to high emissions of methyl N-methylanthranilate [2]. To further investigate on putative kairomones that could mediate the oviposition preference of D. citri we analyzed the leaf waxes and CDCl3 and D2O extracts from the six citrus hosts (N = 2-6/species). GCMS data from leaf waxes were analyzed using MZmine2.10, defining parameters for mass detection, chromatogram building and peak deconvolution. Chromatograms normalized for retention times and peak areas were then aligned (RANSAC tool), generating a matrix of 646x15. NMR data were processed with MestReNova 11.0 (0.04 binning, data from 11 to 0 ppm) generating matrices of 260x12 and 247x12 for CDCl3 and D2O extracts respectively. The three matrices were then subjected to PCA. Chemical profiles of the waxes or the D2O extracts show no distinction between preferred and non-preferred species. However, in the case of CDCl3 extracts, the chemical profiles of the preferred citrus species were more similar among them that the ones not-preferred. These results may point to non-polar compounds as possible cues that D. citri can use to choose oviposition plants.

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INIA: Proyecto CT- 16, 2013 Manejo integrado de Diaphorina citri con énfasis en control biológico.
CSIC, UdelaR: Proyecto CSIC-Grupos, 2015 Ecología Química.
ANII: Beca de maestría (Amorós, M E).

DIFFERENTIAL REMODELLING OF THE LIPIDOME DURING POSTHARVEST OF PEACH FRUITS: ANALYSIS OF CULTIVARS WITH DIFFERENT SUSCEPTIBILITY TO CHILLING INJURY

Bustamante C1, Brotman Y2, Monti L1, Gabilondo J3, Budde C3, Andreo C1, Lara MV1, Fernie A2, Drincovich MF1

1CEFOBI-UNR, Argentina; 2MPI für Molekulare Pflanzenphysiologie, Germany; 3INTA-EE San Pedro, Argentina

E-mail: bustamante@cefobi-conicet.gov.ar

Keywords: chilling injury, fruit, lipidomics, Prunus persica

Peaches ripen and deteriorate quickly at room temperature. Therefore, refrigeration is used to slow these processes and to extend fruit market life; however, several fruits can develop chilling injury (CI) during storage at low temperature. In peaches, CI includes woolliness (lack of juice) and browning, among other symptoms. As the cell membranes are likely sites of primary effects of chilling, in the present work we analyzed the lipidome of six peach cultivars with different susceptibility to CI during ripening and after cold storage. By using ultra-performance liquid chromatography coupled to Fourier-transform mass spectrometry (UPLC-FT-MS), we detected 59 lipid species, including diacyl and triacylglycerides. During fruit ripening, a decrease in the levels of some storage lipids was observed (TAG 52:5 and TAG 54:4), along with changes in the relative amount of several galactolipids (MGDG 36:6, DGDG 36:3 and DGDG 36:6 decreased; MGDG 36:4 increased). After 21 days of cold storage at 0 ºC, all the cultivars accumulated DGDG 36:4 and PC 38:2, and showed a decrease in the level of MGDG 36:5, with respect to harvest. In addition, levels of plastidic glycerolipids were also modified in fruits stored at 0 ºC for a short period, when compared with fruits of the same postharvest age under 20 °C ripening conditions (DGDG 36:3, DGDG 36:6 and MGDG 36:6 increased; MGDG 36:4 and MGDG 36:5 decreased). Finally, the relative abundance of some glycerolipids correlated with the susceptibility to CI, when compared woolly *versus* non-woolly fruits. Overall, the results allowed the identification of lipids linked to the ripening process, lipids that are part of the common response of peach fruit to cold, and lipids that could be used as molecular markers of chilling susceptibility.

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CONICET, ANCYPT, ALEXANDER VON HUMBOLDT FOUNDATION

EFECTO DEL CONSUMO DE MACA NEGRA Y ROJA EN LAS CARACTERÍSTICAS METABOLÓMICAS DEL HABITANTE DE ELEVADAS ALTITUDES. PUNO – PERÚ 2014-15.

Caballero L1*, Flores R1*, Portocarrero H1*, Tapia V2*, Gonzales GF2*,
*1Universidad Nacional del Altiplano Puno – Perú.;.*Círculo de Investigación en Plantas con Efecto en Salud. e-mail: lidsofcaballero@hotmail.com*
2Universidad Peruana Cayetano Heredia
Keywords: lepidium meyenii, N6-acetillisina, lisina, tiramina, N6,N6,N6 trimetillisina, histidina, metionina sulfóxido, homoarginin, dimetiarginina, isocitrato, endocanbinoides.

INTRODUCCIÓN: La maca (Lepidium meyenii) pertenece a la familia Brassicaceae y crece en los Andes centrales del Perú por encima de los 4000 m. de altitud donde se cultiva desde hace más de 2000 años. Se le atribuyen una serie de funciones, que varían según el color y aunque aún no se han identificado completamente los posibles mecanismos de acción, parecería que ejerce un efecto modulador en diferentes funciones biológicas, de allí la importancia de su estudio.

OBJETIVO: Determinar el efecto del consumo del extracto hidroalcohólico atomizado de maca roja, maca negra o placebo sobre las características metabolómicas en hombres y mujeres de 18 a 65 años nativos de Puno (3800 m).

MATERIALES Y MÉTODOS: Es un estudio longitudinal, doble ciego controlado por placebo, con muestreo no probabilístico, error del 20% y nivel de confianza 95%. La muestra fue seleccionada por conveniencia, con distribución aleatoria de los tratamientos: maca negra, roja y placebo. Fueron estudiados hombres (50) y mujeres (50) de 18 a 65 años, quienes consumieron 3g/día del extracto por 3 meses. Para el análisis de las características metabolómicas se aplicó la metodología HPLC- LC-MS/MS. Se utilizaron los estadísticos Wilcoxon y Bonferroni, en el paquete estadístico Stata.

RESULTADOS: Fueron analizados 567 metabolitos correspondientes a procesos metabólicos de los carbohidratos, lípidos, proteínas y cofactores vitamínicos, estableciéndose diferencias independientes del tratamiento, en 85 metabolitos. El análisis inicial-final según tratamiento, determinó diferencias significativas ($p<0.05$, una cola) con respecto al placebo en 9 metabolitos asociados a la maca roja y 45 a la maca negra.

La maca roja y negra reducen los niveles de N6- acetil-lisina y lisina, marcadores de la función renal, siendo más efectiva en mayores de 40 años; este rol es favorable para maca roja. Igualmente induce la modulación de la presión arterial a través de la reducción de los niveles de tiramina. Se estima un efecto modulador de los niveles de glicemia a través de la reducción de lisina, N6,N6,N6 trimetillisina e histidina, reduce la metionina sulfóxido, indicador de un efecto antioxidante, ello por el contenido de catequinas, polifenoles y glucosinolatos de la maca. Esto se asocia al efecto reductor de glicemia de maca negra publicado previamente. Se observa un efecto regulador del nivel de homoarginina y dimetiarginina ADMA+SMDA, aspecto asociado a un menor riesgo cardiovascular. Se ha observado un efecto reductor de los endocanabinoides; palmitoiletanolamida, estearoiletanolamida y 1-araquidonoil GPA (20:4), reducción del picolinato, marcador del aumento de peso. Además muestra un efecto energético por el aumento significativo del isocitrato, un mediador del ciclo de Krebs. **CONCLUSIONES:** La maca negra y roja pueden intervenir en diferentes vías metabólicas, regulando la función renal, presión arterial, glicemia, disminuye el riesgo cardiovascular, presenta alta capacidad energética por lo que se constituyen en potencial nutracéutico asociado al bienestar.

LIPIDOMIC ANALYSIS OF THE FATTY ACID BIOSINTHESYS REGULATOR FASR MYCOBACTERIUM TUBERCULOSIS MUTANT

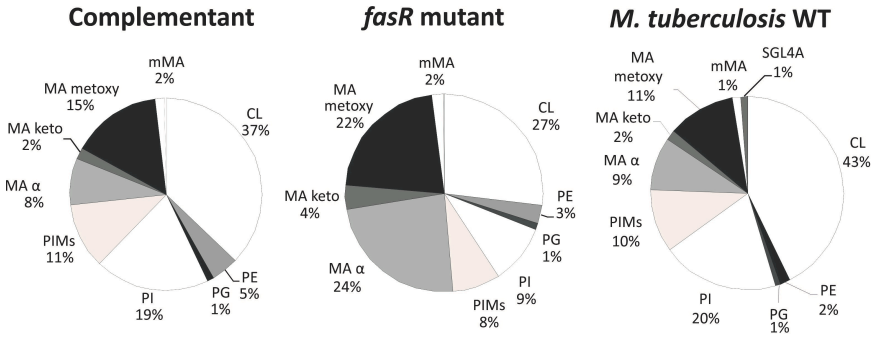
Cabruja ME1, Mondino S, Sala C, Cole ST, Gramajo H and Gago G

IBR, Rosario, Argentina.

cabruja@ibr-conicet.gov.ar

Keywords: Lipidomics, LC-MS-MS, Mycobacterium tuberculosis.

Our interest resides in the study of the regulation of lipid biosynthesis in *Mycobacterium tuberculosis*, the causative agent of human Tuberculosis, a re-emerging disease that is affecting people worldwide, especially those co-infected with HIV [1]. In this work we focused on the role of the transcriptional regulator of *fas* gene (FasR) in lipid biosynthesis [2]. Although we were able to construct a null *fasR M. tuberculosis* mutant strain, the mutant presents severe growth defects compared with the complemented or the wild type strains. We used liquid chromatography coupled to high resolution mass spectrometry (HPLC-HRMS) in data dependent acquisition mode to analyse the lipid profile of this mutant. The analysis of the *fasR* mutant strain presented several changes in the relative amounts of lipids such as phospholipids (Figure 1), and also changes in the instauration level and the acyl chain lengths substituents of several lipids species. Detailed analysis of lipids is of remarkable importance because it allowed to examine the global impact of reduced fatty acid biosynthesis on the composition of the whole mycobacterial outer membrane. Our results, together with proteomic and RNA seq studies that are under progress, will help to start deciphering the regulatory network involved in maintaining lipid homeostasis in *M. tuberculosis* and will eventually provide new tools to combat this re-emerging disease.



Phospholipids		Mycolic acids	
Complementant	73%	Complementant	25%
fasR mutant	49%	*fasR* mutant	50%
M. tuberculosis WT	76%	*M. tuberculosis* WT	22%

Figure 1: Lipid profile in negative mode of *M. tuberculosis* wild type (WT), the *fasR* mutant and the complementant strain. The relative quantification was calculated as the mean of three independent replicates.

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MULTIPLATFORM PLASMA FINGERPRINTING IN CANCER CACHEXIA: A PILOT STUDY

Cala M.P.[1,2], Lopez-Martin J.A.[3], Agullo M.T.[3], Gonzalez-Riano C.[2], Garcia A.[2], Barbas C[2].
[1] *Universidad de Los Andes, Faculty of Sciences, Department of Chemistry, Bogotá, Colombia. e-mail: mp.cala10@uniandes.edu.co*
[2] *CEMBIO (Centre for Metabolomics and Bioanalysis), San Pablo CEU University, School of Pharmacy, Boadilla Del Monte, Spain.*
[3] *12 de Octubre University Hospital & Research Institute, Madrid, Spain.*
Keywords: Cancer cachexia, plasma fingerprinting, LC-MS, GC-MS, CE-MS.

Cachexia is a metabolic syndrome that affects a large amount of cancer patients, especially in advanced stages of the disease. This disorder may affect up to 80% of patients with advanced cancer and it is indirectly responsible for at least 20% of all cancer patients' deaths[1]. Understanding cancer cachexia is a frequent unmet medical need. Its molecular basis has not been extensively studied in humans, and the physiopathology remains poorly understood[2]. In the present study, a pilot multiplatform metabolomics approach was used to obtain a global picture of metabolic alternations that occur in cancer cachexia. Study was performed on plasma samples obtained from 15 cancer (ca) patients (pts), distributed as follows: Cachexia (CX): 8 pts (pancreatic ca: 3, melanoma: 3, biliary duct ca: 2); control (CN): 7 pts (colon ca: 1, esophageal ca: 1, gastric ca: 2, pancreatic ca: 1, melanoma: 1, sarcoma:1). Samples were analyzed by the three platforms commonly used in metabolomics studies (GC-MS, LC–MS and CE–MS), to increase metabolite coverage[3-5]. Differences between profiles from CX and CN groups, obtained within each technique, were evaluated with univariate (UVA) and multivariate (MVA) analysis using a nonparametric *t* test, principal component analysis (PCA) and orthogonal partial least squares regression (OPLS-DA). The metabolite with highest increase was cortisol (fold change 1.67, $p = 0.03$). The largest affected group of metabolites was amino acids and derivatives, all decreased. Glycerophospholipids, sphingolipids, carboxilic acids and derivatives, and indoles were also decreased. Despite tumour type heterogeneity and the small simple size of this study, cancer-related cachexia is significantly associated with a metabolomic signature that represents an increased catabolism. Of note, the increased values of cortisol should lead us to revisit the use of glucocorticoids in this setting. Substitutive therapy for some of the observed deficiencies might deserve clinical exploration.

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PRECONCENTRATION OF HETEROCYCLIC AROMATIC AMINES IN NATURAL WATER SAMPLES USING MULTIWALLED CARBON NANOTUBES AND DETERMINATION BY UPLC-MS/MS

Canales R.[1], Reta M.[2], Cerutti S.[1]

[1]*Institute of Chemistry of San Luis (San Luis CCT). Area of Analytical Chemistry, Faculty of Chemistry, Biochemistry and Pharmacy, National University of San Luis, San Luis, Argentina.*

e-mail:rominacanales66@gmail.com

Keywords: Multiwalled carbon nanotubes, Solid-phase extraction, Preconcentration, Heterocyclic aromatic amines; UPLC-MS/MS.

Heterocyclic aromatic amines (HAAs) are a group of compounds known to be potents mutagens and carcinogens in animals. Human beings are also frequently exposed to them through diet and environmental sources. Recently, the toxicity of these compounds have raised many studies to evaluate their levels and distribution in different environmetal compartments. Many analytical strategies are available for the determination of heterocyclic aromatic amines, mainly focused on their quantification in food. However, there is still scarce information concerning sensitive and selective methodologies for HAAs quantification at ultratrace levels in complex environmental samples. In this sense, multiwalled carbon nanotubes (MWCNTs) have been applied as SPE adsorbents for the enrichment of many kinds of compounds. The hexagonal arrays of carbon atoms in graphene sheets of MWCNTs surface have a strong interaction with other molecules or atoms, which make MWCNTs efficient adsorbent materials.

In this work, a novel solid- phase extraction protocol based on the use of multiwalled carbon nanotubes for the extraction/preconcentration of some HAAs of environmental interest- IQ and PhIP- and, subsequent quantification by UPLC-MS/MS was developed. Thus, the extraction and preconcentration was as follows: 30 mg of MWCNTs were placed into a conical minicolumn, then the column was conditioned with acetonitrile before sample loading. Samples (aliquots 50 mL of drinking water), were passed through the column. After retention, the HAAs were eluted with 0.5 mL of acetonitrile into glass UPLC vials. The obtained extracts were inmediatelly analyzed. The separation was performed by injecting 10 µL of sample into a C_{18} analytical column. Mass analyses were performed on a triple quadrupole mass spectrometer equipped with a electrospray ionization source (ESI) configured in a positive polarity mode. All the experimental variables that affect both, enrichment and separation/determination were optimized. The results showed that the recoveries were close to 100%, indicating that this method is suitable for determination of IQ and PhIP in water samples. The obtained preconcentration factors were aproximately 100-fold. The calculated detection limits were in the picomolar range. The methodology was successfully applied to natural waters samples. These findings indicate that the developed method could be used as a valuable tool for the routine analysis of the two selected HAAs in water samples.

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INQUISAL, CONICET, ANPCyT.

CYTOTOXIC EFECT IN VITRO OF THE ETHANOIC EXTRACTS OF SPECIES BELONG TO THE MORACEAE, EUPHORBIACEAE, ASTERACEAE AND PHYLLANTHACEAE FAMILIES AGAINST CARCINOGENIC CELL LINES

Cardenas, D.[a], Garzon, V[b], Diaz-Barrera, L.E.[b], Coy-Barrera, E.[a]

[a] *Universidad Militar Nueva Granada, Grupo INQUIBIO. Laboratorio Química Biorgánica*

[b] *Universidad de la Sabana. Grupo de Investigación en Bioprospección.*

Keywords: medicinal plants, plant extracts, carcinogenic cell lines.

Introduction: The fight again the cancer is bases in the efforts of multiple investigations in different areas whose goal is to be able to control it. According to WHO the different types of cancer, between men and woman, has taken the life of 202.00 people until 2014. With the purpose of make alternatives of control, one of the more use strategies is the use of different antitumor agents, extract from the plants. The natural therapies using products form the plants can reduce de collateral effects in the cancer patients. Therefore, in our objective of find new agents against the cancer lines, it was proposed than our objective is to evaluate the cytotoxic activities from plants extracts with uses in the traditional Colombian medicine, like the Moraceae, Euphorbiaceae, Asteraceae and Phyllanthaceae families, opposing the tumor cell lines SIHA (cervix cancer) and A549(lung cancer), contrast with an none carcinogenic cell line, the L929(mouse fibroblast).

Methods: Consequently to accomplish what we have said, using an MIT essay, we prepared by maceration 12 ethanoic extracts from different parts of plants and then made cytotoxic tests opposed to the cell lines, then we calculate the IC_{50} of each extract counter the mentioned cell lines. **Results-Discussion**: The most representative values that were obtained, were from the extract of the *Ficus sp.* species with an IC_{50} of 56,59 ppm for the SIHA and 73,07 ppm L929. This indicates a slight cytotoxic activity from the extract of the *Ficus sp.* aimed to the none carcinogenic cell line, despite of the moderate antitumor seen activity, which justify the bio-guided fractionation in search of the cytotoxic phytoconstituents.

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Universidad de la Sabana. Bioprospection Investigation.

QUANTIFICATION OF PLASMA AMINO ACIDS USING UPLC-MRM MS FOR THE CLINICAL DIAGNOSIS OF METABOLIC DISORDERS

Cases G, Calinski G, Uicich R, Mendez M, Gimenez M.
Laboratorio Central – Hospital Italiano de Buenos Aires, Perón 4190, Ciudad Autónoma de Buenos Aires, C1181ACH Buenos Aires, Argentina.
gabriel.cases@hospitalitaliano.org.ar
Keywords: UPLC, MRM, MS, Amino acids.

The analysis of plasma amino acids profile is a useful tool for detection of metabolic disorders. It is also associated with the study of various pathological conditions and nutritional disorders. Quantification is thus required by a selective and sensitive method that allows the clinical laboratory to obtain reliable results for a better profile to help the metabolomic study.

An analytical assay based on Waters MassTrakTM AA Analysis was generated and optimized for using stable isotope labeled internal standards and mass spectrometry as a detection method. Using UPLC-MS/MS in MRM mode associated with stable isotopes dilution (SID) improves the sensitivity and specificity of simultaneous analysis of multiple amino acids. A mixture of twenty 15N enriched amino acids used as internal standard allows to normalize analytical variables like matrix supression and sample storage, and confirms presence of the endogenous analog. By changes in the mobile phase concentration and chromatographic gradient a shorter running time was achieved maintaining the peak resolution of the original method, and allowing the separation of all analytes (42 amino acids).

MS acquisition was divided into five MRM functions in order that each analyte had more scanning time thus improving the method sensitivity. For key analytes detection to the diagnosis of metabolic defect such as Maple Syrup Urine Disease (MSUD) where the presence of alloisoleucine is considered pathognomonic of disease, the emphasis was placed in a optimal peak resolution.

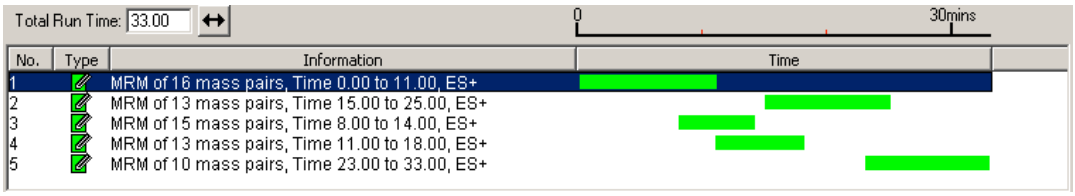

Figure 1: ESI + MRM functions from MS file

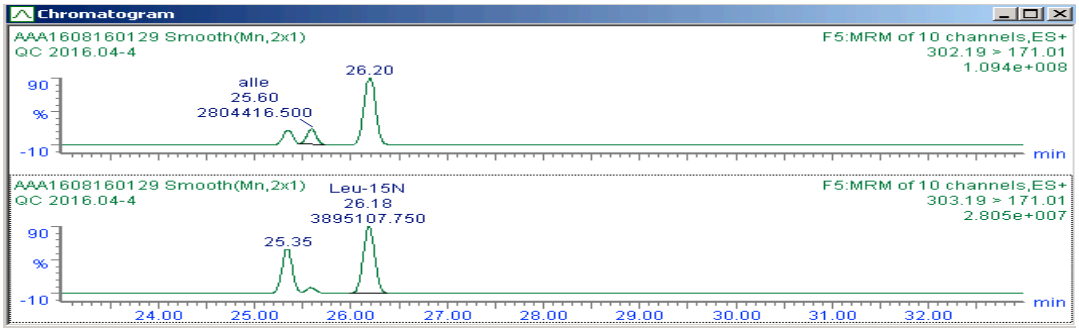

Figure 2: Resolution of alloisoleucine peak

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COMPUTATIONAL CLUSTERING INTEGRATION OF METABOLOMICS, TRANSCRIPTOMICS AND AGRONOMICAL DATA FOR GERMPLASM SELECTION IN A HIGHLY DIVERSE TOMATO LANDRACE COLLECTION

Cernadas RA, Conte M, Pividori M, Insani M, Asís R, D´Angelo M, Zanor M, Boggio S, Valle E, Asprelli P, Peralta I, Milone D, Stegmayer G, Carrari F

Institute of Biotechnology, INTA. Castelar, Argentina

cernadas.andres@inta.gob.ar

keywords: Solanum lycopersicum, Tomato landraces, GC-MS, NMR, HPLC, Metabolites, Computer Clustering

Tomato (*S. lycopersicum*) is a major vegetable crop consumed worldwide that provides a valuable source of vitamins and antioxidants for the human diet. Because of the variability constrains associated with breeding programs, the phenotypic and genetic diversity of heirloom cultivars (landraces) emerges as a landmark to rescue desired agronomic traits for crop improvement. Here, we surveyed a germplasm collection of 68 tomato Andean landraces maintained and cultivated by family farmers. Distinct sets of these accessions were cultivated in the Cuyo region (Mendoza) during several seasons (i.e. 2005-06, 2006-07, 2008-09, 2009-10, 2010-11 and 2011-12) and characterized by morpho-agronomic traits as well as by biochemical characters of the mature fruits. Our analyses undertook a combined approach using, i) GC-MS, NMR and HPLC to identify fruit soluble and volatile metabolites, ii) transcriptomics, and iii) computational biology to integrate the whole dataset. Preliminary results allow to define genotypic clusters according to agronomical traits, including metabolite profiles, antioxidant properties and vitamins accumulation. We also explored organoleptic properties of the different accessions to establish inter-cluster correlations between volatile content and fruit taste. Finally, a multi focus clustering analysis based on accessions diversity and environmental variation along the experimental seasons provides a method to infer the most probable traits to be stable inherited.

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INTA, ANCYPT, CONICET

MONITORING THE TRAINING OF HIGH PERFORMANCE ATHLETES: NMR-BASED METABOLOMIC APPROACH

Costa, C.S.C.1, Fidalgo, T.K.S.2, Pierucci, A.P.T.R.3, Valente, A.P.C.1
[1]*Institute of Medical Biochemistry, CNRMN/ Federal University of Rio de Janeiro*, Rio de Janeiro, Brasil.
[2]*School of Dentistry/ Federal University of Rio de Janeiro,* Rio de Janeiro, Brasil.
[3]*Institute of Nutrition, INJC/ Federal University of Rio de Janeiro,* Rio de Janeiro, Brasil.
e-mail:costa.csd@gmail.com
Keywords: athletes, exercise, serum, metabolomics, nmr

Exercise might not always be healthy in competitive levels, due to dehydration, substrate depletion, muscle damage, inflammation and increased production of damage compounds, like free radicals. During competitive periods athletes have an intense routine of events and training and the goal of athletic training is to make the body adapt to an intensive stimulus, elevate the capacity of the various systems to carry out increased workloads, and, therefore, to enhance the performance. Metabolomics by nuclear magnetic resonance (NMR) is a comprehensive method for metabolite assessment that involves measuring the overall metabolic signature of biological samples. We used this approach to investigate metabolites changes in the serum of athletes over three weeks of training. Six men, modern pentathlon athletes, participated in this study. The athletes were evaluated during three weeks of training. The study protocol was approved by the appropriate Research Ethics Committee and a written consent was obtained. The samples were analyzed by ^{1}H NMR spectroscopy, and multivariate statistical techniques were utilized to process the data. Figure 1 shows representative spectra serum from athletes at baseline and at last day of training.

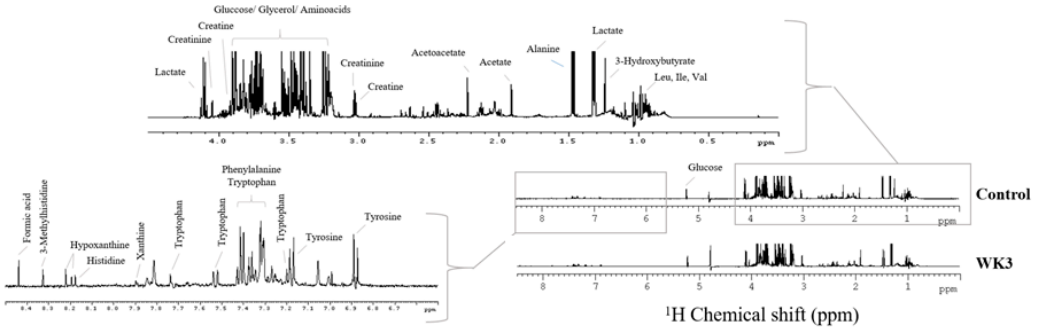

Figure 1 ^{1}H NMR serum representative spectra at baseline (control) and after the last day of training (WK3).

Distinguishing characteristics were observed in the profiles of serum collected before and after exercise (clear separation of groups). The metabolites responsible for such changes were particularly, lactate, valine, leucine, isoleucine, pyruvate, alanine, β-hydroxybutyrate, acetate, glycerol, creatine, citrate, glucose, carnitine and glutamine, metabolites involved in the energy metabolism. In conclusion, the metabolomic assessment by NMR can be used as a research tool to identify a physiological profile after the training period which can assist in recovery and performance strategies.

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FAPERJ, CNPq, INBEB

NMR metabolic profiling for the discovery of new PUTATIVE biomarkers FOR Alzheimer's Disease in a RAT model

Dalmasso M.C., Arán M., Ferrari C.C., Smal C., Morelli L.
Fundación Instituto Leloir – IIBBA - CONICET, C.A.B.A., Argentina.
e-mail:cdalmasso@leloir.org.ar
Keywords: Alzheimer's Disease, early diagnosis, presymptomatic, animal model, McGill-R-Thy1-APP, metabolomics.

Alzheimer's Disease (AD) is the most common form of elderly associated dementia, affecting ~46 million people worldwide. Moreover, most people with dementia have not received a formal diagnosis, and therefore do not have access to treatment, care and organized support. AD starts many years before symptoms appear, defining a presymptomatic stage. A good detection method of this period will provide future patients the opportunity to plan their future, and conduct a preventive behavior to delay the appearance of dementia. Thus our main goal is to perform untargeted NMR metabolomics in an animal model for presymptomatic AD, in order to identify relevant metabolites that could be used as biomarkers for early diagnosis of the disease. To this aim, we use the transgenic hemizygous rat model McGill-R-Thy1-APP (TG+/-). Experiments were performed in male TG+/- and non-transgenic littermate (WT) rats of 9 months of age; conditions that has been well characterized in our laboratory (1, 2). From each animal, CSF and hippocampus were collected to study changes at brain level, and plasma to study changes at systemic level. Samples were processed and analyzed as described by Beckonert O. *et. al.* (3). In contrast to hippocampus and plasma, CSF samples were hard to obtain, often CSF of more than one animal should be pooled to reach detection volume, and they were usually contaminated with plasma, questioning the suitability of CSF samples to our goal. Preliminary results observed by Pincipal Component Analysis (PCA) performed on 1D ^{1}H-NMR results, suggest that metabolic changes occurring in TG+/- rats are more evident in plasma than hippocampus at early stages of the disease (Figure1). Efforts to identify metabolic changes occuring in TG+/- are being carried out.

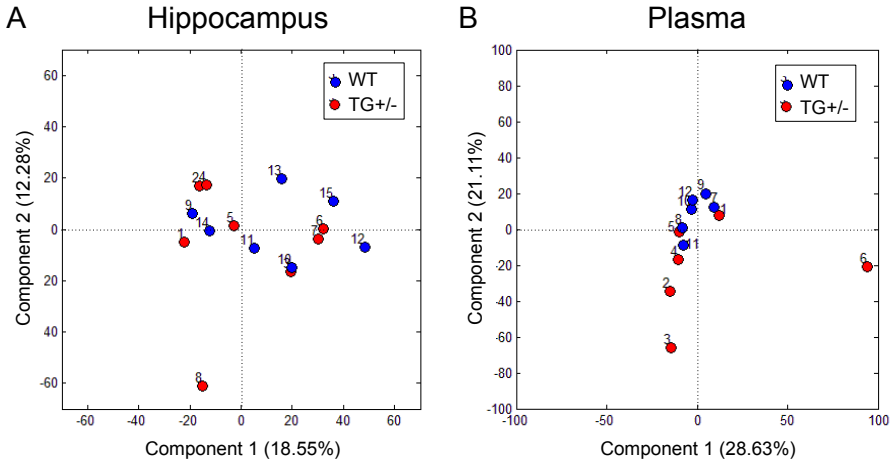

Figure 1: PCA performed on hippocampus (A) and plasma (B) samples.

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CONICET, ANCPYT, ISN

FLAVOR DEVELOPMENT IN *ENTEROCOCCI FAECIUM* GROUP FOR RATIONAL DESIGN OF STRAINS WITH TECHNOLOGICAL IMPACT

D'Angelo M[1], Martino G, Mortera P, Blancato V, Espariz M, Magni C, Alarcón SC.
1IQUIR-CONICET-UNR, Rosario, Argentina.
dangelo@iquir-conicet.gov.ar
keywords: Enterococcus faecium, flavor, amino acid, citrate, cheese.

Enterococci are Lactic Acid Bacteria (LAB) usually found in human and animal gut microbiota and widely distributed in diverse habitats such as dairy and fermented foods. Although they have been associated to nosocomial infections, *Enterococcus* have a desirable technological aspect because their positive contribution to flavor development in madurated cheeses. Citrate, proteins and amino acids (AA) catabolism are the main pathways that influence flavor in cheese.

Here, we analyzed the presence of genes related to amino acids degradation in the genomes of three *E. faecium* strains (GM70, GM75 and IQ110) and one *E. durians* strain (IQ23). These four strains have different citrate properties and diactelyl/acetoin production: IQ23, GM70 and GM75 are a cit^+ strains and produced diacetyl/acetoin when they grown in LB medium supplemented with citrate. IQ110 is a cit^- strain and not significant amount of diacetyl/acetoin production was observed. In this presentation, the volatile organic compounds derived from branched-chain amino acid (BcAAs) degradation by resting cells, were analyzed using solid-phase microextraction (SPME) combined with gas chromatography-mass spectrometry (GC-MS). The volatile profiles obtained were well correlated with the genomic analysis.

This study is part of the first stage of characterization of new isolates for the rational design of strains of technological impact on regional production of cheese.

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PLASMA METABOLIC PROFILE BY NMR FROM PATIENTS WITH PULMONARY TUBERCULOSIS

D'Attilio Luciano[1], Burdisso Paula[2,3], Díaz Ariana[1], Bongiovanni Bettina[1], Santucci Natalia[1], Vila Alejandro[2,3], Rasia Rodolfo[2,3], Bottasso Oscar[1], Bay María L[1].

1 Instituto de Inmunología Clínica y Experimental de Rosario. CONICET-UNR, 2000, Rosario, Argentina. 2 Instituto de Biología Molecular y Celular de Rosario; Área Biofísica, Facultad de Ciencias, Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, 2000, Rosario, Argentina.3 Plataforma de Biología Estructural y Metabolómica (PLABEM), 2000, Rosario, Argentina.

Keywords: TB, metabolomics, NMR.

Tuberculosis (TB) is a major health problem that requires an appropriate cell immune response (IR) to be controlled, however an exacerbated IR could be involved in tissue damage. WHO estimates that one third of the world's population is infected with the bacillus, with less than 10% of these individuals being likely to develop active tuberculosis. Previously we showed that patients with TB show an important immuno-endocrine-metabolic imbalance. This consisted of elevated plasma concentrations of proinflammatory cytokines likes IFNy, IL-6 compared to healthy controls (HCo) correlating with the severity of lung involvement; along with increased levels of cortisol and lowered amounts of dehydroepiandrosterone also associated with disease severity. At the same time patients have a marked decrease of body mass index (BMI) in presence of decreased levels of leptin and increased adiponectin and ghrelin values. Omics sciences have allowed the use of global data analysis to explain biological processes. Among them, metabolomics deals with the study of metabolites, which represent the last product of cellular processes reflecting the identity and the influence of the environment on an organism. NMR allowed the acquisition of data in an effective, reproducible, and high-throughput manner. To achieve a more comprehensive view of the result of all these biological processes, and elucidating distinctive aspects of this disease for the development of diagnostic and prognostic biomarkers, plasma metabolomic profile from TB patients by acquiring NMR spectra were perform. Forty TB patients, with different degrees of pulmonary involvement (Mild, n = 9; Moderate, n = 14 and Severe n = 17), and 31 HCo similar on sex and age, were incorporated. The workflow, including sample preparation, NMR calibration, data acquisition and processing was set up based on the literature. Samples and quality control samples were analyzed, giving high quality ^{1}H-NMR spectra. Multivariate analysis using unsupervised methods as Principal Component Analysis, allowed to get a sample of two main components that managed to distinguish outliers and get an overview of the data matrix showing discrimination between the group of patients with TB and HCo, particularly among those with severe pulmonary TB and HCo. The discriminant analysis was performed using the supervised method (OPLS). From the analysis Loadings plot able to identify several regions discriminating among different groups of samples, including signals corresponding to aromatic amino acids and signals unsaturated lipids were assigned. Using metabolomics through a supervised analysis contributes to discrimination between health and disease in the context of TB. In a disease like TB with a strong metabolic component, this technique would deepen the changes that occur in different metabolic pathways and identify the highest-ranking in relation to the severity of TB.

GRAPE POMACES ANTHOCYANINS AND NON-ANTHOCYANINS PHENOLICS PROFILING: VALORIZATION OF FOOD INDUSTRY BY-PRODUCTS

Fontana AR, D´ Amario Fernández MA, Antoniolli A, Urvieta, R, Bottini R

Laboratorio de Bioquímica Vegetal, Instituto de Biología Agrícola de Mendoza, Consejo Nacional de Investigaciones Científicas y Técnicas-Universidad Nacional de Cuyo, Almirante Brown 500, Chacras de Coria, Argentina.

e-mail:afontana@mendoza-conicet.gob.ar

Keywords: Grape Pomace, Phenolic Compounds, Characterization.

Grape pomace is a rich source of phenolic compounds and represents an important underused residue of the winemaking process. The dry grape by-product consists of pressed skins, seeds and stems and accounts for about 20% of the weight of the grapes used to make wine1. Grape pomace, thus, potentially constitutes a very abundant and relatively inexpensive source of a wide range of phenolic compounds including monomeric and oligomeric flavonols, flavanols, stilbenes and a diversity of anthocyanin glycosides which could be used in pharmaceutical and food industries2. In this way, it is of utmost significance to determine their composition. These data may provide valuable information for the characterization of samples and likewise increase the value of the product.

The main objective of the present study was to determine the profiles and amounts of individual phenolic compounds of grape pomaces coming from different grape varieties cultivated in Mendoza, Argentina (vis. Malbec, Cabernet Sauvingnon, Cabernet Franc, and Merlot). A total of 13 anthocyanins and 18 non anthocyanin compounds of different chemical classes (phenolic acids, flavanols, flavonols, stilbenes and phenylethanol analogs) were quantified. Different chemical profiles for studied grape pomaces were observed. The maximum concentrations of non-anthocyanins corresponded to the flavanols (+)-catechin (5340 μg g-1), (-)-epicatechin (3966 μg g-1), procyanidin B1 (3507 μg g-1) and syringic acid (6664 μg g-1) whereas malvidin-3-glucoside was the most abundant anthocyanin. Malbec and Cabernet Sauvignon presented the highest content of studied non-anthocyanins phenolic compounds with concentrations ranging between 2806 to 14565 μg g-1. Thus, these data may contribute to the selection of suitable plant materials for the extraction of phytochemicals as ingredients of functional foods or other biotechnological applications, as a strategy for sustainable oenology. Knowing the profiles of grape pomaces will give a valuable database for choosing the most appropriate grape variety depending on the phenolic compounds required for a particular use.

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MOLECULAR SIMILARITY PROFILES: A PATENTED APPROACH TO FINGERPRINT NATURAL SOURCE MOLECULES

Freire R. T.1, Selegato D. M.2, Pilon A. C.2, Castro-Gamboa I.2 and Tannús A.1
[1]*Centro de Imagens e Espectroscopia in vivo por Ressoância Magnética, CIERMag, Instituto de Física, Universidade de São Paulo (USP), São Carlos, SP, Brazil;*
[2]*Núcleo de Bioensaios, Biossíntese e Ecofisiologia de Produtos Naturais, NuBBE, Instituto de Química, Universidade Estadual Paulista (UNESP), Araraquara, SP, Brazil;*
e-mail:rafaeltfreire@gmail.com
Keywords: Fingerprint technique, Molecule Profile, Natural Compound, Statistical Methods

Natural products (NP) are compounds derived from natural sources such as plants, animal and microorganisms. It is an important source of diverse molecules in which many have a high pharmacological potential, as well for new drugs designs. In the reductionist view, in which classical phytochemistry studies are included, one of the most exhaustive and difficult steps is the structural elucidation and the identification of NP compounds. There are many techniques to help with rapid characterization of known compounds (dereplication), but the lack of organized data is one of the major drawbacks in NP and medicinal chemistry research [1]. Based on this bottleneck, the aim of this work was to create a new fingerprint technique to be used as an input to the conventional statistical methods such as PCA, HCA and network analysis, to automatically identify the molecular class of an unknown compound. This patented pattern recognition and dereplication [2] methodology was initially applied to ^{1}H, ^{13}C and ^{13}C-HSQC NMR spectroscopic data. To create the molecular similarity profile requires NMR spectra of the NP mixture or a pure compound along with NMR spectra of known standards. In order to do that, we compile two online free NMR database, HMDB and MMCD, data from 15 years of the NMR studies from NuBBE's research group and NMR prediction data of all NuBBE Database compounds, into a final database with more than 5000 compounds. With the application of this new fingerprint technique followed by statistical analysis, it became easier to identify the molecular class of molecules even if they are not present in the database.

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FEASIBILITY OF DETECTING CLEAR CELL RENAL CELL CARCINOMA BY ULTRAPERFORMANCE LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY HUMAN SERUM LIPIDOMICS

Knott ME[1], Puricelli LI[2], Monge ME[1]

1Centro de Investigaciones en Bionanociencias (CIBION), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Ciudad de Buenos Aries, Argentina. 2Instituto de Oncología Ángel H. Roffo, Ciudad de Buenos Aires, Argentina.

e-mail: elena.knott@cibion.conicet.gov.ar

Keywords: metabolomics, lipidomics, ultraperformance liquid chromatography, high resolution mass spectrometry, clear cell renal cell carcinoma, biomarkers.

Renal Cell Carcinoma (RCC) is among the 10 most common cancers in both men and women and accounts for nearly 300,000 cases worldwide. The lifetime risk for developing kidney cancer is about 1 in 63. RCC consists of several histological subtypes with distinct molecular alterations and clinical outcomes. Clear cell renal cell carcinoma (ccRCC), characterized by high lipid and glycogen reserve, is the most common (75%) lethal subtype of kidney cancer.[1] Current research has shown that several metabolic alterations are associated with RCC, such as phospholipid catabolism, sphingolipid, cholesterol, phenylalanine, tryptophan, and arachidonic acid metabolism, and fatty acid beta-oxidation.[2] As well, tumor progression and metastasis have been associated with metabolite increases in glutathione and cysteine/methionine metabolism.[3] However, metabolic alterations induced by ccRCC are still not well understood, and more studies are needed to find robust biomarkers for early diagnosis.

In this pilot study, untargeted lipidomic profiling of age-matched serum samples from 5 patients with advanced ccRCC (stage IV) and 5 healthy individuals was performed using ultra performance liquid chromatography coupled to quadrupole-time-of-flight mass spectrometry. High resolution mass spectra were acquired in both positive and negative ionization modes across the range of *m/z* 50-1200. Metabolites were extracted from the serum samples using isopropanol. Metabolic features (retention time, *m/z* pairs) were obtained via Progenesis QI software, and analyzed using a cross-validated orthogonal projection to latent structures-discriminant analysis (OPLS-DA) model. This supervised classification model classified samples as advanced ccRCC or healthy individuals with high accuracy (R^2Y=0.9985 and Q^2=0.9956). Discriminant metabolic features of this pilot study suggest alterations of glycerophospholipids and cholesterol metabolism in agreement with previous studies. Our current work involves the retrospective analysis of a larger sample cohort (n=240) that includes samples from ccRCC patients with stages I, II, III and IV and healthy individuals, to shed light into the altered metabolic pathways involved in tumor progression and to discover biomarkers useful for early diagnosis, prognosis, and follow-up care.

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SODIUM SALT-SPECIFIC DIFERENTIAL METABOLOME IN THE NATIVE HALOPHYTE PROSOPIS STROMBULIFERA

Llanes A. 1, Arbona V.2, Goméz-Cadenas A.2, Luna V.1

1 Laboratorio de Fisiología Vegetal, Departamento de Ciencias Naturales, Universidad Nacional de Río Cuarto, Route 36 Km. 601, (CP X5804BYA) Río Cuarto-Córdoba, Argentina. 2 Ecofisiologia i Biotecnologia. Departament de Ciencies Agraries i del Medi Natural, Universitat Jaume I, (E-12071) Castelloʹ de la Plana, Spain.

Email: allanes@exa.unrc.edu.ar

Keywords: NaCl, Na2SO4, halophyte, metabolome profiling.

Metabolome analyses enable a clear understanding of alterations in plant responses to salinity. Prosopis strombulifera (Lam) Benth is a shrub abundant in the salinized areas of central Argentina. This species show a halophytic response to NaCl and a strong growth inhibition at relatively low Na2SO4 concentrations. These differential responses to the most abundant salts present in salinized soils make this species an excellent model to study salt tolerance mechanisms. Metabolome changes in plants of P. strombulifera grown hydroponically in iso-osmotic solutions of NaCl, Na2SO4 and their combination at medium Ψo of -1, -1.8 and -2.6 MPa, were studied by means of GC/MS and UPLC/ESI-QqTOF-MS metabolite profiling. Principal Component Analysis of results indicated differences in metabolite profiles associated to organ and Ψo. Results revealed a rapid induction of numerous metabolites in response to sodium salt stress. These results were correlated with modifications in growth parameters. A total of 108 significantly altered compounds including 18 amino acids, 19 secondary metabolites, 23 carbohydrates, 13 organic acids, 4 indole acids, among others. These primary metabolites analyzed by GC/MS showed a differential response under the salt treatments, which was dependent on salt type and concentration, organ and age of plants. The intensity of each compound varied depending on the type of salt, salt concentration, organ analyzed and age of plants. Indeed, at the highest salt concentration in the medium (Ψo: -2.6 MPa) the highest intensity in most of the identified compounds was observed in Na2SO4-treated plants and this response was correlated with the damaging effect of sulfate anion on plant growth. In general, roots showed stronger variations in secondary metabolites (analyzed by UPLC/ESI-QqTOF-MS) than leaves. Moreover, a low degree of overlapping between metabolites altered in roots and leaves of NaCl and Na2SO4-treated plants was found. Roots of NaCl-treated plants showed a higher number of altered mass chromatographic features compared to other treatments, while leaves of Na2SO4-treated plants showed the highest number of altered signals. However, when both NaCl and Na2SO4 salts were present in the medium, plants always showed a low number of altered mass chromatographic features. Three compounds were tentatively identified: tryptophan, lysophosphatidylcoline and 13-hydroxyoctadecadienoic acid. The present results contribute to better understanding of physiological responses of woody halophytic plants to soil salinity (NaCl + Na2SO4) and provide the basis for future molecular studies for generating salt-tolerant, economically important species for salinised soils throughout the world.

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METABOLIC REPROGRAMMING DURING LEAF SENESCENCE IS AFFECTED BY CHLOROPLAST REDOX STATE

Mayta ML1, Hajirezaei, MR2, Lodeyro, A1, Carrillo, N1

[1]*Institute of Molecular and Cellular Biology of Rosario (IBR-CONICET), Rosario, Argentina. e-mail:mayta@ibr-conicet.gov.ar*

[2]Leibniz Institute for Plant Genetics and Crop Research (IPK), Gatersleben, Germany.

Keywords: Senescence, Chloroplast, Reactive oxygen species, Flavodoxin, Metabolite profilling

Senescence is a genetically programmed sequence of biochemical and physiological changes that comprise chloroplast dismantling and degradation of macromolecules such as proteins, nucleic acids and lipids (1) in order to mobilize nutrients from senescing leaves to other parts of the plant, such as younger leaves, developing seeds or storage tissues. Hence, it is vital for plant survival and reproduction. The primary physiological purpose is the recycling of N-containing compounds (e.g. amino acids) and sugars, which mainly derive from chloroplasts (2). Therefore, senescence involves substantial metabolic reprogramming, and metabolite profiling analysis has been used to characterize plants with delayed senescence (3). Transgenic tobacco plants expressing the electron carrier protein flavodoxin (Fld) from Anabaena in their chloroplasts (pfld plants) display a "stay-green" phenotype in leaves and lower reactive oxygen species accumulation in the organelle during leaf senescence. Under environmental stress conditions, Fld has been shown to preserve central metabolic pathways presumably by acting as an electron donor for critical reactions in the chloroplast (4, 5). To evaluate if a similar role is played during senescence we determined the presence and quantity of several amino acids, soluble sugars, starch and phosphorylated metabolites involved in primary processes in young and senescent leaves from wild-type and transgenic lines. Fld expression in chlororplasts was able to delay the inhibition of central metabolic routes in senescent leaves, as reflected by higher levels of key aminoacids and phosphorylated sugars. Sucrose levels were also higher and starch and monosaccharides were lower in pfld plants. Additionally, several forms of citokinins (CKs) and auxins (two important senescence-related phytohormones) were measured. No differences were observed for auxins in old leaves of all lines. Instead, transgenic pfld lines displayed higher levels of a major active CKs form and lower levels of a major CKs reserve form. Taken together, the results indicate that signals responding to chloroplast redox state modulate the progress of metabolic changes during leaf senescence.

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TOWARDS AN UNDERSTANDING OF CONSUMER PREFERENCE FOR MANDARIN VARIETIES USING A METABOLOMIC APPROACH

Migues I[1,2], López A[2], Hodos N[3], Lado J[4], Rodríguez C[1], Moyna G[2], Gambaro A[3], Rivas F[4], and Heinzen H[1,2].

[1]Farmacognosia y Productos Naturales, Facultad de Química, *Universidad de la República*, Montevideo, Uruguay.

[2]*Departamento de Química del Litoral, CENUR Litoral Norte, Universidad de la República, Paysandú, Uruguay.*

[3]*Sección Evaluación Sensorial, Ciencia y Tecnología de Alimentos, Facultad de Química, Universidad de la República, Montevideo, Uruguay.*

[4]*Instituto Nacional de Investigación Agropecuaria – Salto Grande, Salto, Uruguay.*

e-mail: imigues@fq.edu.uy

Keywords: [1]H NMR; Citrus reticulata; Consumers' acceptability; Metabolomics.

The biosynthetic origin of the compounds responsible for the organoleptic and nutraceutical properties of mandarins is diverse. While the genotypes of different mandarin varieties can be established unequivocally, the mechanism by which these genotypes are expressed to yield the different varieties has not been fully elucidated. Nevertheless, their metabolome, due to the phenotypic expression, allows mandarin differentiation and can be used to characterize them unequivocally. In an effort to correlate phytochemical markers and quality of different mandarin varieties with consumers' acceptability, we conducted a study of four mandarin germoplasm sources and some cultivars derived from them using NMR-based metabolomic methods.

The correlation between ^{1}H NMR metabolic profiles [1], general physicochemical properties (pH, color, and texture), and consumers' surveys of mandarin acceptability criteria was studied using multivariate analysis. A principal component analysis (PCA) of the ^{1}H NMR fingerprints obtained for each organ was sufficient not only to differentiate between varieties, but also to follow the transfer of phenotype traits from parental to hybrid varieties. These preliminary results demonstrate, in principle, that the non-targeted analysis of the ^{1}H NMR metabolic profile of mandarin extracts can be correlated with acceptability among consumers.

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EFFECT OF LIGHT ON GROWTH AND METABOLISM IN *AZOSPIRILLUM BRASILENSE* AZ39.

Molina R.[1]; Llanes A.[1]and Cassán Fabricio[1].
[1]*Universidad Nacional de Río Cuarto. Río Cuarto. Córdoba. Argentina. *Contact: rmolina@exa.unrc.edu.ar*
Keywords: Azospirillum, metabolome, photoreceptors.

In the 80s, an intense program of INTA was performed to select strains of *Azospirillum* sp. based on their ability to promote the growth of certain grasses of economic interest, such as corn and wheat. This program allowed to select *A. brasilense Az39* strain as the best in agronomic performance and for more than four decades has been used and recommended for inoculant formulation of Argentina. Recently, we have sequenced and analyzed the Az39 genome and we have confirmed these and other mechanisms related to plant growth promotion and rhizosphere life adaptation. Light is an essential signal that controls growth, development and behavior of many organisms. The recent discovery of bacterial photoreceptors has led researchers to an active seek of its role in bacterial metabolism. Given this, the objective of this work was to determine the effect of different lengths of light on the growth and metabolism of *A. brasilense* Az39. Pure growths of A. *brasilense* Az39 with a DO_{595} approx. 0.1 (early exponential phase) was fractionated into sterile Petri dishes (20 ml) and incubated in a culture chamber at 37 °C under different lengths of light, blue PAR38, red PAR38, white 75 w, and control under conditions of darkness. After 24, 48 and 72 of exposure from each treatment the growth (DO_{595} and ufc.ml^{-1}) and quantification of indole-3-acetic acid (IAA), as a marker of secondary metabolism, was determined. The complete metabolic profile (metabolomics) was evaluated by RPLC-QqTOF-MS. Our results indicate that exposure to 3 light sources, white 75 w, blue PAR38 and red PAR38 determined a differential behavior in *A. brasilense* Az39. Exposure light blue and white to a significant increase of biomass production at 24 hours compared to the control treatment in darkness. The white light was the only treatment that maintained a tendency of continuos and significant increase after 48 and 72 h incubation comparable to the control in the dark, however this is not evident in the number of viable cells (ufc.ml-1) and only could be a process of accumulation of biomass. The concentration of secondary metabolite AIA in the culture medium was greater than the value in the dark after exposure for 24 h to white light. In the presence of white light it was observed a significant increase in the synthesis of AIA at 24 and 48 h and then it was held for 72 h exposure. In the presence of blue light, a decrease was found in the concentration of the hormone. As for each light source used, light blue and especially white, generated greater development of biomass, with respect to red light and treatment in the dark; however, no significant differences were observed in the number of viable cells in the presence of any type of light over time. With these results, we propose that this condition could alter its ability to survive and interact with plant species in inoculant formulations intended for agricultural use.

FEASIBILITY OF EARLY DETECTION OF ACUTE PULMONARY EXACERBATIONS BY EXHALED BREATH CONDENSATE METABOLOMICS

Monge ME,[1] Zang X,[2] McCarty NA,[3] Stecenko A,[3] and Fernández FM[2]

[1]Centro de Investigaciones en Bionanociencias (CIBION), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Ciudad de Buenos Aires, Argentina. [2]School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, USA, [3]Emory+Children's Center for Cystic Fibrosis and Airways Disease Research and Department of Pediatrics, Emory University School of Medicine and Children's Healthcare of Atlanta, Atlanta, USA.

e-mail: maria.monge@cibion.conicet.gov.ar

Keywords: cystic fibrosis, acute pulmonary exacerbations, untargeted metabolomics, ultraperformance liquid chromatography, mass spectrometry

Progressive lung function decline and ultimately respiratory failure is the most common cause of death in cystic fibrosis (CF).[1] This decline is punctuated by acute pulmonary exacerbations (APEs) and, in many cases there is failure to return to baseline lung function. In this study, we utilize a discovery-based metabolomics approach to analyze exhaled breath condensate (EBC) samples from 17 clinically stable CF patients, 9 CF patients with an APE, 5 CF patients during recovery from an APE event (termed post-APE), and 4 CF patients who were clinically stable at the time of collection but in the subsequent 1 to 3 months developed a severe APE (termed pre-APE), using ultraperformance liquid chromatography-quadrupole-time-of-flight mass spectrometry in combination with supervised multivariate classification models. A panel containing 2 metabolic discriminant features identified as 4-hydroxycyclohexylcarboxylic acid and pyroglutamic acid differentiated the APE from the stable CF samples with 84.6% accuracy. Pre-APE EBC samples were distinguished from stable CF EBC samples by lactic acid and pyroglutamic acid with 90.5% accuracy, and in general matched the "APE signature" when projected into the APE vs. stable CF model. Post-APE samples were on average more similar to stable CF samples in terms of their metabolomic signature. These results show feasibility for detecting APEs, and even predicting an oncoming APE, or monitoring APE treatment using EBC metabolites.

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OPTIMIZED TECHNIQUE APPLIED TO THE DETERMINATION OF AMINO ACID CONTENT IN FOOD AND BIOLOGICAL HUMAN FLUIDS

Müller GL[1], Alvarez CE, Wiggenhauser J, Andreo CS, Lara MV, Drincovich MF
Cefobi, Rosario, Argentina.
muller@cefobi-conicet.gov.ar
Keywords: amino acid, Inborn error of metabolism, food amino acid content.

Recent studies have indicated that amino acid analysis is a fundamental analytical technique, which can be used for the determination of amino acid composition in the content of proteins, peptides and other pharmaceutical or biological sample fractions.

Specifically, since proteins and various amino acids are needed in the human diet to promote protein synthesis and amino acid circulation, amino acid analysis can be used to monitor or detect the metabolic states by analyzing the content of free amino acids in biological fluids such as urine, blood or plasma. Also, the determination of amino acid content is an essential information provided in the label of food products.

In this research we optimized the use of a reverse Phase C18 HPLC of derivatized amino acids which provides highly reproducible quantitative analysis of samples for the estimation of amino acid composition in specific samples such as human blood or tomato samples. Such technique uses internal standards of known amount to gain reliable quantitative results, enabling the accurate quantification of each amino acid. Most importantly, this technique is dedicated to drive the variety of amino acid analysis methods with more effective applications. As an integral part of analytical biochemistry, amino acid analysis is continuously showing its functions on scientific studies.

METABOLOMIC STRATEGIES FOR STUDIES OF THE EFFECT OF POLIFENOLS IN URUGUAYAN DIET

Panzl V.[1], Rufo C.[2], Rodriguez-Haralambides A.[1],
[1]*Quimica Bioanalítica, Instituto Polo tecnológico de Pando, Facultad de Química, Universidad de la República, Montevideo, Uruguay.* victoriapanzl@fq.edu.uy
Keywords: polifenols, metabolomics, Ilex paraguariensis.

Dietary sources of polyphenolic compounds have become important because of their potential health benefits. Data from the First National Survey of Chronic Diseases (ECNT, 2006)[1] clearly shows that the Uruguayan average diet is low in fruits and vegetables. On the other hand, consumption of water infusions based on "yerba mate" *(Ilex paraguariensis)* is very high. According to the aforementioned survey, 85% of the Uruguayan population consume infusions of "yerba mate" at least once a week. Although there are several studies on various aspects of "yerba mate", controversy persists over its potential benefits and risks to health. It is of great interest to characterize this food from the functional point of view [2], and advance in the studies to determine the effects on human health. The research strategy involves the use of metabolomic tools, both to determine chemical profiles infusions of "yerba mate", and to study some of their possible biological effects. In this research proposal, metabolomic strategies will be implemented in *in vitro* models (HepG2 cells), and in human subjects [3, 4]. The working hypothesis for the study in liver cells establishes that there is a cytotoxic effect of "yerba" infusions in HepG2 cells[5], and that is possible to identify the components that are metabolized. The study in humans will allow us to establish biomarkers for yerba mate polyphenols, in order to study their bioavailability and metabolism. This objective would be achieved through the identification and relative quantification of the excreted metabolites.

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PEDECIBA, CSIC, PROYECTO ENLACES-EU-URY-2003-5906 Y PROYECTO INNOVA-EU-URY II.

DEREPLICATION OF NATURAL PRODUCTS USING GC-TOF MASS SPECTROMETRY: IMPROVED METABOLITE IDENTIFICATION BY SPECTRAL DECONVOLUTION RATIO ANALYSIS

Pilon A.C.1, Carnevale Neto F.2, Selegato D.M.1, Freire R.T.3, Gu H.4,5, Raftery D.4,6, Lopes N.P.2, Castro-Gamboa I.1

1Núcleo de Bioensaios, Biossíntese e Ecofisiologia de Produtos Naturais (NuBBE), Universidade Estadual Paulista UNESP, Araraquara, Brazil. 2Núcleo de Pesquisas em Produtos Naturais e Sintéticos (NPPNS), Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, Brazil. 5Centro de Imagens e Espectroscopia in vivo por Ressonância Magnética (CIERMag), Instituto de Física de São Carlos, Universidade de São Paulo, São Carlos, Brazil. 4Northwest Metabolomics Research Center, University of Washington, Seattle, USA. 5Jiangxi Key Laboratory for Mass Spectrometry and Instrumentation, East China Institute of Technology, Nanchang, China. 6Public Health Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, USA.

e-mail:pilonac@gmail.com

Keywords: GC-MS, plant metabolomics, compound identification, peak deconvolution, ratio analysis, Databases.

Dereplication based on hyphenated techniques has been extensively applied in plant metabolomics, avoiding re-isolation of known natural products. However, due to the complex nature of biological samples and their large concentration range, dereplication requires the use of chemometric tools to comprehensively extract information from the acquired data. In this work we developed a reliable GC-MS-based method for the identification of non-targeted plant metabolites by combining the Ratio Analysis of Mass Spectrometry deconvolution tool (RAMSY) with Automated Mass Spectral Deconvolution and Identification System software (AMDIS). Plants species from Solanaceae, Chrysobalanaceae and Euphorbiaceae were selected as model systems due to their molecular diversity, ethnopharmacological potential and economical value. The samples were analyzed by GC-MS after methoximation and silylation reactions. Dereplication initiated with the use of a factorial design of experiments to determine the best AMDIS configuration for each sample, considering linear retention indices and mass spectral data. A heuristic factor (CDF, compound detection factor) was developed and applied to the AMDIS results in order to decrease the false-positive rates. Despite the enhancement in deconvolution and peak identification, the empirical AMDIS method was not able to fully deconvolute all GC-peaks, leading to low MF values and/or missing metabolites. RAMSY was applied as a complementary deconvolution method to AMDIS to peaks exhibiting substantial overlap, resulting in recovery of low-intensity co-eluted ions. The results from this combination of optimized AMDIS with RAMSY attested to the ability of this approach as an improved dereplication method for complex biological samples such as plant extracts.

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TARGETED METABOLOMIC STUDY TO DISCRIMINATE ARGENTINEAN WHEAT VARIETIES

Podio NS, Baroni MV and Wunderlin DA
ICYTAC-Facultad de Ciencias Químicas/CONICET-UNC, Córdoba, Argentina.
e-mail:npodio@fcq.unc.edu.ar
Keywords: wheat, secondary metabolites, polyphenol profile, statistical.

Targeted metabolomics are important for assessing the behavior of a specific group of compounds in the sample under determined conditions. In this context, the study of polyphenols has gained much attention because they have the ability to prevent oxidative stress. The aim of this study was to evaluate the polyphenol profile of 12 Argentinean wheat varieties (ACA 303, ACA 315, ACA 320, ACA 903 B, BAGUETTE PREMIUM 11, BIOINTA 3004, BUCK 75 ANIVERSARIO, CRONOX, KLEIN CAPRICORNIO, KLEIN GUERRERO KLEIN YARARÁ and LE 2330) from different regions in 2 seeding years. Polyphenol extraction was performed in two fractions (free fraction-FF and bound fraction-BF) and the polyphenol profile was studied by HPLC-DAD-ESI-QTOF. Twenty-five compounds were identified in wheat extracts: 11 of them were identified in the FF, while 14 compounds were identified in the BF. Three hydroxybenzoic acid derivative compounds, tryptophan (included because it contributes to the total polyphenol values), 3 flavones, *p*-coumaroyl-feruloylputrescine, *trans*-ferulic acid and 3 dimers of ferulic acid (isomers 3, 4 and 6) were found in FF of wheat extracts. The most abundant compound in this fraction was hydroxybenzoic acid glucoside and BUCK 75 ANIVERSARIO variety showed significantly highest values ($P < 0.05$) of this compound, while the other varieties did not show significant differences between them. With respect to the BF of wheat extracts, *p*-coumaric acid; *trans*-ferulic acid; *cis*-ferulic acid and 8 dimers and 1 trimer of ferulic acid (DFAs and TFA, respectively) were identified. The main compound quantificated in BF was *trans*-ferulic acid, followed by isomer 9 of DFA. Both compounds showed significant differences ($P < 0.05$) between wheat varieties. To assess whether the profile of polyphenols is able to differentiate between varieties of wheat, principal component analysis (PCA) was applied taking into account the 25 compounds identified. PCA model was obtained using four principal components (PCs), which explained 84% of the variability found in the analyzed data. PC 1 explained 49.4% of the variability found among different wheat varieties studied, while CP 2 explained 14.5% of it. DFAs from BF were the largest contributors to the CP1 allowing differentiate both ACA 315 and KLEIN CAPRICORNIO varieties from both LE 2330 and BIOINTA 3004 varieties. Furthermore, 2-hydroxy-3-O-β-D-glucopyranosylbenzoic acid, followed by compounds ferulic acid derivative, *p*-coumaroyl-feruloylputrescine, hydroxybenzoic acid glucoside, 6-C-glucosyl-8-C-arabinosyl-apigenin, *trans*-ferulic acid, *cis*-ferulic acid and chrysoeriol-6,8-di-C-pentoside explained the variability found in the PC 2, which allowed separate BAGUETTE PREMIUM 11 variety from CRONOX. This analysis shows that the polyphenol profile depends of the wheat variety studied, being the ferulic acid derivatives (DFAs, *cis* and *trans*-ferulic acids), hydroxybenzoic acids (2-hydroxy-3-O-β-D-glucopyranosylbenzoic acid and hydroxybenzoic acid glucoside) and flavones (chrysoeriol-6,8-di-C-pentoside and 6-C-glucosyl-8-C-arabinosyl-apigenin) the most important compounds for their differentiation.

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CONICET, SECYT and FONCYT.

ASSOCIATION BETWEEN PLASMA LACTATE LEVELS WITH INSULIN SECRETION/SENSITIVITY INDEXES DERIVED FROM ORAL AND INTRAVENOUS GLUCOSE TOLERANCE TESTS IN CHILEAN NORMOGLYCEMIC WOMEN

Santos JL (1), Cataldo LR (1), Bravo C (1), Díaz-Casanova L (1), Martinez JA (2), Milagro F (2), Galgani J (1)

1. Departamento de Nutrición y Diabetes, Facultad de Medicina, Pontificia Universidad Católica de Chile, Santiago (Chile), 2. Centro de Investigación en Nutrición, Universidad de Navarra (Pamplona, España).

Keywords: lactate, insulin secretion, insulin sensitivity.

Introduction: Metabolomic studies have identified plasma lactate as an important circulating molecule associated with cardiometabolic traits and type 2 diabetes mellitus. It has been reported an increased lactatemia after an oral glucose load, in a magnitude that may be exacerbated in subjects with insulin resistance. In this context, the increased plasma lactate levels are possibly linked to reduced mitochondrial respiratory capacity in insulin-sensitive tissues followed by enhanced derivation to anaerobic glycolysis. Since mitochondrial function has been related both to insulin secretion and sensitivity, the aim of this study was to evaluate the association between blood lactate and insulin indexes after an oral or intravenous glucose administration.

Subjects and Methods: 65 Chilean normoglycemic women with no family history of diabetes (age: 26.9 ± 6.3 years; BMI: 23.6 ± 3.2 kg / m2) were studied. Standard 75-grams Oral Glucose Tolerance Test (OGTT) was carried out to calculate the Matsuda-ISICOMP index as a measure of insulin sensitivity, and the ratio of AUC-Insulin/AUC-Glucose at 30 minutes (AUCRIG-30; and its equivalent using c-peptide) as indexes of insulin secretion. In addition, an intravenous glucose load of 0.3 g/kg were administered in the Abbreviated Minimal Model (AMM) to calculate the index of insulin sensitivity CSi and the Acute Insulin Release index (AIR). Disposition Indexes (IDs) were calculated as the product of secretion x insulin sensitivity measurements. We measured basal plasma lactate levels, the area under the curve for lactatemia during OGTT (AUCL-OGTT),the change of plasma lactate during AMM and Non-Esterified Fatty Acids (NEFAs) before-after OGTT. On the other hand, fasting plasma levels of leptin, adiponectin and high-molecular-weight leptin were also measured.

Results: AUCL-OGTT showed a significant inverse correlation with the Matsuda-ISICOMP index (rho = -0.60; p <0.0001) and the HOMA-S index (rho = -0.41; p = 0.0007). The change of lactate during MMA showed also a negative slope in their relation with CSi, without reaching statistical significance (rho = -0.22; p = 0.11). A significant positive association was found between AUCL during OGTT with surrogates of insulin secretion such as the AUCRIG-30 (rho = 0.49; p<0.0001) or its equivalent using peptide-c (rho = -0.29; p = 0.02), reflecting the inverse known hyperbolic relationship between secretion and insulin sensitivity. In this context, a significant inverse association was found between AUCL-OGTT with the ID defined as AUCRGI-30 x Matsuda-ISICOMP; rho = -0.28; p = 0.02) or its equivalent using the peptide-c (rho = -0.43; p = 0.0006). AUCL also significantly correlated with the leptin/adiponectin ratio (rho = 0.28; p = 0.03). On the other hand, no significant association was found between AUCL with NEFAs change during OGTT (rho = -0.02; p=0.86).

Conclusions: The area under the curve of plasma lactate during OGTT shows an inverse significant association with different common indexes of insulin sensitivity (Matsuda-ISICOMP, HOMA-S, leptin/adiponectin ratio) as well as with the glucose disposition index, without being associated with changes in plasma NEFAs. These results support the use of plasma lactate as a biomarker of insulin resistance and future diabetes risk.

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EVALUATION BY LC/MS AND PRINCIPAL COMPONENT ANALYSIS OF FUNGAL CULTURES WITH HISTONE DEACETYLASE INHIBITORS AS EPIGENETIC MODIFIERS

Siless G.E.[a], Rodriguez M.A.[b], Godeas A.M.[b], Cabrera G.M.[a]
[a] *UMYMFOR, DQO, CONICET, FCEyN, UBA,* Buenos Aires, Argentina.
[b] *PRHIDEB, DB, CONICET, FCEyN, UBA,* Buenos Aires, Argentina.
e-mail: gsiless@qo.fcen.uba.ar
Keywords: LC/MS, Fungal Natural Products, PCA.

Mass spectrometry (MS) has become one of the most valuable tools on natural products research, allowing complex mixture analysis by hyphenation with chromatographic techniques such as liquid chromatography (LC/MS)[1]. The study of fungal metabolome is still a challenging task, these organisms often yield compounds of unprecedented structures, which requires intensive structure elucidation by MS and NMR. However, as metabolite production of fungal cultures are dependent on environment conditions, different culture methods[2] and dereplication[3] techniques has been developed.

The use of suberoylanilide hydroxamic acid (SAHA) and other histone deacetilase inhibitors as epigenetic modifiers was evaluated in order to observe changes in the metabolite production of the dark septate fungal endophyte *Drechslera sp.*, isolated from the roots of rye grass (*Lollium sp.*). LC/MS and LC/MS/MS were employed for the analysis of the cultures. Unsupervised and supervised principal component analysis of LC/MS data were performed using two different software packages, *Bruker Profile Analysis 2.0* and *MZmine 2.21,* which differ mainly on data treatment prior to PCA itself. Results on both platforms were compared.

Several differences in the metabolite production were detected. The release of hexosyl phytosphyngosine(HPS) to the culture medium was observed using SAHA as an additive of the cultures, **figure 1**. The biotransformation of the inhibitors was observed in addition to the production of antifungal metabolites, showing the ability of this endophytic strain to control xenobiotics.

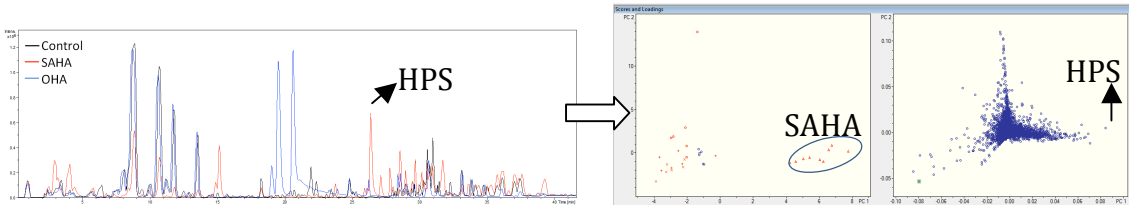

Figure 1: LC-MS of medium extracts of *Drechslera sp.* cultures using different HDACIs, and unsupervised PCA analysis performed on *Bruker Profile Analysis 2.0.*

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Universidad de Buenos Aires, CONICET, ANPCyT.

CONTRIBUTION OF MEMBRANE LIPIDS' UNSATURATION TO ACQUISITION OF CHILLING-TOLERANCE IN TOMATO FRUITS

Sossi ML[1], Valle EM[1], Boggio SB[1]

[1]*Institute of Molecular and Cellular Biology of Rosario (CONICET-UNR)*, Ocampo 210 bis, 2000 Rosario, Argentina.

e-mail:sossi@ibr-conicet.gov.ar

Keywords: Unsaturated lipids, chilling injury, tomato.

Chilling injury is a physiological disturbance that causes significant losses of fruits and vegetables during the postharvest refrigerated storage. Although this practice is commonly used to extend the tomato fruit commercialization period [1], it could produce the undesirable appearance of chilling symptoms, depending on the tomato variety. Tomato cv. "Micro-Tom" fruit is tolerant to chilling in contrast with tomato cv. "Minitomato" fruit [2]. To understand the mechanisms of generation of chilling injury and its influence on the tomato fruit quality, it was analyzed the effect of low-temperature storage on the lipidome of tomato fruit from the two contrasting varieties.

Fruits were harvested at the mature green stage, refrigerated for four weeks at 4ºC and then transferred during *n* days to a shelf in the growing cabinet. The pericarp tissue was processed for lipid extraction and the lipid composition was analyzed by gas chromatography-mass spectrometry (GC-MS).

The tolerance of Micro-Tom fruit to postharvest chilling injury was associated with an increase of lipids containing linolenic acid after cold storage concomitantly with a decrease in lipids containing saturated, mono- and diunsaturated acids. Unsaturation degree of fruit lipids returned to its initial values once Micro-Tom fruits were returned from growing-temperature. These changes occurred mainly in galactolipids and were not observed in the Minitomato fruit.

The increase of linolenic acid observed during cold storage of fruit, and the subsequent decline when transferred to room temperature may be related to an adjustment of fluidity of the fruit membrane. This membrane lipid remodeling of the fruit could help to maintain the fruit integrity when they are stored at low temperature.

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CONICET, ANCYPT

MERCURY BIOTRANSFORMATION MONITORING IN VITIS VINIFERA CV MALBEC BY LIQUID CHROMATOGRAPHY COUPLED TO ATOMIC SPECTROMETRIES

Adrián Spisso[a], Federico J.V. Gomez[a], Fernanda Silva[a], Pablo Pacheco[b], Dante Martinez[b]
[a]*Instituto de Biología Agrícola de Mendoza (IBAM), Alte.Brown 500, Chacras de Coria, CP 5505 Mendoza, Argentina*
[b]*Instituto de Química de San Luis (INQUISAL), Chacabuco y Pedernera, CP 5700 San Luis, Argentina*
e-mail: aaspisso@gmail.com
Keywords: Mercury, vitis vinifera, phytochelatins.

The present study evaluates the changes in Hg distribution for a two-year period in vines (*Vitis vinifera cv Malbec*) by coupling different liquid chromatography [LC] modes to atomic spectrometries; inductively coupled plasma mass spectrometry [ICP MS] and atomic fluorescence spectrometry [AFS]. To this end, vines were administered with 100 mg L^{-1} Hg solution for 3 days, simulating acute poisoning by watering with Hg contaminated waters. Sampling of roots, stems and leaves was performed one week after administration and at regular periods for two years, depending on availability. Total Hg was evaluated by microwave assisted digestion [MAD] of plant organs followed by ICP MS determination. Hg distribution according to molecular weight was evaluated by coupling size exclusion chromatography [SEC] to ICP MS. Hg metabolites like Hg-phytochelatins [Hg-PCs] complexes; or methyl mercury, phenyl mercury, dimethyl mercury and ethyl mercury were evaluated by reversed phase chromatography [RPC] coupled to AFS, by two dimension chromatography.

Results showed that vines uptake Hg translocating it from roots through stems to leaves. Roots accumulated the highest Hg concentration. Hg in stems and leaves was accumulated mostly as organic Hg, bind to different moieties [1]. SEC showed that Hg is distributed mainly in high molecular weight [HMW] fractions of 669 kDa in vine plants. In stems and leaves, Hg–S associations were found in 669 and 66 kDa fractions. Hg–S association at medium molecular weight [MMW] fraction, 66 kDa, which suggests a possible protein or peptide binding affecting vines normal physiology. From these outcomes, Hg-PCs complexes were evaluated by RPC-AFS in this fraction, in a second dimension chromatography. Chromatograms showed Hg-PC_2, Hg-PC_3 and Hg-PC_4 presence only in roots [2]. Since Hg contamination through organomercurials is more harmful than Hg^{2+} itself, methyl mercury, dimethyl mercury, and phenyl mercury, more toxic Hg species were evaluated by RPC-AFS with negative results.

The initial Hg concentration of 86.43 ± 15.98 μg g^{-1} absorbed by vines was reduced to 32% after six months, and to 11% after one year post administration. After two years Hg was only detectable in roots, at concentrations close to control plants (0.06 ± 0.01 μg g^{-1}). Regard Hg distribution according to molecular weight, after one year the only detectable fraction detected was the HMW fractions of 669 kDa. No MMW or low molecular weight [LMW] fractions associated to Hg were determined one year after administration; however sulfur fractions of MMW and LMW determined at initial stages were continuously detected. These observations, along with PCs detection, show elevated Hg metabolism in MMW and LMW fractions. Hg associations to HMW fractions probed to be more conserved since they were continuously detected after one year post Hg administration.

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PHENOLIC PROFILING OF MALBEC WINES FROM MENDOZA: INFLUENCE OF TERROIR

Urvieta R[1,2], Buscema F[2], Bottini R[1], Fontana A[1].
1 Instituto de Biología Agricola de Mendoza, Mendoza, Argentina.
2 Catena Institute of Wine, Mendoza, Argentina.
e-mail:rurvieta@catenainstitute.com
Keywords: Phenolic compounds, Malbec, Wine

Malbec is the most cultivated variety in Argentina with 34.559 ha in Mendoza[1]. In the international market a wide price gap of Malbec wines can be found, and one of the most important factor defining the quality and final prices is the origin of the grapes, also denominated with the name of "terroir". The concept of "terroir" in viticulture refers to a complex interplay of factors, covering the vine and its surrounding environment, highlighting the importance of climate, soils, plant material, and human intervention[2]. Mendoza´s environmental conditions are highly variable within its territory, from cold areas as Gualtallary in Tupungato, to warmer zones such as the East of the province. Phenolic compounds are products of secondary metabolism present in the vacuoles of berry skins that are extracted during vinification[3]. The profiling and relative concentration of phenolics can be used as markers of wines origin, thus giving information about the "terroir"[4].

The objective of the present study was to investigate the influence of geographical location on phenolic composition of Malbec wines from grapes of 25 parcels distributed in 6 regions of Mendoza, Argentina. Each parcel was selected based on their uniformity and regional representativeness, and the same winemaking technique without oak aging was used. Phenolics, anthocyanins and non-anthocyanins, were analyzed by HPLC-UV after 6 months of bottle aging. Thirty phenolics were characterized and their relative amounts analyzed using chemometrics. Results show that Mendoza´s Malbec wines have phenolic compositions associated to geographic origin. In the so called "First zone" and "Uco Valley", wines from different appellation have similar phenolics profile, while wines of "East zone" are separated from the other two regions. Discriminating by Department, similar characteristics for San Carlos and Luján de Cuyo were obtained; Tunuyán and Tupungato were fairly similar, while the Departments of Maipú and Rivadavia were clearly differenced. By performing cluster analysis, a wide variability in the phenolics profiles of wines from each zone was observed, where parcels located in the same vineyard are quite different among them, although they may be assimilated to wines from other region. The results suggest that the actual "terroir" denomination in Mendoza defined as political limits may not be the proper way for classification of wines, since a great influence of climatic and soil characteristics of each parcel were observed.

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