Special Issue
Selected Abstracts of the III International Caparica Symposium on Profiling (ISPROF 2017)

The content published in this issue was previously selected by the scientific committee to be presented at the III International Caparica Symposium on Profiling, from 4 - 7 September 2017 in Caparica, Almada, Portugal. Only the Proceeding Abstracts with a copyright transfer agreement were reproduced.

Commitees

Conference Chairs

***Carlos Lodeiro Espiño*, PhD. FRSC**
UCIBIO-REQUIMTE, Chemistry Department, Universidade NOVA de Lisboa, Portugal.
***José Luis Capelo Martínez*, PhD. FRSC**
UCIBIO-REQUIMTE, Chemistry Department, Universidade NOVA de Lisboa, Portugal.

Scientific Committee

Carlos Lodeiro Espiño, PhD. FRSC
UCIBIO-REQUIMTE, Chemistry Department, Universidade NOVA de Lisboa, Portugal.
José Luis Capelo Martínez, PhD. FRSC
UCIBIO-REQUIMTE, Chemistry Department, Universidade NOVA de Lisboa, Portugal.
Gilberto Igrejas, PhD
Universidade de Trás-os-Montes e Alto Douro, Portugal.
Niels Morling, PhD
Faculty of Health and Medical Sciences, University of Copenhagen, Denmark.
Péter Horvatovich, PhD
Department of Pharmacy, University of Groningen, Groningen, The Netherlands.
Francisco Amado, PhD
School of Health Sciences, University of Aveiro, Portugal.
Karlheinz Peter, PhD
Monash University and Royal Melbourne Institute of Technology (RMIT), Australia.
Marco Silva, PhD
FCT-UNL University NOVA of Lisbon, Portugal.

Conference Secretariat

José Luis Capelo Martínez, PhD. FRSC
UCIBIO-REQUIMTE, Chemistry Department, Universidade NOVA de Lisboa, Portugal.
Marta Silva, MSc
UCIBIO-REQUIMTE, Chemistry Department, Universidade NOVA de Lisboa, Portugal.

JOURNAL OF INTEGRATED OMICS

A methodological Journal

SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Tolerance to caspofungin in *Candida albicans* is associated with at least three distinctive mechanisms that govern expression of *FKS* genes and cell wall remodeling 1

Profiling the dynamics of the metabolism of human cells: A unique approach to illuminating human disorders 3

Combining activity-based and substrate-based profiling approaches in the characterization of a/b-hydrolase domain (ABHD) containing serine hydrolases. 4

Coffee Consumption, Obesity and Reduced Risk of Type-2 Diabetes. 5

Fluorescence lifetime based assays in drug discovery. 6

MALDI TOF MS Profiling: advances in species identification and future prospects. 7

Sugar Rush: New LC-MS method to quantify the plant regulator Trehalose-6-Phosphate. 8

Using multi-omics profiling to identify target pathways for the treatment of age-related neurodegenerative diseases. 9

Profiling and identifying compounds from the aquatic fern *Azolla*: Why?. 10

Effect of adaptive changes in lipids on conformation of OmpF porin from *Yersinia pseudotuberculosis*. 11

Metabolite profiling of rice volatile compounds from Portuguese varieties. 12

Statistical Inference of Gene Regulatory Network from Gene Expression Profiles. 13

Next generation toxicology risk-assessment applied to novel nicotine delivery products. 14

Identification of new proteins associated with atherosclerotic plaque instability in the tandem stenosis mouse model. 15

Immunochemical characteristic of antigenic structure of OmpF porin from *Yersinia pseudotuberculosis*. 17

Untargeted profiling of phenylalanine derived metabolites in wheat ears during *Fusarium graminearum* infection by ^{13}C-labeling, LC-HRMS and custom data processing 18

Interaction of OmpF porin from *Yersinia pseudotuberculosis* with antibodies to human thyroid-stimulating hormone receptor. Study *in vitro* and *in silico*. 19

Development of a transcriptomic protocol combining bovine somatic cells and OpenArray® technology to trace the ab(use) of somatotropin in dairy cattle. 20

GC-TOF-MS Metabolite Profiling of Drought Tolerant *Quercus ilex*. 21

Fattyacidomics and minerals: profiling the effects of recombinant bovine somatotropin on milk composition. 22

New Protocols for NMR-based Metabolic Profiling.. 23

About the origin of the matrix mechanism and the genetic code. 24

Regarding Core Issues of the Origin of Life: About protocells synthesis and biological asymmetry. 25

Evaluation of a probable regulatory network between CYP1A1- CYP1A2 fragment and AHR on Coffee Consumption. 26

Mass spectrometry-based metabolomics as a tool to study *Casuarina glauca* salt stress tolerance. 27

New human plasma type 2 diabetes mellitus biomarkers. 28

Unveiling the role of human cardiac stem cells in acute myocardial infarction. 29

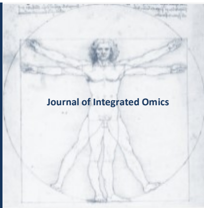
Keratinolytic activity of Bacillus subtilis AMR wilde type and mutants. 30

Using metabolic approach to uncover plant-bacteria interactions that affect antibiotic production. 31

Epigenetic variability among saffron crocus (*Crocus sativus L.*) accessions characterized by different phenotypes 32

Understanding the importance of KIX domain proteins in different systems 33

A proteogenomics approach for identification of molecular determinants of the oncosuppressive actions of Estrogen Receptor beta in breast cancer 34



JOURNAL OF INTEGRATED OMICS
A METHODOLOGICAL JOURNAL
HTTP://WWW.JIOMICS.COM

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Tolerance to caspofungin in *Candida albicans* is associated with at least three distinctive mechanisms that govern expression of *FKS* genes and cell wall remodeling

Elena Rustchenko [1]*, Hironao Wakabayashi [1], Sumanun Suwunnakorn [1], Feng Yang [1], Lulu Zhang [2], Yuanying Jiang [2], Yongbing Cao [2], David S. Perlin [3]

[1] Department of Biochemistry and Biophysics, University of Rochester Medical Center, Rochester, New York 14642, USA; [2]Center for New Drug Research, School of Pharmacy, Second Military Medical University, Shanghai, 200433, P.R. China; [3]Public Health Research Institute, New Jersey Medical School, Rutgers Biomedical and Health Sciences, 225 Warren Str. Newark, NJ 07103, USA

Available Online: 2 October 2017

ABSTRACT

Fungal infections are currently found to be a leading cause of global infectious disease, resulting in death tolls surpassing those from such diseases as drug-resistant tuberculosis or malaria. Infections involving *Candida* species are responsible for most systemic invasive fungal infections (1). Despite an increase in the diversity of *Candida* species isolated from clinical samples, *C. albicans* is still the predominant cause of infections causing up to 50% of candidemia (2, 3). *C. albicans* also prevails among isolates from blood stream, from 42% to 50.7% (4). Currently, echinocandins (ECNs) that prevent biosynthesis of cell wall are recommended for treatment of *Candida* infections, as front-line drugs. Expanding echinocandin use to prevent or treat invasive fungal infections has led to an increase in the number of breakthrough infections due to resistant Candida species. ECN resistance is well documented for *C. albicans*. The genome of *C. albicans* contains three homologous *FKS* (FK506 sensitivity) genes: *FKS1* (*GSC1*) (orf19.2929), *FKS2* (*GSL2*) (orf19.3269), and *FKS3* (*GSL1*) (orf19.2495). The only generally accepted mechanism of *C. albicans* clinical resistance to ECNs involves point mutations in FKS1 (5). Such clinically resistant strains usually exhibit high minimum inhibitory concentration (MIC) values for ECNs. However, the mutations in *FKS1* gene are not sufficient to explain elevated ECN MICs that are observed in many clinically-derived *C. albicans* strains with no *FKS1* mutations (6). A better understanding is needed to assess the cellular factors that promote decrease of susceptibility to ECNs and ultimate breakthrough.

We find that, following direct selection on caspofungin (CAS) from the ECN class of drugs, laboratory resistance (tolerance) can be conferred by at least three independent mechanisms: 1) reversible loss of one chromosome 5 (Ch5); 2) conversion of one Ch5 into a chromosome with two right arms, and 3) an aneuploidy-independent mechanism that downregulats 2-fold or more ~9.6% of genes on disomic Ch5s that are also downregulated on the monosomic Ch5 (6; E. Rustchenko, unpublished RNA-seq data). Mutants selected for CAS tolerance possessed remodeled cell walls with elevated chitin and showed overall downregulation of *FKS* genes that were free of mutations (7). We also found that Ch5 carries at least three genes *CSU51* (orf19.1105.2), *CHT2* (orf19.3895), and *PGA4* (orf19.4035) for negative regulation of ECN susceptibility. These genes were downregulated in all kinds of mutants irrespectively of Ch5 ploidy. The final number of genes dor negative control of ECN susceptibility is yet to be established (8). In addition, Ch5 carries at least two genes *CNB1* and *MID1* involved in calcineurin signaling pathway that encode positive regulators of ECN susceptibility. These genes are expressed at the disomic level in all kinds of mutants irrespectively of Ch5 ploidy. The final number of Ch5 genes for positive control of ECN susceptibility is also not known yet. Apparently, multiple mechanisms can impact the relative expression of genes residing on Ch5 or outside Ch5 controlling the genes in a similar manner. Although mutations in *FKS1* have previously been associated with CAS resistance, we find mechanisms of CAS tolerance that are independent of mutations in *FKS1* suggesting it is an earlier event in resistance development.

Correspondence: Email - elena_bulgac@urmc.rochester.edu

Acknowledgments: This work was supported in part by National Institutes of Health grant AI110764 to E.R. and grant AI109025 to D.S.P; Merck Sharp & Dohme Inc. MISP award 51184; and The University of Rochester Funds to E. Rustchenko, and National Natural Science Foundation of China (no. 81173100, 81273556) to Yongbing Cao.

References:

1] D. Li, R. Calderone, Virulence 0 (2016) 1-10. doi: 10.1080/21505594.2016.1188235

2] J. Guinea, Clin Microbiol. Infect. 20 Suppl 6 (2014) 5-10. doi: 10.1111/1469-0691.12539

3] S.R. Lockhart, Clin. Microbiol. 36 (2014) 131-136. doi: 10.1016/j.clinmicnews.2014.08.001

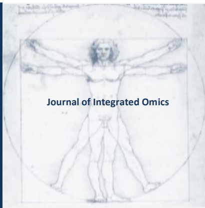
4] E. Matsumoto, L. Boyken, Sh. Tendolkar, J. McDanel, M. Castanheira, M. Pfaller, D.Diekema, Diagnostic Microbiol. Infect. Dis. 79 (2014) 205-208. doi: 10.1016/*j*.diagmicrobio.2014.02.016

5] D.S. Perlin, Ann. N.Y. Acad. Sci. 1354 (2015) 1-11. doi: 10.1111/nyas.12831

6] M.A. Pfaller, SA Messer, D.J. Diekema, R.N. Jones, M. Castanheira, J. Clin. Microbiol. 52 (2014) 108-14. doi: 10.1128/JCM.02481-13

7] F. Yang, L. Zhang, H. Wakabayashi, J. Myers, Y. Jiang, Y. Cao, C. Jimenez-Ortigosa, D.S. Perlin, E. Rustchenko, Antimicrob. Agents Chemother. 61 (2017) e00071-17.

8] S. Suwunnakarn, H. Wakabayashi, E. Rustchenko, Antimicrob. Agents Chemother. 60 (2016) 7457-7467.



JOURNAL OF INTEGRATED OMICS
A METHODOLOGICAL JOURNAL
HTTP://WWW.JIOMICS.COM

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Profiling the dynamics of the metabolism of human cells: A unique approach to illuminating human disorders

Charles E. Schwartz [1], Chin-Fu Chen [1], Rini Pauly [1], Luigi Boccuto [1]

[1] J.C. Self Research Institute, Greenwood Genetic Center, Greenwood, S.C. USA, 29646

Available Online: 2 October 2017

Abstract

We employed plates PM M1-M8 of the Phenotype MicroArrays (PM) platform from Biolog (Haywood, CA) to explore cell utilization of single compounds as an energy source or metabolic effectors in patients with various human genetic disorders with known gene mutations. Doing so, allowed us to generate kinetic profiles which created distinct fingerprints for some human disorders.

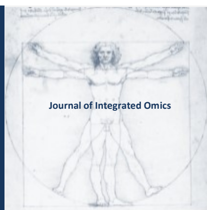
We have successfully applied the PM methodology to establish: 1) Abnormal utilization of critical cytokines mediating the immune response of both fibroblasts and lymphoblasts from patients with a duplication of *MECP2* and recurrent infections; 2) Low utilization of Krebs cycle compounds and high utilization of sugars in patients with Snyder-Robinson syndrome; 3) Patients with the FG syndrome and with Lujan syndrome exhibited different metabolic profiles even though both have mutations in the same gene, *MED12*; 4) Abnormal response to growth factors of fibroblasts from affected tissue from patients with segmental overgrowth. Based on these observations we have explored the utilization of specific compounds to treat somatic overgrowth conditions and have obtained some success in vitro.

The PM assay represents a novel approach to investigate the cellular metabolism and evaluate the functional impact of genetic alterations on metabolic pathways. The assay does not require any prior knowledge of how a particular gene may affect a metabolic pathway. Most importantly, the findings may point to an avenue of potential treatment otherwise not evident from the information known on the function of the gene.

Keywords: Metabolic profiling; human genetic syndromes; Phenotype Microarray

Acknowledgments: Grants from NICHD (R21HD072473), NINDS (R01NS073854) and South Carolina Dept. of Disabilities and Special Needs (SCDDSN)

Correspondence: Email - ceschwartz@ggc.org



JOURNAL OF INTEGRATED OMICS
A METHODOLOGICAL JOURNAL
HTTP://WWW.JIOMICS.COM

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Combining activity-based and substrate-based profiling approaches in the characterization of a/b-hydrolase domain (ABHD) containing serine hydrolases

Jarmo T. Laitinen [1]*

[1] Institute of Biomedicine, University of Eastern Finland (UEF), Kuopio Campus, Finland

Available Online: 2 October 2017

Abstract

The human metabolic serine hydrolases (mSHs) comprises a large family of enzymes and utilize a conserved serine nucleophile to hydrolyze e.g. amide, ester and thioester bonds. The majority of mSHs contains an α/β-hydrolase domain (ABHD) fold and use a Ser-His-Asp (SHD) triad for catalysis. Genes encoding ABHD proteins are found throughout the reported genomes and conserved structural motifs shared by these proteins are predictive for common roles in cell signaling and lipid metabolism. Although a handful of these enzymes are well-known, many still remain poorly characterized with respect to their physiological substrates, products and metabolic functions. Recent research have brought mSHs such as monoacylglycerol lipase (MAGL), acyl protein thioesterase 1 (APT1/LYPLA1) and KIAA1363 to the center stage of cancer research. Various aggressive cancer cells show heightened serine hydrolase activity, supporting high migratory, invasive, and protumorigenic activity. Therefore, small molecule inhibitors targeting the mSH family may open new avenues for anticancer therapies. In addition, newly discovered inhibitors could be useful in characterizing biochemical and physiological function of unknown mSHs. Hydrolase activity can be readily monitored using activity-based protein profiling (ABPP), a chemoproteomic approach that relies on the use of catalytic serine-targeting fluorophosphonate probes. The ABPP requires no *a priori* knowledge of the identity of the target and enables parallel activity measurement of many enzymes in complex native proteomes. In competitive ABPP, the proteome is first treated with the inhibitor candidate after which the mSHs are labeled with the fluorescent probe. Protein bands targeted by the inhibitor can be visualized using in gel fluorescence imaging after SDS-PAGE separation. In this presentation, I will highlight the applicability of ABPP when combined together with natural or artificial substrate-based activity profiling in our recent efforts to characterize human ABHD family members, such as ABHD11 [1], ABHD16A/BAT5 [2], ABHD6 and ABHD12 [3] and the cysteine mutants of MAGL [4].

Acknowledgments: Supported by the Academy of Finland, Research grants #139620 and #278212

References:

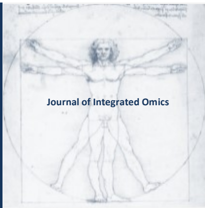
1] D. Navia-Paldanius, J.Z. Patel, M. López Navarro, H. Jakupovic, S. Goffart, S. Pasonen-Seppänen, T.J. Nevalainen, T. Jääskeläinen, T. Laitinen, J.T. Laitinen, J.R. Savinainen, Eur J Pharm Sci 93 (2016) 253-263. doi: 10.1016/j.ejps.2016.08.031

2] J.R. Savinainen, J.Z. Patel, T. Parkkari, D. Navia-Paldanius, J.J.T. Marjamaa, T. Laitinen, T. Nevalainen, J.T. Laitinen, PLoS ONE 9(10): e109869 (2014). doi: 10.1371/journal.pone.0109869

3] D. Navia-Paldanius, J.R. Savinainen, J.T. Laitinen, J Lipid Res 53 (2012) 2413-2424. doi: 10.1194/jlr.M030411

4] T. Laitinen, D. Navia-Paldanius, R. Rytilahti, J. Marjamaa, J. Kařízková, T. Parkkari, T. Pantsar, A. Poso, J.T. Laitinen, J.R. Savinainen, Mol Pharmacol 85 (2014) 510-519. doi: 10.1124/mol.113.090795

Correspondence: Email - jarmo.laitinen@uef.fi



JOURNAL OF INTEGRATED OMICS
A METHODOLOGICAL JOURNAL
HTTP://WWW.JIOMICS.COM

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Coffee Consumption, Obesity and Reduced Risk of Type-2 Diabetes

Roseane Maria M Santos [1]*, Darcy Roberto Andrade Lima [2]

[1] Associate Professor, Dept. Pharmaceutical Sciences, South University School of Pharmacy; [2] Instituto de Neurologia Deolindo Couto, Universidade Federal do Rio de Janeiro

Available Online: 2 October 2017

Abstract

Diabesity [1] is the term used to describe the coexistence of type-2 diabetes and obesity, and it is responsible for more than 90% of the world's 382 million people with type-2 diabetes. Sadly, this number is set to rise beyond 592 million in less than 25 years. Innumerous reports from epidemiological studies have been published claiming that regular coffee intake, caffeinated or decaffeinated, averaging 3-4 cups a day, reduces significantly the risk of developing diabetes type 2 [2, 3]. Caffeine is present in the brewed coffee on average at 0.5-2% and has well- known properties, such as thermogenesis and ergogenic properties [4] that could be used to explain part of the effect of coffee in reducing obesity. However, coffee is a very complex mixture and the presence of chlorogenic acids, phenolic antioxidant compounds, is found in much higher concentration than caffeine (7-12%); depending on the beans blend (percentage of basically arabica and robusta species) and the roasting process (the higher temperature and longer roasting process, the lower the amount of those compounds in coffee) [5]

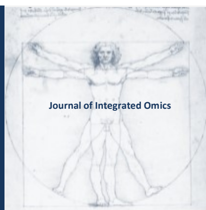
As of today, there are mounting evidences of the reduced risk of developing type-2 diabetes by regular coffee drinkers of 3-4 cups a day. The effects are likely due to the presence of chlorogenic acids and caffeine; the two constituents of coffee in higher concentration after the roasting process [6].

Acknowledgments: The authors would like to acknowledge the fundamental support from Mrs. Valerie Yaughn, Director of the Library at South University, Savannah Campus in helping to find peer-reviewed papers that were of interest. Dr. Darcy Lima passed away last July 2015 and I wanted to acknowledge his infinite encouragement to write this and many other papers, book chapters and books and as research partner and enthusiast. He will be forever remembered.

References:

1] J. Allmer, C.H. Markert, E.J. Stauber, M. Hippler, FEBS Letters 562 (2004) 202–206. doi: 10.1016/S0014-5793(04)00212-1
2] Astrup A, Finer N Journal of the International Association for the Study of Obesity (2000) 1:57-59. doi: 10.1046/j.1467-789x.2000.00013.x
3] Kahn SE, Hull RL, Utzschneider KM Nature (2006) 444:840-846. doi: 10.1038/nature05482
4] van Dam RM, Hu FB Jama (2005) 294:97-104. doi: 10.1001/jama.294.1.97
5] Nehlig A, Debry G International journal of sports medicine (1994) 15:215-223. doi: 10.1055/s-2007-1021049
6] van Dijk AE, Olthof MR, Meeuse JC, Seebus E, Heine RJ, van Dam. Diabetes care (2009) 32:1023-1025. doi: 10.2337/dc09-0207
7] Santos, RMM & Lima, DRA. Eur J Nutr (2016) 55:1345–1358. doi: 10.1007/s00394-016-1206-0

Correspondence: Email - rsantos@southuniversity.edu



JOURNAL OF INTEGRATED OMICS
A METHODOLOGICAL JOURNAL
HTTP://WWW.JIOMICS.COM

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Fluorescence lifetime based assays in drug discovery

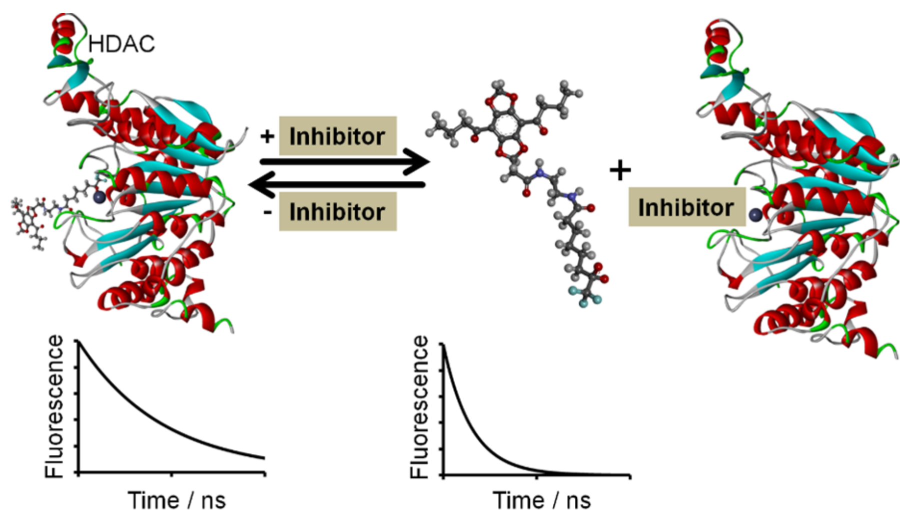
Franz-Josef Meyer-Almes [1]*

[1] University of Applied Sciences Darmstadt, Germany

Available Online: 2 October 2017

Abstract

High-throughput assays for drug screening applications have to fulfill particular specifications. Besides the capability to identify even compounds with low potency, one of the major issues is to minimize the number of false-positive hits in a screening campaign in order to reduce the logistic effort for the subsequent cherry picking and confirmation procedure. In this respect, fluorescence lifetime (FLT) appears as an ideal readout parameter that is supposed to be robust against autofluorescent and light -absorbing compounds, the most common source of systematic false positives. The extraordinary fluorescence features of the recently discovered [1,3]dioxolo[4,5-f][1,3] benzodioxole dyes were exploited to develop FLT-based binding assays for several bacterial and human isoforms of the histone deacetylase (HDAC) family.[1-2]



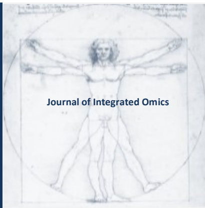
Acknowledgments: The work was supported by the Deutsche Forschungsgemeinschaft (DFG, Grant ME 3122/2-1).

References:

1] C. Meyners, M. Mertens, P. Wessig, F.J. Meyer-Almes, A fluorescence lifetime based binding assay for class IIa histone deacetylases, Chemistry, (2016). doi: 10.1002/chem.201605140

2] C. Meyners, R. Wawrzinek, A. Kramer, S. Hinz, P. Wessig, F.J. Meyer-Almes, A fluorescence lifetime-based binding assay for acetylpoly-amine amidohydrolases from Pseudomonas aeruginosa using a [1,3]dioxolo[4,5-f][1,3]benzodioxole (DBD) ligand probe, Anal. Bio-anal. Chem., 406 (2014) 4889-4897. doi: 10.1007/s00216-014-7886-5

Correspondence: Email - franz-josef.meyer-almes@h-da.de



JOURNAL OF INTEGRATED OMICS
A METHODOLOGICAL JOURNAL
HTTP://WWW.JIOMICS.COM

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

MALDI TOF MS Profiling: advances in species identification and future prospects

Jayaseelan Murugaiyan [1]*, Uwe Roesler [1]

[1] Institute of Animal Hygiene and Environmental Health, Centre for Infectious Medicine, Freie Universität Berlin, Robert-von-Ostertag-Str. 7-13, Berlin 14163, Germany

Available Online: 2 October 2017

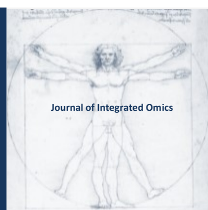
ABSTRACT

Matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) based microbial species identification has emerged as an important tool in modern diagnostic laboratories, due to its rapidness, easy handling, cost-effectiveness, high-throughput analysis and reliability as that of genome-based techniques. This technique involves direct transfer of intact microbial colonies on to a target plate, co-crystallised with UV absorbing chemicals referred to as matrix, generation of MALDI spectra and deduction of species through pattern matching with the reference spectra library of well-defined microorganisms. Despite the advancement over past two decades, none of the available reference databases are complete in terms of all known genus/species of microorganisms. Therefore, the commercial software tools include a provision for database extension by creation of additional reference spectra either to improve the identification confidence or to include missing species information. This provision has been reported to be successful in identification of several microbial species, higher organisms such as parasites and their biological vectors, investigation of geographical origin of food products such as mozzarella cheese or differentiation of immune cells. In our laboratory, we extended the commercial database with reference spectra to enable species identification up-to the genotypes of the colourless microalgae genus *Prototheca*, the only known plant like infectious agent associated with rare but severe infections in humans and animals. Subsequently, MALDI based *Prototheca* identification has been included as a rapid tool in our diagnostics, which was otherwise, time-consuming and tedidious [1, 2]. Furthermore, database extension resulted in enhanced identifcation confidence and differentation of the members of the *Staphylococcus intermedius-group* (SIG: *S. intermedius*, *S. pseudintermedius* and *S. delphini*), an important opportunistic pathogens in animals and occasionally in humans [3]. The standard formic acid/acetonitrile producure recommended for microbial protein extraction was successfully applied to compile a reference database for different body parts of the insect Tsetse fly (*Glossina*), biological vector of African sleeping sickness causing trypanosomes [4]. Our work and other recent publications indicate the possibilities of utilising MALDI profiling towards universal identification of microorganisms, insects and other higher organisms. In future, a single MALDI TOF MS measurement might be useful in deriving multiple informations such as species, host or geographical specificity, antimicrobial resistance pattern and presence of pathogens within biological vectors.

References:

1] J. Murugaiyan, J. Ahrholdt, V. Kowbel, U. Roesler, Clinical Microbiology and Infection 18 (2012) 461–467. doi: 10.1111/j.1469-0691.2011.03593.x
2] J. Ahrholdt, J. Murugaiyan, R.K.Straubinger, T. Jagielski, U. Roesler, Medical mycology 50 (2012) 234-243. doi: doi.org/10.3109/13693786.2011.597445
3] J. Murugaiyan, B. Walther, I. Stamm, Y. Abouelnaga, S. Brueggemann-Schwarze, S. Vincze, L. Wieler, A. Luebke-Becker, T. Semmler, U. Roesler, Clinical Microbiology and Infection, 20 (2014) 1007-1015. doi: 10.1111/1469-0691.12662
4] A. Hoppenheit, J. Murugaiyan, B. Bauer, S. Steuber, P.H. Clausen, U. Roesler, PLOS Neglected Tropical diseases 7(2013) e2305. doi: 10.1371/journal.pntd.0002305

Correspondence: Email - jayaseelan.murugaiyan@ fu-berlin.de



JOURNAL OF INTEGRATED OMICS
A METHODOLOGICAL JOURNAL
HTTP://WWW.JIOMICS.COM

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Sugar Rush: New LC-MS method to quantify the plant regulator Trehalose-6-Phosphate

Carla António *

Plant Metabolomics Laboratory (ITQB NOVA), Instituto de Tecnologia Química e Biológica António Xavier, Avenida da República, 2780-157 Oeiras, Portugal

Available Online: 2 October 2017

Abstract

Metabolomics is one 'omics' approach that can be used to acquire comprehensive information on the composition of a metabolite pool providing a functional screen of the cellular state. By quantifying the changes taking place inside cells at specific times and under specific conditions, metabolomics offers new insight into cellular biology and a new path of research into the development of abiotic stress tolerant crops [1-2].

Studies of the plant metabolome include the analysis of a wide range of chemical species with diverse physical properties, from ionic inorganic compounds to biochemically derived hydrophilic sugars, organic and amino acids, and a range of hydrophobic lipid-related compounds. Current plant metabolomics studies therefore combine robust on-line chromatographic separations with the high sensitivity and specificity provided by mass spectrometry (e.g., LC-MS, GC-MS) in an effort to acquire more comprehensive metabolite coverage [3].

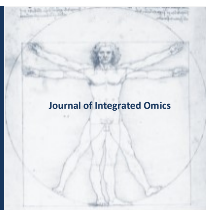
Trehalose 6-phosphate (Tre6P), the intermediate of trehalose biosynthesis, is an essential signal metabolite in plants. Tre6P is the phosphorylated intermediate of trehalose biosynthesis. It is a signal of Suc status in plants and influences many metabolic and developmental processes, including responses to stress conditions [4]. However, the almost undetectable levels of T6P together with the complex plant matrix and the presence of Tre6P isomers makes the detection of this metabolite challenging. In this presentation, details of our highly sensitive and reliable LC-MS method to detect and quantify Tre6P in the picomole range in Medicago truncatula roots and leaves subjected to water deficit will be discussed [5]. This new analytical tool is fully validated and can now be used to measure low-abundant Tre6P in other biological systems to better understand the regulation of this signaling metabolite.

Acknowledgments: C. António gratefully acknowledges support from the FCT Investigator Programme (IF/00376/2012/CP0165/CT0003) by Fundação para a Ciência e a Tecnologia (FCT) and funding from the ITQB NOVA R&D Unit GREEN-it 'Bioresources for sustainability' (UID/Multi/04551/2013).

References:

1] O. Fiehn, J. Kopka, P. Dörmann, R.N. Trethewey, L. Willmitzer, Nat. Biotechnol. 2000, 18, 1157-1161. doi: 10.1038/81137
2] O. Fiehn, Plant Mol. Biol. 2002, 48, 155-171.
3] T.F. Jorge, J. A. Rodrigues, C. Caldana, R. Schmidt, J.T. van Dongen, J. Thomas-Oates, C. António, Mass Spectrom. Rev. 2016, 35, 620-649. doi: 10.1002/mas.21449
4] J.E. Lunn, I. Delorge, C.M. Figueroa, P. Van Dijck, M. Stitt, Plant J. 2014, 79, 544-567. doi: 10.1111/tpj.12509
5] A.T. Mata, T.F. Jorge, J. Ferreira, M.R. Bronze, D. Branco, P. Fevereiro, S. Araújo, C. António, J Chromatogr. A 1016, 1477, 30-38.

Correspondence: Email - antonio@itqb.unl.pt



JOURNAL OF INTEGRATED OMICS
A METHODOLOGICAL JOURNAL
HTTP://WWW.JIOMICS.COM

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Using multi-omics profiling to identify target pathways for the treatment of age-related neurodegenerative diseases

Pamela Maher [1*], Antonio Currais [1], Daniel Daughtery [1], David Schubert [1]

[1] Salk Institute for Biological Studies, La Jolla, CA USA

Available Online: 2 October 2017

Abstract

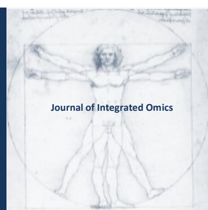
There is currently no drug to prevent of slow the progression of Alzheimer's disease (AD) pathology. Because age is the greatest risk factor for sporadic AD, phenotypic screens based on old age-associated brain toxicities were used to develop new drug candidates based on the natural products curcumin and fisetin (1-4). Since certain aspects of aging may be the primary cause of AD, we hypothesized that these drug candidates would be effective in rapidly aging SAMP8 mice. These mice show a progressive, age-associated decline in brain function similar to human AD patients. To determine if the drug candidates could prevent the progression of age-associated declines in brain function, 8 month old SAMP8 mice were fed the drug candidates in their diet for 4 months. At 12 months of age, changes in behavior, protein expression, the levels of metabolites and the whole transcriptome in mice fed the drug candidates were compared to 8 month old mice and 12 month old mice fed a control diet. Using this inclusive and integrative multi-omics approach, we identified a subset of metabolic changes associated with aging that may be relevant to sporadic AD as well as other forms of dementia. We found that some of these changes were prevented by all of the drug candidates. In addition, other changes were only affected by one of the drug candidates suggesting that these compound-specific changes might be able to provide a specific fingerprint for drug efficacy in future studies.

Acknowledgments: This work was supported by the Salk Institute Pioneer Fund Postdoctoral Scholar Award and the Salk Nomis Fellowship Award to AC and grants from the Alzheimer's Association, Burns Foundation and National Institutes of Health (grant numbers RO1AG046153, RO1AG035055 and R42AI104034) to PM and DS.

References:

1] Chiruta C, Schubert D, Dargusch R, Maher P. Chemical modification of the multi-target neuroprotective compound fisetin. J Med Chem. 2012;55:378-389. doi: 10.1021/jm2012563
2] Prior M, Dargusch R, Ehren J, Chiruta C, Schubert D. The neurotrophic ocmpound J147 reverses cognitive impairment in aged Alzheimer's disease mice. Alzheimer's Res Ther. 2013;5:25. doi: 10.1186/alzrt179
3] Prior M, Chiruta C, Currais A, Goldberg J, Ramsey J, Dargusch R, et al. Back to the future with phenotypic screening ACS Chem Neurosci. 2014;5:503-513. doi: 10.1021/cn500051h
4] Currais A, Goldberg J, Farrokhi C, Chang M, Prior M, Dargusch R, et al. A comprehensive multiomics approach toward understanding the relationship between aging and dementia. Aging. 2015;7:937-955. doi: 10.18632/aging.100838

Correspondence: Email - pmaher@salk.edu



JOURNAL OF INTEGRATED OMICS
A METHODOLOGICAL JOURNAL
HTTP://WWW.JIOMICS.COM

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Profiling and identifying compounds from the aquatic fern *Azolla*: Why?

Ana L. Pereira [1,2*]

[1] Centre for Functional Ecology, Department of Life Sciences, Faculty of Sciences and Technology, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal; [2] Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos, s/n, 4450-208, Porto, Portugal

Available Online: 2 October 2017

ABSTRACT

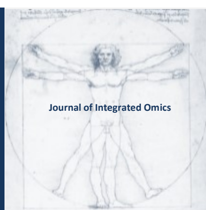
The search for novel compounds or new sources of bioactive compounds from plants are normally made in angiosperms not in ferns and so their potential is not fully investigated. The genus *Azolla* is composed by seven species that is unique since it is the only fern that harbours a permanent symbiosis with the cyanobacterium *Anabaena azollae*. The research of their natural compounds is spread in time and possible bioactivity is sparse. These ferns have several compounds such as fatty acids [1], phenols as luteolinidin, caffeic acid, aesculetin, chlorogenic acid [2], α-asarone and isoeugenol [3], terpenoids such as lupeol, cholesterol [2], lycoxanthin, α-sitosterol [3], cycloartenol, campesterol, sitosterol [4], volatiles [5] and much more. Despite the scarce data on this symbiosis, it has been indicated as having medicinal potential to cure sore throat and cough [6]. Further, the lipophilic extract had activity against *P. expansum*, while the hydrophilic extract presented activity against A. vitis and the crustacean A. salina [7]. Also, the methanolic extract of *A. microphylla* has antimicrobial activity against *Xanthomonas* sp. [8]. In a wide survey with six *Azolla* species, [9] establish that organic extracts of *A. caroliniana*, *A. rubra* and *A. filiculoides* inhibited the growth of *B. subtilis* whereas *A. caroliniana* and *A. microphylla* extracts inhibited the growth of *S. aureus*. Hence, this ferns seems to have the potential to be used as antibacterial, but nothing is known about the compounds or compounds that induce the bioactivities.

Acknowledgments: The European Social Funding (FSE) under the Human Potential Operational Program (POPH) of National Strategic Reference Board (QREN) supported the fellowship SFRH/BPD/44459/2008 to Ana L. Pereira.

References:

1] T. Nakane, T. Matsuhisa, Y. Arai, K. Masuda, A. Takano, K. Shiojima, Natural Medicines 57 (2003) 201.
2] G.M.M. Teixeira, Ph. D. Thesis, University of Lisbon (1999).
3] M.D. Greca, P. Monaco, M. Onorato, L. Previtera, Gazzeta Chimica Italiana 119 (1989) 553-556.
4] Y. Arai, T. Nakagawa, M. Hitosugi, K. Shiojina, H. Ageta, O.B. Abdel-Halim, Phytochemistry 48 (1998) 471-474.
5] A.L. Pereira, A.C. Figueiredo, J.G. Barroso, L.G. Pedro, F. Carrapiço, Plant Biosystems 143 (2009) 268-274. doi: 10.1080/11263500902722477
6] G.M. Wagner, Botanical Review 63 (1997) 1-26.
7] R. Piccardi, A. Frosini, M.R. Tredici, M.C. Margheri, Journal of Applied Phycology 12 (2000) 543-547.
8] G. Abraham, Vegetos 26 (2013) 200-204. doi: 10.5958/j.2229-4473.26.1.029
9] A.L. Pereira, L.J. Bessa, P.N. Leão, V. Vasconcelos, P.M. Costa, Symbiosis 65 (2015) 17-21. doi: 10.1007/s13199-015-0316-4

Correspondence: Email - anapereira271268@yahoo.com



JOURNAL OF INTEGRATED OMICS
A METHODOLOGICAL JOURNAL
HTTP://WWW.JIOMICS.COM

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Effect of adaptive changes in lipids on conformation of OmpF porin from *Yersinia pseudotuberculosis*

Sanina N.M.1*, Davydova L.A.1, Bakholdina S.I. [2], Novikova O.D. [2]

[1] Far Eastern Federal University, Vladivostok, Russia; [2] G.B. Elyakov Pacific Institute of Bioorganic Chemistry, FEB RAS, Vladivostok, Russia

Available Online: 2 October 2017

ABSTRACT

Bacteria contact with the environment through the cell membrane lipid matrix. Compensatory changes in the fatty acid (FA) composition and the head groups of membrane lipids during adaptation provide unique dynamic and structural properties of the membrane that are necessary to support the ability of microorganims to live in the new conditions of existence. Probably, the same processes can influence resistance of bacteria to antibiotics and immune system of the host organism. It is assumed that lipid environment provides optimal conformational state of membrane proteins in the certain conditions. Therefore, changes in the physico-chemical properties of lipids should correlate with their effects on conformation, and therefore, on functions of membrane proteins. Thus, the understanding of the dynamic processes in membrane proteins in their native hydrophobic environment allows for a fresh look at their functioning. OmpF porin (YOmpF) is the dominant protein of the outer membrane (OM) of *Yersinia pseudotuberculosis* which performs an important role in the exchange processes between the cell and the environment. It is known also that porins are the channels for penetration of some antibiotics into the bacterial cell, and hence responsible for development of resistance to these drugs. Therefore, our work was aimed to study the influence of adaptive changes in lipids of Y. pseudotuberculosis on conformation of YOmpF, as well as establishing their role in the resistance of bacteria to antibiotic ampicillin. It was shown that stress predominantly induces accumulation of unsaturated form of lysophosphatidylethanolamine (LPE) which unlikely saturated form increases thermal stability of YOmpF compacting protein monomers [1]. Mostly LPE is localized in OM of *Y. pseudotuberculosis*, where YompF is situated too [2]. DSC and intrinsic fluorescence were shown that total lipids from *Y. pseudotuberculosis* enriched with LPE increased thermal stability of the protein, associated with the compaction of its hydrophobic region, to a greater extent than the total lipids with a low content of LFE [3]. An adaptive changes in the level of LPE correlates with the sensitivity of bacteria to β-lactam antibiotic ampicillin. So, the accumulation of LPE in membranes is the part of adaptive response of bacteria to stress.

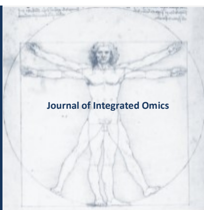
Acknowledgments: This work was supported by Russian Science Foundation Grant 15-15-00035.

References:

1] L.A. Davydova, N.M. Sanina, O.D. Novikova et al., Protein & Peptide Letters 22 (2015) 1060-1065
2] L. Davydova, S. Bakholdina, M. Barkina et al., Biochimie 123 (2016) 103-109. doi: 10.1016/j.biochi.2016.02.004
3] N. Sanina, L. Davydova, S. Bakholdina et al., FEBS Lett. 587 (2013) 2260-2265. doi: 10.1016/j.febslet.2013.05.056

Correspondence: Email - sanina.nm@dvfu.ru

JIOMICS | VOL 7 | ISSUE 2 | DECEMBER 2017 | 1-34



JOURNAL OF INTEGRATED OMICS
A METHODOLOGICAL JOURNAL
HTTP://WWW.JIOMICS.COM

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Metabolite profiling of rice volatile compounds from Portuguese varieties

Carla Brites [1]*, Mariafe Calingacion [3], Pedro Sampaio [1,2], Ana Castanho [1], Ana Almeida1, Melissa Fitzgerald [3]

[1] INIAV-Instituto Nacional de Investigação Agrária e Veterinária, Oeiras, Portugal; [2] Faculty of Engineering, Lusophone University of Humanities and Technology, Lisboa, Portugal; [3] University of Queensland, Brisbane, Australia

Available Online: 2 October 2017

ABSTRACT

The identification of metabolite profiling of rice volatile compounds affecting flavour and taste and can be used in breeding programs to produce locally adapted varieties that answer consumer preferences.

Six rice genotypes, including two commercial varieties, Ariete (japonica), Sprint (indica); two new Portuguese varieties, Maçarico (OP1001), Ceres (OP1203) and two advanced lines OP1109, OP140 were selected to analyse metabolite profiles and assess the stability of detected associations in 6 environments: 3 different locations (Alcácer do Sal-AS, Salvaterra de Magos-SM and Bico da Barca-BB) and harvested in two years. Volatile compounds were measured by GCxGC-TOF-MS, and multivariate analysis was conducted. The resulting data were represented through Principal Components Analysis (PCA).

The volatile compounds from the rice grains led to clustering based on the growing location, which is most unusual. Generally, the volatile compounds from grains lead to clustering based on genotype. This suggests significant genotype by environment interactions. While there were 220 compounds in the volatile headspace of the rice varieties, only a few show discrimination that explains the clustering. The most discriminating compounds are mainly alkanes and alcohols responsible for separating the samples grown at AS (Figure 1, Group 1) than those grown at SM and BB

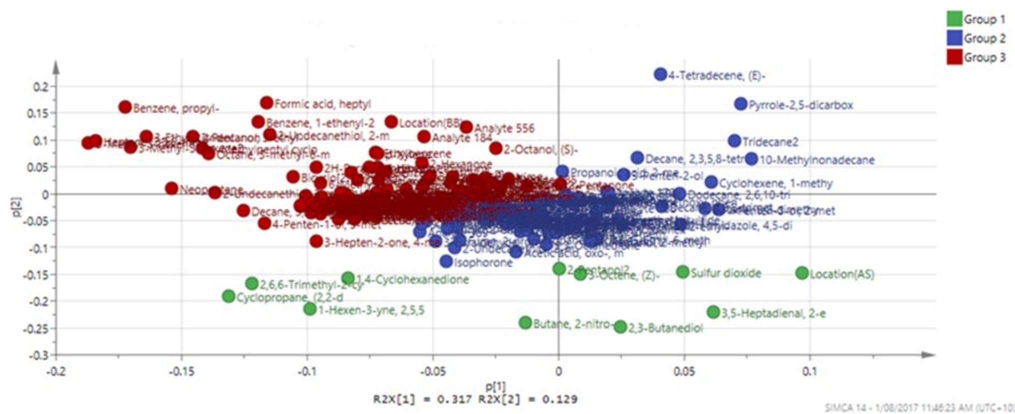
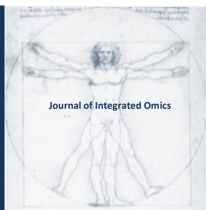


Figure 1 | Volatile compounds obtained from six rice genotypes harvested in 6 environments.

Acknowledgments: Funding from the Portuguese Fundação para a Ciência e Tecnologia (FCT) under the grant agreement RECI/AGR-TEC/0285/2012, BEST-RICE-4-LIFE.

Correspondence: Email - carla.brites@iniav.pt

JIOMICS | VOL 7 | ISSUE 2 | DECEMBER 2017 | 1-34



JOURNAL OF INTEGRATED OMICS
A METHODOLOGICAL JOURNAL
HTTP://WWW.JIOMICS.COM

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Statistical Inference of Gene Regulatory Network from Gene Expression Profiles

Sachiyo Aburatani [1,3*], Tomohiro Tamura [2]

[1] Com. Bio Big Data Open Innovation Lab. (CBBD-OIL), AIST; [2] Bioproduction Research Institute, AIST; [3] Biotechnology Research Institute for Drug Discovery, AIST

Available Online: 2 October 20177

ABSTRACT

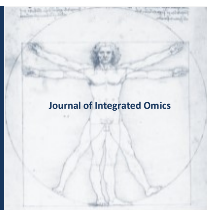
he rapid advances in experimental technologies means that we can obtain the several types of omics information as profiling data in living cells. Those profiling data is the results of some of cell activity for surviving or adapting under several conditions, and we can uncover the cell activity mechanism via disclosing these profile data. Among the several types of profile data, gene expression profiles are obtained by complex functional transcriptional regulations in cells. To clarify the mechanism of those complex regulations, inference of gene regulatory network is one of the useful approach. Actually, various algorithms including Boolean and Bayesian networks, have been developed to infer complex gene networks [1][2]. In our previous investigation, we developed an approach based on graphical Gaussian modeling (GGM) combined with hierarchical clustering to infer the huge network among all of the genes [3]. Although all of these approaches are feasible for establishing relationships among genes, it is difficult to reveal the critical interactions between genes and the other cellular components, owing to the insufficient information about the other cellular components in the gene expression profiles. Since the underlying mechanism for transcriptional regulation in living cells, regulations of genes and the effects from other cellular components should be considered. Estimation of regulatory networks among genes and the other cellular components is absolutely essential to uncover the mechanism of gene expression control, and an alternative approach is needed.

We developed a statistical approach to obtain better insights of the transcriptional regulatory mechanism from gene expression profiles [4]. Our approach based on Structural Equation Modeling (SEM) in combination with factor analysis and new algorithms for initial model assumption and model optimization. SEM can include the latent variables within the constructed model and infer the relationships among the latent and observed variables, as a network model. We improved a method for construction of initial models for SEM calculation, and applied our approach to estimate the regulatory network for several types of gene transcriptional controls. In this new approach, we combined cross-correlation and partial correlation to summarize the temporal information and to extract the direct interactions from gene expression profiles. This approach allowed us to reconstruct a model of transcriptional regulation that involves protein-DNA interactions and the RNA division effects from maternal cell to daughter cells from only the gene expression profiles, in the absence of protein information. In this presentation, we'll show you the details of SEM approach for detecting the causality between genes and other cellular components

References:

1] Friedman N, Linial M, Nachman I and Pe'er D, J. Comput. Biol. (2000) 7:601-620. doi: 10.1089/106652700750050961
2] Akutsu T, Miyano S and Kuhara S, J. Comput. Biol. (2000) 7:331-343. doi: 10.1089/106652700750050817
3] Aburatani S, Saito S, Horimoto K, Algebraic Biology (2005), 157-171
4] Aburatani S, Gen. Reg. Sys. Biol. (2011) 5:75–88. doi: 10.4137/GRSB.S7569

Correspondence: Email - s.aburatani@aist.go.jp



JOURNAL OF INTEGRATED OMICS
A METHODOLOGICAL JOURNAL
HTTP://WWW.JIOMICS.COM

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Next generation toxicology risk-assessment applied to novel nicotine delivery products

Emmanuel Minet [1]* and Marianna Gaça [1]

[1] British American Tobacco R&D Centre, Regents Park Road, Southampton, SO15 8TL, UK

Available Online: 2 October 2017

Abstract

In the past decade, novel nicotine delivery devices (e-cigarettes, heated tobacco products) have emerged as a substitute for cigarette smoking with the potential to be significantly less harmful than combustible products [1], yet, epidemiological studies are lacking. Due to intrinsic limitations of traditional toxicology testing, there has been a shift towards holistic alternative systems biology approaches. The NRC's "Toxicity Testing in the 21st Century" [2] outlines the utility of such approaches using human tissues, high throughput profiling screens and omics to dissect the cellular-response networks to support the mechanistic understanding of toxicity pathways. We have developed a systems toxicology strategy to assess novel nicotine delivery products in vitro and in clinical samples. In this presentation, we outline how RNA-seq toxicogenomics is used in combination with downstream causal reasoning to assess the effect of e-cigarette aerosols *in vitro* on a reconstituted human 3D airway tissue [3]. We further describe how a multiplatform metabolomics/lipidomics/lipoprotein/miRNA profiling method was applied to map biological changes in the serum of smokers [4]. Finally, we will place those results in the context of building adverse outcome pathways (AOPs) [5] relevant to tobacco-related diseases that can support the assessment of novel nicotine products.

Acknowledgments: The authors are grateful to Fios Genomics and Metabometrix Ltd for their support with the RNA-seq and metabolomics data analysis, respectively. This work was funded by British American Tobacco. Part of this work was contracted to Fios Genomics and Metabometrix Ltd.

References:

1] McNeill, A. et al. E-cigarettes: an evidence update (Public Health England). https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/457102/Ecigarettes_an_evidence_update_A_report_commissioned_by_Public_Health_England_FINAL.pdf, 1-113. 2015.

2] Krewsk, D. et al., Toxicity testing in the 21st century: a vision and a strategy. J. Toxicol. Environ. Health Perspect. 124, A60-A63 (2016). doi: 10.1080/10937404.2010.483176.

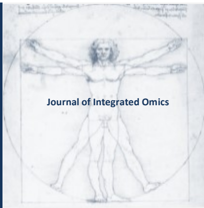
3] Banerjee, A. et al. Differential gene expression using RNA-seq profiling in a reconstituted airway epithelium exposed to conventional cigarette smoke or electronic cigarette aerosols. Applied in vitro Tox. (2017), in press.

4] Kaluarachchi, M. et al. Multiplatform serum metabolic phenotyping combined with pathway mapping to identify biochemical differences in smokers. Bioanalysis 19, 2023-2043 (2016). doi: 10.4155/bio-2016-0108

5] OECD, http://www.oecd.org/chemicalsafety/testing/adverse-outcome-pathways-molecular-screening-and-toxicogenomics.htm (2016).

Correspondence: Email - emmanuel_minet@BAT.com

JIOMICS | VOL 7 | ISSUE 2 | DECEMBER 2017 | 1-34



JOURNAL OF INTEGRATED OMICS
A METHODOLOGICAL JOURNAL
HTTP://WWW.JIOMICS.COM

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Identification of new proteins associated with atherosclerotic plaque instability in the tandem stenosis mouse model

Yung-Chih Chen [1*], Ya-Lan Ying [1,2], Manousos Makridakis [3], Harald Mischak [4], Antonia Vlahou [3], Karlheinz Peter [1,2]

[1] Baker Heart and Diabetes Institute; [2] Department of Medicine, Monash University, Melbourne; [3] Biomedical Research Foundation, Systems Biology, Academy of Athens, Greece; [4] Mosaiques-diagnostics GmbH, Germany

Available Online: 2 October 2017

ABSTRACT

Strokes and particularly myocardial infarction (MI), as the most frequent single cause of death, are typically caused by abrupt rupture of atherosclerotic plaques in arteries such as the coronary arteries. Despite major advances in the treatment of MI, improvements in mortality and morbidity are substantially limited by our inability to foresee plaque rupture and the resulting thrombotic vessel occlusion. We hypothesized that using our newly created tandem stenosis mouse model of plaque instability/ rupture we will identify new proteins that might particípate in plaque progression or plaque vulnerability. Plaques samples from 120 ApoE deficient mice were collected after tandem stenosis surgery. Plaques were allocated into five segments according to the histological evaluation of plaque stability [1]. Segment I showed thin cap fibroatheromas as typically seen in human unstable plaque (Figure 1). Each segment was homogenized in lysis buffer and then LC-MS/MS was performed. Data analyses were performed using Proteome Discoverer software (Thermo Scientific) and mouse Swiss-Prot database (Mus musculus, including 16.717 reviewed canonical entries). Following serial selection criteria, only the high confidence peptides were included for further analysis. We identified multiple protein targets that had a strong correlation with plaque instability and intraplaque hemorrhage. These newly identified proteins were validated by immunohistochemistry in tandem stenosis mouse plaques as well as in human carotid endarterectomy plaques. Finally, these proteins belong into diverse functional categories, including cell adhesion, protein metabolic processes and celular component morphogenesis. The gene ontology shows an association of the integrin signalling pathway and the cytoskeletal regulation pathway with plaque instability. Overall, proteomic analysis of unstable atherosclerotic plaques in comparison to stable atherosclerotic plaques holds promise to identify proteins involved in plaque instability and thus to define potential novel targets for plaque stabilization.

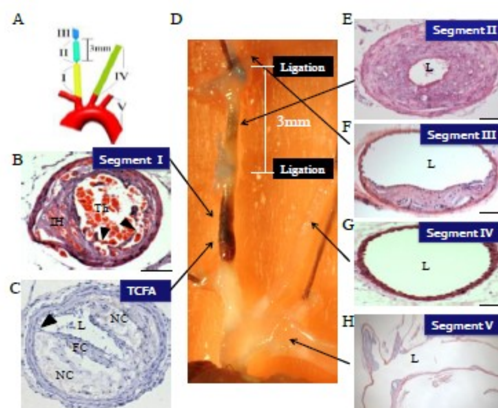
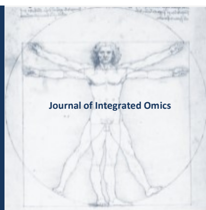


Figure 1 | (A) Schematic drawing of each segment. (B, C) Segment I showed thin cap fibroatheroma and intraplaque hemorrhage (D) Gross anatomy of TS model (E) Segment II showed positive outward vascular remodelling. (F, H) Segment III and V showed stable atherosclerotic plaques containing thick caps and small necotic cores. (G) Segment IV represented healthy vessel. L: Lumen; FC:Fibrous Cap; NC:Necrotic Core; Th:Thrombus; IH: Intraplaque hemorrhage.

Correspondence: Email - yungchih.chen@baker.edu.au

References:

1] Chen YC, Bui AV, Diesch J, Manasseh R, Hausding C, Rivera J, Haviv I, Agrotis A, Htun NM, Jowett J, Hagemeyer CE, Hannan RD, Bobik A, Peter K. Circ Res 2013;113:252-265.



JOURNAL OF INTEGRATED OMICS

A METHODOLOGICAL JOURNAL

HTTP://WWW.JIOMICS.COM

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Immunochemical characteristic of antigenic structure of OmpF porin from *Yersinia pseudotuberculosis*

Olga D. Novikova*, Olga Yu. Portnyagina, Valentina A. Khomenko, Marina P.Isaeva, Elena F. Solov'eva, Tamara F. Solov'eva

Available Online: 2 October 2017

Abstract

Many authors consider antigenic mimicry as the main reason of autoimmune diseases. The immunodominant antigens (namely non-specific porins) of bacteria of *Yersinia* species are proved to be similar to antigens of human organ tissues (including thyroid gland (TG) tissue) that are precondition for the development of autoimmune process in the organism infected. Outer membrane (OM) non-specific porins of Gram-negative bacteria belong to the β-structured integral membrane proteins. Structural elements of the porins corresponding to the outer loops connecting β-strands are shown to coincide with hydrophilic maxima and to form porin antigenic determinants. The present work is aimed to determine the role of external loops of recombinant OmpF porin from OM of *Yersinia pseudotuberculosis* (YpOmpF-r) in antigenic structure of the protein. Mutant porin monomers of YpOmpF-r with deletions of the loops L1, L4, L6 and L8 (hereinafter - del1, del4, del6 and del8) and specific antisera to full-sized and mutant porins studied were used for immunochemical characteristic of the protein. According ELISA results, the greatest number of B-epitopes was lost following the deletion of loops L1 and L4. Analysis of interaction between mutant porins and the pool serum of patients with Graves' disease and monoclonal antibodies (Abs) to hormone thyrotropin receptor (TSHR) showed that the porins without one of the outer loops (del1, d el4, del6, del8) bind to these antibodies differently (Figure A and B).

According the data of Figure A and B, del1 porin interacted very weakly with pool serum of patients with Graves' disease and did not practically interact with mAbs to TSHR. Thus, it can be assumed that outer loop L1 of YpOmpF-r comprises the region of amino acid (AA) sequence of the protein having a certain degree of homology with some immunodominant site(s) of TSHR molecule. However, a comparative analysis of the AA sequences of YpOmpF and the TSHR subunit A showed not only a sufficiently low degree of their overall homology, but also the lack of extended homologous regions in the primary structure of both proteins. Only some motifs containing two and three AA residues corresponding to fragments of the L1 sequence were found. In this regard, the most likely cause of cross-reactivity of YpOmpF and TSHR is the presence of the distinct segments of AA sequence with similar spatial organization at the level of the protein tertiary structure.

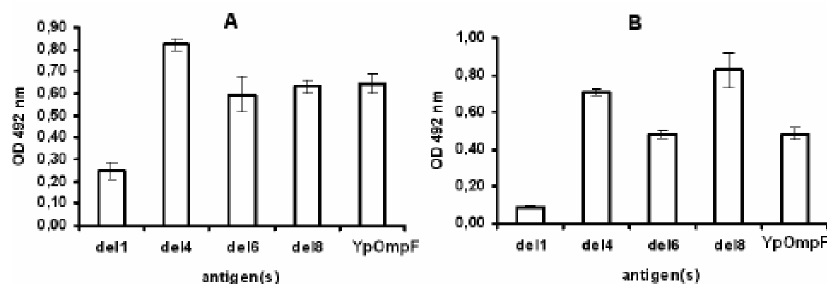
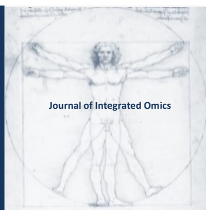


Figure 1 | ELISA analysis of interaction between deletion mutants of YpOmpF-r (del1, del4, del 6, del8) and full-sized YpOmpF-r with: (A) pool serum of patients with Graves' disease and (B) with mAbs to TSHR (dilution 1/500).

Acknowledgments: This work was partly supported by RFBR grant № 16-04-00424.

Correspondence: Email - novolga_05@mail.ru



JOURNAL OF INTEGRATED OMICS
A METHODOLOGICAL JOURNAL
HTTP://WWW.JIOMICS.COM

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Untargeted profiling of phenylalanine derived metabolites in wheat ears during *Fusarium graminearum* infection by ^{13}C-labeling, LC-HRMS and custom data processing

Christoph Bueschl [1]*, Maria Doppler [1], Bernhard Kluger [1], Marc Lemmens [2], Rainer Schuhmacher [1]

[1] Center for Analytical Chemistry, Department of Agrobiotechnology (IFA-Tulln), University of Natural Resources and Life Sciences, Vienna, (BOKU), Austria; [2] Institute of Biotechnology in Plant Production, IFA-Tulln, BOKU, Austria

Available Online: 2 October 2017

ABSTRACT

Fungi and their host plants have large arsenals of secondary metabolites, many of which have designated functions during pathogen-host interaction. For example, the fungus *Fusarium graminearum* produces deoxynivalenol (DON), which is an inhibitor of protein synthesis [1] and a toxic contaminant of food and feed [2], while wheat plants synthesize secondary metabolites to defend themselves against the pathogen (e.g. phenylpropanoids [3]).

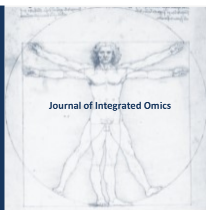
To investigate the response of wheat plants to *Fusarium* infection, we have established an untargeted metabolomics workflow designed for detecting biotransformation products of uniformly isotopically labeled tracer compounds using LC-HRMS and an in-house developed data processing software. We have started from the aromatic amino acid phenylalanine, which is further synthesized into many different compound classes such as courmarins, hydroxycinnamic acid amides, and flavonoids. Although many compounds are already known, our workflow allowed probing these compounds in an untargeted manner using the highly specific isotopolog pattern of the ^{13}C-labeled phenylalanine units. In total, we have detected 144 phenylalanine-derived metabolites, of which around 70 could be annotated with previously known phenylpropanoids. We have then tested their abundance levels in DON- and mock- (water) treated samples and found that these metabolites clearly separated the two experimental groups in a multivariate statistical analysis. In a univariate comparison using t-tests (p-value $\leq$ 0.05; mean-fold-change $\geq$ 2) approximately 50% of all biotransformation products had increased levels in the DON-treated samples further suggesting their involvement in defense-related mechanisms of wheat against *Fusarium graminearum.*

Acknowledgments: This work was supported by the Austria Science Fund (projects SFB Fusarium 3706/3715 and T2/HT2 P26213) and the Government of Lower Austria (projects NovAlgo/NoBiTUM).
.

References:

1] Shifrin VI, et al. (1999) Trichothecene mycotoxins trigger a ribotoxic stress response that activates c-Jun N-terminal kinase and p38 mitogen-activated protein kinase and induces apoptosis. J Biol Chem 274: 13985–92.
2] Pestka JJ (2010) Deoxynivalenol: mechanisms of action, human exposure, and toxicological relevance. Arch Toxicol 84: 663-79. doi: 10.1007/s00204-010-0579-8
3] La Camera S, et al. (2004) Metabolic reprogramming in plant innate immunity: the contributions of phenylpropanoid and oxylipin pathways. Immunol Rev: 198: 267-84.

Correspondence: Email - christoph.bueschl@boku.ac.at



JOURNAL OF INTEGRATED OMICS
A METHODOLOGICAL JOURNAL
HTTP://WWW.JIOMICS.COM

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Interaction of OmpF porin from *Yersinia pseudotuberculosis* with antibodies to human thyroid-stimulating hormone receptor. Study *in vitro* and *in silico*

Elena A. Zelepuga [1]*, Olga Yu. Portnyagina [1], Olga D. Novikova [1]

[1] G.B. Elyakov Pacific Institute of Bioorganic Chemistry, Far Eastern Branch, Russian Academy of Sciences, Prospect 100 let Vladivostoku, 159, Vladivostok, Russia, 690022

Available Online: 2 October 2017

ABSTRACT

Previously, it was shown that monomeric form of *Yersinia pseudotuberculosis* OmpF porin (YpOmpFm) could interact with the pool serum of patients with Graves' disease and with monoclonal antibodies (mAbs3B12) to thyroid-stimulating hormone receptor (TSHR). In present work, we carried out molecular modeling of the interaction of YpOmpFm antigen epitopes with a stimulating antibody (Abs, M22 Pdb ID 3g04) to TSHR. At the first stage of our study using different online resources and based on the primary and spatial structure of the YpOmpFm, a set of probable antigenic B-epitopes that satisfy the requirement of availability to the solvent was predicted. There were two small conformational epitopes: the first one was formed as a result of the convergence of the regions comprising two outer loops L4 (D158) and L6 (246NK247), as well as two β-strands, β11 (230ETQ232) and β13 (D276). The second region was formed with the participation of loop L2 (66EDS68, 70AGD72) and adjacent β-strands: β2 (36G, 38F) and β3 (W56). At the second stage of our study the participation of the amino acid (AA) residues forming the predicted B-epitopes of YpOmpFm in the interaction with Abs to TSHR was confirmed by molecular docking performed using MOE CCG® program (Fig. A). It should be noted that in the AA sequence of YpOmpFm and TSHR there are no extended homologous regions. However, for both proteins AA residues with similar properties and position on the binding surface in the interaction area with the Abs were revealed (Fig. B, C). According our results, E107, K209 and D232 of TSHR were overlapped with E66 K163 and D158 of the porin with binding energy contribution -11.1, -25.86 and -7.65 kcal/mol, respectively. Hydrophobic cluster F130, F153, I155 of TSHR was overlapped also with W56, Y58 and F38 of porin (total contribution -11.71 kcal/mol). Thus, a similarity of the spatial structures of YpOmpFm and TSHR that determines their cross-interaction as antigens has been established. Consequently, antibodies to porin may interact with TSHR inducing excessive stimulation of thyroid gland and thereby causing the autoimmune disorder with the symptoms of hyperthyroidism that is characteristic of Graves' disease.

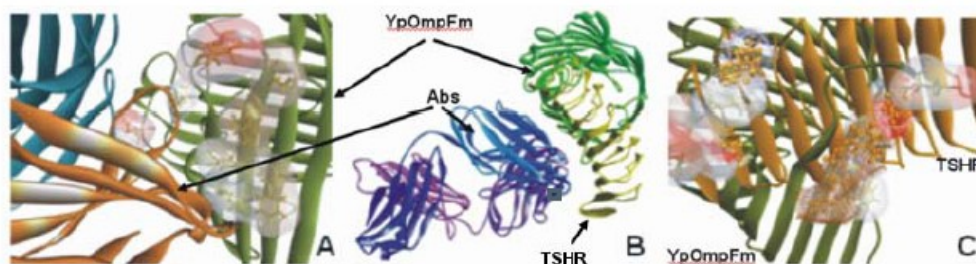
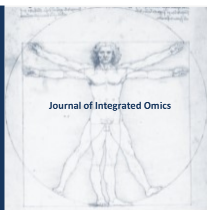


Figure 1 | Ribbon diagrams of: Abs to TSHR interaction with YpOmpFm B-epitopes (A); superposition of complexes Abs to TSHR with YpOmpFm and TSHR (B) and overlapped epitopts of YpOmpFm and TSHR (C).

Acknowledgments: this work was supported by RFBR grant № 16-04-00424 .
.

Correspondence: Email - zel@piboc.dvo.ru



JOURNAL OF INTEGRATED OMICS
A METHODOLOGICAL JOURNAL
HTTP://WWW.JIOMICS.COM

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Development of a transcriptomic protocol combining bovine somatic cells and OpenArray® technology to trace the ab(use) of somatotropin in dairy cattle

Alexandre Lamas [1*], Patricia Regal [1], Beatriz Vázquez [1], Alberto Cepeda [1], Carlos Manuel Franco [1]

[1] Department of Analytical Chemistry, Nutrition and Bromatology. Faculty of Veterinary of Lugo, University of Santiago de Compostela

Available Online: 2 October 2017

Abstract

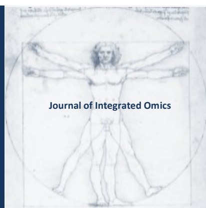
The administration of exogenous recombinant somatotropin (rbST) in dairy cattle is used to increase the milk yield. Since its approval in dairy production in 1993, the use of rbST has been controversial due it possible harmful effects in animal welfare and human health. While their use is approved in countries as United States of America, Mexico or Brazil, the European Union (EU) banned their use in 1999. In spite of this ban, in the year 2013 the illegal use of somatotropin was detected in Spain, making the authorities aware of the need of control the illegal use of this substance in the EU. For this purpose, the development of analytical methods to detect their fraudulent use is necessary in the EU. Analytical chemistry methods based on the direct detection of the banned substance as Liquid Cromatography Tandem Mass Spectrometry are the first option to detect their use [1]. However, some commercially available rbST has the same amino acid composition that the natural bST, rendering impossible their differentiation. Therefore it is of great importance to develop indirect methods that allows detecting the use of rbST in cattle [1]. In the last years the transcriptomic technology experiences a boom due to the development of RNA-seq, microarrays or High-Throughput Real-Time PCR. Real-Time PCR is considered the gold standard for quantification purposes, allowing the measurement of small differences between samples. Recently, transcriptomic has been used as a tool to detect the use of growth promotors in beef cattle [2]. However, these studies are focused in the use of target tissues as liver or muscle obtained *post-mortem*. In the case of rbST, it is key to control their misuse *in vivo*, during the period of lactation. Therefore, target samples have to be easy to collect, the method of collection should be non-invasive and it has to be economically viable. The goal of this study was to develop a transcriptomic protocol based in the combination of High-Throughput Real-Time PCR and milk somatic cell (MSCs) to detect the ab(use) of rbST. MSCs were collected from 6 treated and 3 control cows and different time points to analyze the expression of selected genes. To represent real conditions, cows were housed in a semi-intensive farm and administred with 500mg of rBST every two weak (Lactotropina®, Elanco®, Eli Lilly, México). The results showed that MSCs are an optimal alternative to isolate RNA with good quality using a simple protocol for their collection. The use of OpenArray® technology allowed the simultaneous analysis of 18 selected genes for 48 samples taken at different treatments points. With this technology it was possible to stablish a transcriptomic profile of treated and control cows that could be used to detect the illegal use of rBST in dairy cattle in EU.

Acknowledgments: Authors acknowledge the financial support of the Spanish Innovation Program "Programa *Estatal de Investigación, Desarrollo e Innovación Orientada a los Retos de la Sociedad*" (project AGL2014-58881-R).

References:

1] P. Regal, A. Lamas, C. Fente, C.M. Franco, A. Cepeda, Journal of Liquid Chromatography and Related Technologies (2017) 1-8.
2] I. Riedmaier, MW. Pfaffl, and Heinrich HD Meyer, Drug testing and analysis 4.S1 (2012) 70-74.

Correspondence: Email - alexandre.lamas@usc.es



JOURNAL OF INTEGRATED OMICS
A METHODOLOGICAL JOURNAL
HTTP://WWW.JIOMICS.COM

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

GC-TOF-MS Metabolite Profiling of Drought Tolerant *Quercus ilex*

Ana M. Rodrigues [1]*, Jesús Rodríguez-Calcerrada [2], Luis Gil [2], Carla António [1]

[1] Plant Metabolomics Lab, Instituto de Tecnologia Química e Biológica António Xavier (ITQB-NOVA), Av. da República, 2780-157 Oeiras, Portugal; [2] Forest History, Physiology and Genetics Research Group, School of Forestry Engineering, Technical University of Madrid, 28040 Madrid, Spain

Available Online: 2 October 2017

Abstract

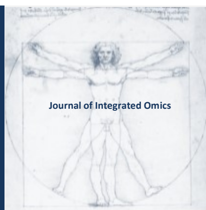
Holm oak (*Quercus ilex* L.) is the dominant tree species in natural forest ecosystems of the Western Mediterranean Basin. This species is well adapted to summer droughts but may not be able to cope with future increases in drought intensity, duration, and/or frequency as the climate becomes warmer and the water availability decreases [1]. In order to better understand the mechanisms underlying drought tolerance in *Q. ilex*, a metabolite profiling analysis was performed with gas chromatography time-of-flight mass spectrometry (GC-TOF-MS) using leaves of two-year-old *Q. ilex* seedlings subjected to increasing drought severity. Leaf sampling was carried out at mild, moderate, severe and very severe water stress conditions. Primary metabolites were extracted and analysed using a well-established protocol for metabolite profiling described by [2]. A set of 31 primary metabolites were detected in *Q. ilex* leaves. Among these, amino acids and derivatives were the most abundant metabolites, followed by sugars and sugar-alcohols and organic acids. Mild water stress caused most sugars and sugar alcohols to increase, suggesting a role of these metabolites in stress signaling and osmoregulation. These metabolites continued to increase through moderate to very severe water stress conditions. At very severe water stress conditions most amino acids dramatically increased, especially γ-aminobutyric acid (GABA) and proline suggesting enhanced protection against oxidative damage. These results indicate that high drought tolerance of *Q. ilex* relies on early water stress signaling and osmoregulation by hexoses and polyols, and enhanced protection against oxidative damage by amino acids at severe water stress. *Q. ilex* has shown mechanisms of acclimation to drought, which can be useful for its persistence under a future drier climate.

Acknowledgments: The authors are thankful to Guillermo G. Gordaliza and Meng Li. A.M. Rodrigues acknowledges FCT for the PhD fellowship (PD/BD/114417/2016) and the ITQB NOVA International PhD Programme "Plants for Life" (PD/00035/2013). J. Rodríguez-Calcerrada acknowledges funding by the project OLMOS (AGL2012-35580). C. António acknowledges support from FCT Investigator Programme (IF/00376/2012/CP0165/CT0003), ITQB NOVA R&D GREEN-it 'Bioresources for sustainability' (UID/Multi/04551/2013), and LabMet metabolomics facility at CTBE (Campinas, Brazil) for GC-TOF-MS metabolite profiling services..

References:

1] A. Barbeta, J. Peñuelas, Plant Ecol Divers (2016) doi: 10.1080/17550874.2016.1212288
2] J. Lisec, N. Schauer, J. Kopka, L. Willmitzer, A.R. Fernie, Nat Protoc. 1 (2006) 387-396. doi: 10.1038/nprot.2006.59

Correspondence: Email - amrodrigues@itqb.unl.pt



JOURNAL OF INTEGRATED OMICS
A METHODOLOGICAL JOURNAL
HTTP://WWW.JIOMICS.COM

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Fattyacidomics and minerals: profiling the effects of recombinant bovine somatotropin on milk composition

Patricia Regal [1]*, Alexandre Lamas [1], Rocío Barreiro [1], Carlos M. Franco [1], Alberto Cepeda [1]

[1] Department of Analytical Chemistry, Nutrition and Bromatology; Facultade de Veterinaria, Universidade de Santiago de Compostela (Spain)

Available Online: 2 October 2017

Abstract

Growth hormone or somatotropin (ST) is a species-specific polypeptide hormone produced in the pituitary gland of vertebrates. When administered exogenously to dairy cattle, it has galactopoietic effects and is capable of increasing the milk yield. The commercial production of recombinant bovine ST (rbST) enabled large-scale applications in farms, enhancing significantly milk production. While it is banned in the European Union (EU), several countries permit the trade and use of recombinant somatotropins in animal husbandry. The literature reflects the existence of effective analytical methods to trace rbST presence in milk and other matices [1]. However, very little effort has been invested into defining the impact this treatment may have on the quality of the dairy products obtained. In this context, *profiling* and *omic* technologies offer a good opportunity to assess various components simultaneously in food [2]. This study is meant to profile the measurable effects or rbST on the nutritional properties of milk.

A group of nine cows was separated; 6 animals were treated every two weeks with a dose of Lactotropina® (i.e. 500 mg of rbST), for a period of 8 months, while the other 3 were used as controls. Milk samples (> 400 milk samples) were collected freshly, at different time points (first milking of the day). Fifty fatty acids were measured using GC-FID, gross composition was obtained by infrared spectroscopy in a certified Spanish laboratory (*Laboratorio Interprofesional Galego de Análise do Leite* – LIGAL) and minerals (Ca, P, K, Na, Mg) were measured by ICP-MS.

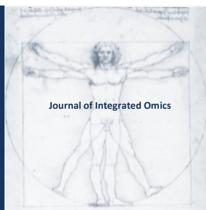
The implementation of univariate and multivariate statistics showed a tendency towards a less saturated fatty-acidome in the milk collected from animals treated with rbST, with higher concentrations of omega-6 and monounsaturated fatty acids. Conversely, the short chain fatty acids and various omega-3 were higher in controls. In addition, less calcium and protein content and more potassium was observed in milk from treated animals, in comparison to the control population. Thanks to this multi-component profiling of milk, a clear impact of somatotropin treatment on milk qualities was observed. The obtained results should be particularly interesting for those countries that permit the use of this hormone in dairy production.

Acknowledgments: This work is funded by the Spanish Innovation Program "*Programa Estatal de Investigación, Desarrollo e Innovación Orientada a los Retos de la Sociedad*" (project AGL2014-58881-R).

References:

1] P. Regal, A. Lamas, C.A. Fente, C.M. Franco, A. Cepeda, Journal of Liquid Chromatography & Related Technologies (2017) 1-8.
2] A. Cifuentes (Editorial), Electrophoresis 37 (2016) 1747. doi: 10.1002/elps.201670114

Correspondence: Email - patricia.regal@usc.es



JOURNAL OF INTEGRATED OMICS
A METHODOLOGICAL JOURNAL
HTTP://WWW.JIOMICS.COM

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

New Protocols for NMR-based Metabolic Profiling

Lei Bruschweiler-Li [3], Bo Zhang [1], Cheng Wang [1], Dawei Li [3], Rafael Brüschweiler [1,2,3]

[1] Department of Chemistry and Biochemistry; [2] Department of Biological Chemistry and Pharmacology; [3] Campus Chemical Instrument Center, The Ohio State University, Columbus, Ohio 43210, U.S.A

Available Online: 2 October 2017

Abstract

NMR spectroscopy is extremely powerful for the identification and monitoring of individual metabolites in complex mixtures encountered in metabolomics.[1] It can be applied to a wide range of biological samples, such as urine, serum, tissue extracts, cell lines, etc. Protocols will be presented for optimal sample preparation to ensure reproducible, high quality NMR data.

In order to be able to monitor a maximal number of metabolites in these samples, we use 2D NMR experiments, including 13C-1H HSQC, 13C-1H HSQC-TOCSY, and homonuclear TOCSY. We have streamlined the analysis of these spectra by our COLMAR suite of web servers that permit the uploading of peak lists extracted from these spectra or the entire 2D data sets for query against the COLMAR spectral databases, which have been customized for the different types of 2D spectra. First, the HSQC query provides a rank-ordered list of metabolite candidates along with quantitative metrics. These hits are then validated using either one or both TOCSY-type experiments providing spin-connectivity information across the molecules. This workflow has been implemented in COLMARm (http://spin.ccic.ohio-state.edu/index.php/colmarm/index) alllowing the user to carry out this process in a computer-assisted, semi-automatic fashion (Figure 1). The identification step is followed by quantification of individual metabolites, which can be performed on a cohort of samples for profiling applications. Examples we will be provided for selected systems.

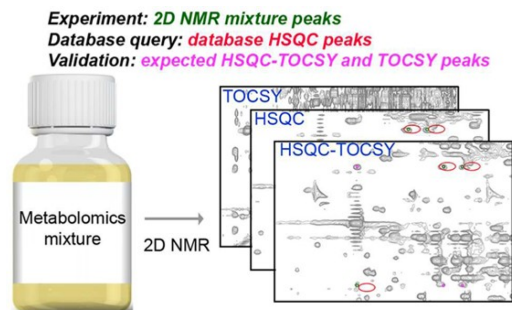


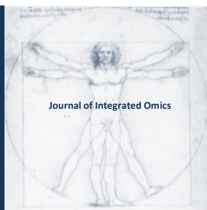
Figure 1 | Flow chart of the semi-automated COLMARm web server for metabolite identification in a complex mixture. COLMARm enables high-throughput metabolomics studies.

Acknowledgments: This work was supported by the National Institutes of Health.

References:

1] K. Bingol, L. Bruschweiler-Li, D. Li, B. Zhang, M. Xie, and R. Brüschweiler, Bioanalysis 2016, 8:557-73. doi: 10.4155/bio-2015-0004

Correspondence: Email - bruschweiler-li.1@osu.edu



JOURNAL OF INTEGRATED OMICS
A METHODOLOGICAL JOURNAL
HTTP://WWW.JIOMICS.COM

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

About the origin of the matrix mechanism and the genetic code

Eduard Y. Kostetsky

Far Eastern Federal University, Russian Federation

Available Online: 2 October 2017

Abstract

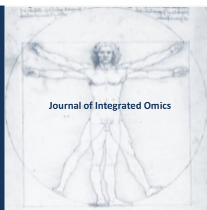
The matrix synthesis mechanism of transcription and translation can be originated in the defective zone of the crystalline lattice of co-crystallizing minerals: apatite, carbonate-apatite and calcite in mutually coordinated ratio, with the assistance of the gas-phase radicals and ions (NH_3, CH_4 and CO_2)[1]. This is the area of rhythmic changes in chemical composition, of isomorphic substitutions and vacancies and of occurrence of significant energetic fluctuations of thermal vibrations of atoms in the structure of this minerals. This led to the emergence of the growing center of the disorder and to appearance of the need to reset the energy potential in the form of stratified zones. All this is implemented in the structure of the emerging organo-mineral nucleoproteid complex. The ternary complex - DNA, RNA, protein - organized themselves in the area of apatite and carbonate-apatite according to the principle of stereospecific complementarity "spire in the spire". The double helix of DNA is a basis of this. Already at this stage all the necessary proteins for replication, transcription and translation related with their RNA were present, although the ribosomal apparatus has not been yet formed. The transition from a defect-free region in the apatite crystal to the occurrence of the disorder was realized in sizes and specificity of the genes in the DNA in the form of a gradual transition from the satellite zone (uninformative part of the DNA in eukaryotes) to the multiple repeated zone of the pre-tDNK, the moderately repeated zone of the pre-rDNA and the unique zone of genes of the pre-mDNK. The RNA-protein complex formed in the area of carbonate-apatite complementary to these zones. Each pre-tRNA in the crystal matrix interacted with its protein (aminoacyl-tRNA synthetase in future) stereospecifically. Each this protein already had amino acid and ATP in its composition. Each pre-rRNA also was associated with its proteins which were much due to the larger length of the fragments compared with the pre-tRNA and further which will be part of a ribosomes. Other proteins complementary associated with the first DNA- and RNA-associated proteins followed them further. Each pre-mRNA also had its own complementarily bound proteins. In the second stage, at occurrence water, crystal organomineral complex passed into the liquid-crystalline metastable state which could be disrupted by the change in ion concentration, by the appearance of protocells and by the matrix mechanisms starts. DNA is a virtual carrier of the genetic information. The true carrier of such information is the aminoacyl-tRNA-synthetase (AA-tRNA-synthetase) which stereospecifically complementarily associated with its pre-tRNA, an amino acid and ATP and determines not only anticodon structure in pre-tRNA (there may be up to the six of different these) but also the amino acid for its pre-tRNA which will be presented to the codon of DNA. Various pre-tRNA structure for the same amino acids acquire a degenerate anticodon via its synthetase via the compensatory loop in tRNA. In the unit cell of apatite structure the three base pairs (purin-pyrimidine) are formed at a height of 0, 50, 100 (the area of CaI), wherein the third pair is always at the border of cells. In our opinion, this area of increased energetic fluctuations is the cause of the genetic code degeneracy.

Acknowledgments: This work was supported by Russian Science Foundation (Grant 14-50-00034).

References:

1] E. Y. Kostetsky, Journal of Biological Physics 31 (2005) 607–638. doi: 10.1007/s10867-005-2383-x

Correspondence: Email - kostetskiy.yeya@dvfu.ru



JOURNAL OF INTEGRATED OMICS
A METHODOLOGICAL JOURNAL
HTTP://WWW.JIOMICS.COM

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Regarding Core Issues of the Origin of Life: About protocells synthesis and biological asymmetry

Eduard Y. Kostetsky

Far Eastern Federal University, Russian Federation

Available Online: 2 October 2017

Abstract

The author discusses some principal problems of biochemistry and molecular biology concerning the origin of life on the Earth. It is based on his theory about the origin of protocells of pro- and eukaryotic types with the assistance of gas-phase elements (NH_3, CH_4 and CO_2), apatite matrix and its co-crystallizing minerals (carbonate apatite, calcite, mica) in mutually coordinated ratio [1]. This theory describes in detail the possibilities of synthesis of purine-pyrimidine bases, DNA chains, RNA chains and nucleoproteid complexes; the formation of transcription–translation apparatus and matrix mechanism, tRNA and rRNA; the appearance of the first proteins in the ribosomes; the reason of code degeneracy according to the third nucleotide; and also considers how the structural asymmetry of molecules could arise and other fundamental questions of organization and of functioning of living cells.

Synthesis of protocells without violating the second law of thermodynamics. Cells forming through the transition from the crystalline matrix to the liquid-crystalline organo-mineral matrix is accompanied by a loss of the crystal energy by changing the bond types to hydrogen bonds and the emergence of stereospecific complementarity. Hydrogen bonds are much weaker than covalent one and coordination bonds of the crystal lattice. As a result, a rigid system of bonds of the mineral crystal lattice becomes modified. In general, the entropy of the new complex is much larger than the entropy of the crystal lattice.

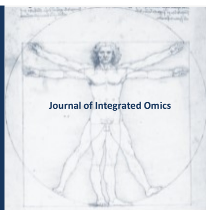
The problem of biological asymmetry. Molecular asymmetry (chirality – right- or left-handedness) is one of the fundamental characteristics of living matter. The question about L-amino acids and D-sugars remains unanswered rather than about their racemic mixture [2]. In our opinion, the cause of the chirality is associated with an external asymmetrical (unidirectional from the center of the Earth to its surface) influence of temperature on forming of the complex chiral minerals and on structures of future protocells respectively. "When certain causes produce certain effects, the symmetry elements of the reasons should be manifested in the consequences caused by them" [3]. Synthesis of nucleic acids and proteins on crystals proceeded unidirectionally (upward) simultaneously by the principle "spire in the spire". This virtually excluded the possibility of racemic mixter forming, but did not prevent the emergence of the right or left helix. Apparently this determined structural asymmetry of monomeric units of nucleic acids and proteins.

Acknowledgments: This work was supported by Russian Science Foundation (Grant 14-50-00034).

References:

1] E. Y. Kostetsky, Journal of Biological Physics 31 (2005) 607–638. doi: 10.1007/s10867-005-2383-x
2] V. A. Avetisov, V. I. Goldanskii, Proc. Natl. Acad. Sci. USA V. 93, N 21 (1996) 11435–11442.
3] M. Curie, P. Curie, M.: Nauka (1968) 28.

Correspondence: Email - kostetskiy.yeya@dvfu.ru



JOURNAL OF INTEGRATED OMICS
A METHODOLOGICAL JOURNAL
HTTP://WWW.JIOMICS.COM

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Evaluation of a probable regulatory network between CYP1A1- CYP1A2 fragment and AHR on Coffee Consumption

Roseane Maria M Santos [1*], Brown Will [2], Lily Bellicini [2],Darcy Roberto Andrade Lima [3]

[1] Associate Professor, Dept. Pharmaceutical Sciences, South University School of Pharmacy; [2] PharmD Candidates at South University School of Pharmacy; [3] Instituto de Neurologia Deolindo Couto, Universidade Federal do Rio de Janeiro

Available Online: 2 October 2017

Abstract

A previous pilot study examined if the genetic variability of caffeine metabolism could influence coffee consumption [1]. CYP1A2 is encoded by a gene located at 15q24 and presents polymorphism that can determine a decrease in the enzyme inducibility. Carriers of variant CYP1A2*1F allele are slow caffeine metabolizers, whereas individuals who are homozygous for CYP1A2*1A allele are fast metabolizers [2, 3]. Genomic-wide association studies (GWAS) of coffee drinking suggest a strong association with CYP1A1/CYP1A2 and AHR genes. According with their findings [4, 5], an increased intake of caffeine was associated with having a T-allele for CYP1A1-CYP1A2-rs2472297. They also found that another intergenic loci at 7p21 that corresponds to aryl hydrocarbon receptor (AHR), has a regulatory role in basal and substrate-induced expression of CYP1A1 and CYP1A2. The objective of the study is to examine if there is a relationship between coffee consumption and CYP1A1, CYP1A2 and AHR genotypes in the population of our previous pilot study. Our hypothesis is that the wide variability seen in caffeine levels between fast CYP1A2 metabolizers might have a correlation with changes in the genotype of the AHR and a regulatory region between the genes CYP1A1 and CYP1A2.

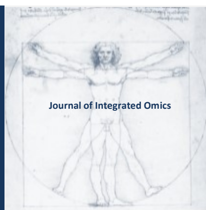
In our previous pilot study, we found that 8 out of 11 healthy volunteers presented a fast metabolizer phenotype and showed a large variability in their caffeine levels (0-0.67 mg/L) The objective of the present study is to examine if there is a relationship between coffee and caffeine consumption and CYP1A1/ CYP1A2 and AHR genotypes in the same population.

Acknowledgments: The authors acknowledge the fundamental support from Mrs. Valerie Yaughn, Director of the Library at South University, Savannah Campus in helping to find peer-reviewed papers that were of interest. Dr. Darcy Lima passed away last July 2015 and I wanted to acknowledge his infinite encouragement to write this and many other papers, book chapters and books and as research partner and enthusiast. He will be forever remembered.

References:

1] Santos RM, Cotta K.; Jiang, S.; Lima, D.R.A. Austin J Pharmacol Ther (2014) 3: id 1065.
2] Gu L, Gonzalez FJ, Kalow W, Tang BK. Pharmacogenetics. (1992) 2:73-7
3] Amin N, Byrne E, Johnson J, Chenevix-Trench G, Walter S, Nolte IM, et al. Mol Psychiatry. (2012) 17:1116-29. doi: 10.1038/mp.2011.101
4] Josse AR, Da Costa LA, Campos H, El-Sohemy A. Am J Clin Nutr. (2012) 96:665-71. doi: 10.3945/ajcn.112.038794
5] Sulem P, Gudbjartsson DF, Geller F, Prokopenko I, Feenstra B, Aben KK, et al. Human molecular genetics (2011) 20:2071-7. doi: 10.1093/hmg/ddr086

Correspondence: Email - rsantos@southuniversity.edu



JOURNAL OF INTEGRATED OMICS

A METHODOLOGICAL JOURNAL

HTTP://WWW.JIOMICS.COM

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Mass spectrometry-based metabolomics as a tool to study *Casuarina glauca* salt stress tolerance

Tiago F. Jorge [1*], José C. Ramalho [2,3], Ana I. Ribeiro-Barros [1,2,3], Alisdair R. Fernie [4], and Carla António [1]

[1] Plant Metabolomics Laboratory, Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa (ITQB NOVA), Av. da República, 2780-157 Oeiras, Portugal; [2] PlantStress&Biodiversity Group, Linking Landscape, Environment, Agriculture and Food (LEAF), Dept. Recursos Naturais, Ambiente e Território (DRAT), Instituto Superior de Agronomia (ISA), Universidade de Lisboa (ULisboa), Tapada da Ajuda, 1349-017 Lisboa, Portugal; [3] GeoBioTec, Faculdade de Ciências e Tecnologia (FCT), Universidade Nova de Lisboa (UNL), 2829-516 Caparica, Portugal; [4] Central Metabolism Group, Max Planck Institute of Molecular Plant Physiology, D–14476 Potsdam-Golm, Germany

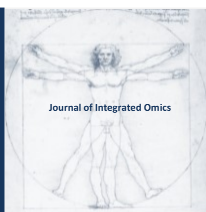
Available Online: 2 October 2017

ABSTRACT

Casuarina glauca is a model actinorhizal plant characterized by its ability to establish symbiosis with nitrogen-fixing Frankia bacteria. This plant species grows naturally in coastal zones and is able to thrive under extreme salinity environments. *C. glauca* tolerance to high salinity is associated to biochemical and physiological adjustments such as low tissue dehydration, osmotic adjustments, and high membrane integrity. Mass spectrometry (MS)-based plant metabolomics has emerged as a powerful tool to address biological questions related to plant environment and agriculture. To date, there is almost no information on the *C. glauca* metabolome. In this study, a modern metabolomics approach that combines two MS-based analytical platforms, namely LC-QIT-MSn target analysis and GC-TOF-MS metabolite profiling, is being applied to study the impact of salt stress in nodulated and non-nodulated *C. glauca* plants. Our most recent results agree with those previously obtained from morpho-physiological analysis, and provide new knowledge on the primary metabolome of *C. glauca*, its symbiosis with *Frankia Thr*, and its metabolic readjustments under increasing salt concentrations. Furthermore, the divergent metabolite responses particularly found in the amino acid metabolism suggest root and root-nodule specific metabolite responses, and support the fact that from 200 mM NaCl upwards, symbiosis was turned off. Based on these results, a second independent biological experiment is currently ongoing to assess, at the physiological and metabolite levels, the performance of non-nodulated C. glauca plants under a combined salt and heat stress.

Acknowledgments: This work was supported by Fundação para a Ciência e a Tecnologia (FCT) through the project PTDC/AGR-FOR/4218/2012, the FCT Investigator Programme (contract IF/00376/2012/CP0165/CT0003 C. António), and the research units UID/GEO/04035/2013 (GeoBioTec), UID/AGR/04129/2013 (LEAF), and UID/Multi/04551/2013 (ITQB NOVA R&D unit GreenIT). T.F. Jorge further acknowledges FCT (PD/BD/113475/2015) and the ITQB NOVA International PhD Programme 'Plants for Life' (PD/00035/2013) for the PhD grant.

Correspondence: Email - tiagoj@itqb.unl.pt



JOURNAL OF INTEGRATED OMICS
A METHODOLOGICAL JOURNAL
HTTP://WWW.JIOMICS.COM

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

New human plasma type 2 diabetes mellitus biomarkers

Alena Soboleva [1*], Gregory Mavropulo-Stolyarenko [1], Anna Bystrova [2,3], Domenika Thieme [5], Wolfgang Hoehenwarter [5], Vasily Stefanov [1], Tatiana Grishina [1,4], Tatiana Karonova [2,3], Andrej Frolov [6]

[1] Saint-Petersburg State University, Biological Faculty, Department of Biochemistry, Russian Federation ; [2] Federal Almazov North-West Medical Research Centre, Russian Federation ; [3] The First Pavlov State Medical University of St. Petersburg, Russian Federation ; [4] Saint-Petersburg State Pediatric Medical University, Russian Federation ; [5] Leibniz Institute of Plant Biochemistry, Proteome Analytics Research Group ; [6] Leibniz Institute of Plant Biochemistry, Department of Bioorganic Chemistry, Germany

Available Online: 2 October 2017

Abstract

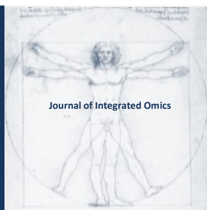
One of the central problems of modern medicine is the development of new approaches for diagnosis, treatment and monitoring of diabetes mellitus and its complications. Glycation is a post-translational modification formed by interaction of reducing sugars (e.g. glucose) with protein amino groups, yielding early glycation products (Amadori compounds). The latter undergo oxidative degradation, accompanied by formation of advanced glycation end products (AGEs). The abundance of glycation products in human plasma strongly correlates with hyperglycemia, characteristic for type 2 diabetes mellitus (T2DM). Currently, glycated proteins (hemoglobin and albumin) are routinely employed as clinical T2DM markers. However, these species show low sensitivity to short-term changes in blood glucose concentration.

In contrast, individual glycation sites in blood plasma proteins with different half-life time might be considered as alternative T2DM markers [1]. To prove this concept, the patterns of potential biomarkers were compared in cohorts (n = 20) of T2DM patients (women 45-75 years old with the HbA1C levels of 7.5-10%, not undergoing insulin therapy and not smoking) and age-matched normoglycemic controls. Individual glycation sites were analyzed by the LC-based bottom-up proteomic approach, including protein digestion, enrichment of early glycation products by affinity chromatography on boronic acid (BAC) and solid phase extraction prior to RP-HPLC-QqTOF-MS. Sequence assignment of differentially abundant glycated peptides relied on RP-HPLC-ESI-LIT-Orbitrap-MS/MS, whereas their biomarker potential was addressed by label-free relative quantification. Statistical analysis relied on the Mann-Whitney test and linear discriminant analysis (LDA). Totally, 51 differentially glycated protein lysine residues were identified, among them 42 individual glycation sites worked as biomarkers of type 2 diabetes. Thereby 32 glycated peptides were characterized as new potential biomarkers of T2DM [1]. The LDA approach showed variable set containing 12 biomarkers distinguished T2DM patients from the normoglycemic controls that might be useful for diabetes prediction, early diabetes diagnostic, therapy control, and patient stratification according to the concept of personalized medicine.

References:

1] A. Frolov, M. Bluher, R. Hoffmann, Anal. Bioanal. Chem. 406 (2014) 5755-5763. doi: 10.1007/s00216-014-8018-y

Correspondence: Email - oriselle@yandex.ru



JOURNAL OF INTEGRATED OMICS
A METHODOLOGICAL JOURNAL
HTTP://WWW.JIOMICS.COM

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Unveiling the role of human cardiac stem cells in acute myocardial infarction

M.J. Sebastião [1,2], R. Pereira [1,2], I. Palacios [3], M. Serra [1,2], P. Gomes-Alves [1,2], P.M. Alves [1,2]

[1] Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. Da República, 2780-157 Oeiras, Portugal; [2] iBET, Instituto de Biologia Experimental e Tecnológica, Apartado 12, 2780-901, Oeiras ,Portugal; [3]Coretherapix, S.L.U., Parque Tecnológico de Madrid, Tres Cantos, 28760 Madrid, Spain

Available Online: 2 October 2017

Abstract

After an Acute Myocardial Infarction (AMI), Ischemia-Reperfusion (I/R) injury is characterized by a substantial decrease in the number of cardiomyocytes (CMs). Human myocardium harbors a population of endogenous cardiac stem cells (hCSCs) that is activated upon I/R injury, contributing to myocardial repair through the establishment of an auto/paracrine crosstalk between hCSCs and CMs in stress.

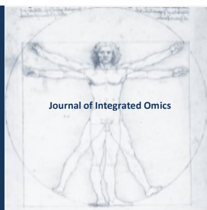
Cardiomyocytes response to I/R has been extensively studied, while CSC role in myocardial I/R is still lacking characterization. In this study, we set up an in vitro human cellular model of myocardial I/R injury using donor derived hCSCs and CMs differentiated from human induced pluripotent stem cells (hiPSC-CMs) to further decipher the action mechanisms of hCSCs upon injury.

Monocultures and co-cultures of hCSCs and hiPSC-CMs were established. Ischemia was mimicked by culturing the cells at 0% O2 in Ischemia Mimetic Solution. In the reperfusion step, cells were placed back in their physiological conditions of oxygen (3%) and nutrients. The effect of I/R injury in hCSCs was accessed by total proteome analysis at different time points using nanoLC-MS (*Eksigent LC4500* & *TripleTOF 6600*) and evaluated by IPA software. Growth factor secretion, cell viability, as well as hCSC proliferation were also evaluated.

Important features of I/R injury were successfully captured, namely CM viability loss, hCSC proliferation activation upon insult and the protective role of hCSCs on hiPSC-CMs. The culture readouts obtained together with the proteins identified in the different time points of the insult, allowed us to propose new possible players on hCSC regeneration response upon injury including activation of pathways related with cell proliferation, paracrine signaling, stress response and metabolism.

The human cellular model established in this work will allow further profiling and understanding on the molecular landscape of AMI, namely regarding hCSC regenerative response. This will potentiate the development of novel cell-and/or molecular-based therapies for myocardium regeneration.

Correspondence: Email - msebastiao@itqb.unl.pt,



JOURNAL OF INTEGRATED OMICS
A METHODOLOGICAL JOURNAL
HTTP://WWW.JIOMICS.COM

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Keratinolytic activity of Bacillus subtilis AMR wilde type and mutants

Selma Soares de Oliveira [1], Daniel Pereira de Paiva [1], Samara Sant'Anna Oliveira [1], Ana Maria Mazzoto [1], Alane Beatriz Vermelho [1]

[1] Microbiology Institute Paulo de Góes , Federal University of Rio de Janeiro (UFRJ) , Rio de Janeiro , Brazil

Available Online: 2 October 2017

ABSTRACT

Keratin is a insoluble protein found in feathers, wool, horns and hair. They represent important residues in industry. Keratinases have been described to be produced by different microorganisms and can have application in food, animal feed, leather, detergent, pharmaceutical, cosmetic and textile industries.

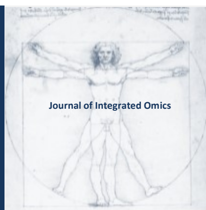
The *Bacillus* gender has been used in different biotechnology applications, since most species are considered as GRAS (Generally recognized as safe), been used as probiotic. Previous study from our group selected bacilli with high keratinolytic activity. One of these strains, *Bacillus subtlis* AMR was isolated from agro-industrial waste of poultry industry.

In this work, the strain *Bacillus subtilis* AMR was mutagenized to improve the keratinolytic activity. The cells were incubated with ethyl methanosulfonate (EMS) at a final concentration of 1%. Gelatinolytic and keratinolytic mutants were selected on gelatin and keratin agar and compared with the activity of the wilde type.

The selected mutants were evaluated by zymography. The results showed that the mutants had a higher activity than the wilde type strain. Detected bands on proteic substrates maintained the same molecular weight only showing variation in the intensity. However, some differences were observed in some peptidases of these mutants. Peptidase activity was inhibited by PMSF and EDTA and were considered to belong to serine peptidase group. Analysis of the degradation products by HPLC showed few differences in the profiles. These mutants and its peptidases can have applications in improving the nutritional value of animal feed, cosmetics, food, textile and leather industries. As a consequence, the degradation of poultry industries residues can reduce the environmental impact of those industries, generating a more clean production.

Acknowledgments: We would like to thank to CNPq and FAPERJ for the financial support .

Correspondence: Email - selma@micro.ufrj.br,



JOURNAL OF INTEGRATED OMICS
A METHODOLOGICAL JOURNAL
HTTP://WWW.JIOMICS.COM

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Using metabolic approach to uncover plant-bacteria interactions that affect antibiotic production

Ilana Kolodkin-Gal [1]*, Eliane-Hadas Yardeni [1], Arik Ogran-Nachum [1]

[1] Department of Molecular Genetics, Weizmann Institute of Science

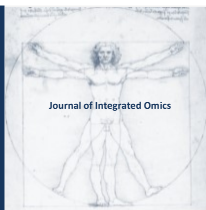
Available Online: 2 October 2017

ABSTRACT

Plant roots harbor many bacterial species that are in constant competition over space and nutrients, and are frequently found to exist as micro-colonies or biofilms. Both the Gram-positive *Bacillus subtilis* and Gram-negative c*ompetitors* can form biofilms on plant roots, presenting an intriguing model system to explore interspecies interaction between biofilms. During the interaction, *B. subtilis* advances towards its competitors while activating antibiotic synthesis and mining dead cell debris from the competing colony. Following this engulfment, cell-contact on the solid surface promoted engulfment and killing of the competitors biofilm. Our overall results indicate a mechanism that allows biofilms of *B. subtilis* to overcome biofilms formed by Gram-negative bacteria during colonization of plants. Strikingly, the plant host produces secondary metabolites that influences the efficiency of the killing by affecting the synthesis of bacterial antibiotics. Using a metabolome screening, we are now unravelling plant secondary metabolites that specifically activate the synthesis of antibiotics in the rhizosphere community. These results suggest that the composition of the plant microbiome can be orchestrated by higher-order interactions where the host directly regulates bacterial antibiotic production.

Acknowledgments: This research was supported by the ISF I-CORE grant 152/1, Mr. and Mrs. Dan Kane, Ms. Lois Rosen, by the Larson Charitable Foundation, by Ruth and Herman Albert Scholars Program for New Scientists, by the Ilse Katz Institute for Materials Sciences and Magnetic Resonance Research grant, by the Ministry of Health grant 712376 for alternative research methods, and by the France-Israel Cooperation - Maimonide-Israel Research Program grant 3-13021, and by the Israeli Science Foundation (No. 119/16). IKG is a recipient of the Rowland and Sylvia Career Development Chair.

Correspondence: Email - ilana.kolodkin-gal@weizmann.ac.il



JOURNAL OF INTEGRATED OMICS
A METHODOLOGICAL JOURNAL
HTTP://WWW.JIOMICS.COM

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Epigenetic variability among saffron crocus (*Crocus sativus L.*) accessions characterized by different phenotypes

Matteo Busconi [1], Licia Colli [1], Giovanna Soffritti [1], Marcello Del Corvo [1], Silvia Fluch [2], Elisabeth Wischnitzky [2], Eva Maria Sehr[2], Marcelino de los Mozos Pascual[3], José Antonio Fernandez [4]

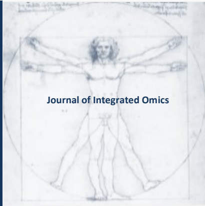
[1] Faculty of Agricultural, Food and Environmental Sciences, Research Center BioDNA, Università Cattolica del Sacro Cuore, Piacenza (Italy); [2] Austrian Institute of Technology GmbH, Tulln (Austria); [3] Centro de Investigación Agraria de Albaladejito, Consejería de Agricultura de Castilla–La Mancha, Cuenca (Spain); [4] IDR-Biotechnology, Universidad de Castilla—La Mancha, Albacete (Spain)

Available Online: 2 October 2017

Abstract

Saffron (*Crocus sativus L.*) is a sterile triploid (2n=3x=24), initially assumed to be of autotriploid origin, although a growing number of evidences support alloploidy as the most probable mechanism to have occurred. The crop vegetatively multiplies year by year by means of corms. Corm multiplication does not generate genome variations with the exception of some spontaneous mutations that in a triploid saffron population are not easily detectable. At the present time, the real level of genetic variability inside saffron is still debated and in literature it is possible to recover contradictory articles providing contrasting results about if the species is monomorphic or not. In a preliminary characterisation of 50 saffron accessions of the WSCC (World Saffron and Crocus Collection, located in the Bank of Plant Germplasm of Cuenca), characters related to phenology (date of sprouting and flowering, duration of flowering), floral morphology (length and width of tepals, and length of stamen filaments and anthers) and saffron production (percentage of flowering corms, number of flowers per corm, saffron spice weight per flower) were measured and a big variation detected. This raises the question about the origin of such variability, and, considering that gene expression can be influenced both by genetic and epigenetic changes, epigenetic variation could be a possible origin of the alternative phenotypes. In order to have a deeper insight in the epigenetic of saffron, the present study was devoted to the analysis of the cytosine methylation among saffron accessions with different geographic origin and cultivated for at least three consecutive years in the same conditions inside the saffron “CrocusBank” collection. The analysis of the methylation was carried out by using the High C+G Patch (HCGP) Filtration method coupled with high throughput sequencing. The accessions have been selected based on geographic origin, different phenotypes, and different agronomic characters and were characterized by high or low saffron production, early and late flowering time. The presence of high epigenetic variability in DNA regions associated with gene expression was detected. Finally, in order to gain information on the stability along the years of the epigenetic in a vegetatively propagated plant, saffron epigenetics of 17 different accessions stored in the “CrocusBank” collection was analysed in 4 consecutive years from 2013 to 2016. Each accession, despite the cultivation in proximity in the same field and despite the presence of intra- and inter-accession variability, tended to maintain a proper epigenotype clearly different from the other accessions.

Correspondence: Email - matteo.busconi@unicatt.it



JOURNAL OF INTEGRATED OMICS
A METHODOLOGICAL JOURNAL
HTTP://WWW.JIOMICS.COM

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Understanding the importance of KIX domain proteins in different systems

Jitendra K. Thakur

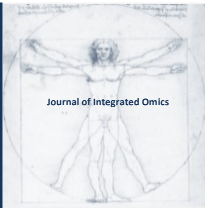
National Institute of Plant Genome Research, Aruna Asaf Ali Marg, New Delhi 110067, New Delhi, India

Available Online: 2 October 2017

Abstract

The KIX domain, important for protein-protein interaction, was first discovered as a part of the large multidomain transcriptional activator histone acetyltransferase p300/CBP. Later on, this domain was identified in Mediator subunit MED15. We found that in CBP, disorder region following the KIX domain has evolved from Med15 KIX domain. In both of these proteins, the KIX domain has been shown to be a target of activation domains of diverse transcription activators and found to be essential in several specific gene-activation pathways in fungi and metazoans. However, not much is known about KIX domain proteins in plants. We made an attempt to characterize all the KIX domain proteins coded by *Arabidopsis* and rice genomes. Interestingly, KIX domain was found not only in p300/CBP- and MED15-like plant proteins as known earlier, but also in F-box containing proteins in rice and DNA helicase in *Arabidopsis*. These findings suggest new roles of KIX domain in ubiquitin mediated proteasomal degradation of protein and genome stability. In *Arabidopsis*, we have found more than twenty proteins interacting with the KIX domain of MED15. In rice, expression analysis revealed overlapping expression of *OsKIX_3, OsKIX_5* and *OsKIX_7* in seeds of different stages of development, suggesting their individual or combined role during rice seed development. Moreover, the association analysis using the genotyping data of 136 *in silico* mined SNP loci in 23 contrasting rice genotypes and their grain length-specific phenotypic information identified three non-synonymous SNP loci in these three rice genes showing strong association with long- and short-grain differentiation. Interestingly, these SNPs are located within KIX domain encoding genomic regions. It, thus, indicates the additional importance of novel SNP loci/alleles identified in KIX domain containing genes for determining seed size in rice.

Correspondence: Email - jthakur@nipgr.ac.in



JOURNAL OF INTEGRATED OMICS
A METHODOLOGICAL JOURNAL
HTTP://WWW.JIOMICS.COM

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

A proteogenomics approach for identification of molecular determinants of the oncosuppressive actions of Estrogen Receptor beta in breast cancer

Alessandro Weisz [1*], Giovanni Nassa [1,2], Giorgio Giurato [1,2], Annamaria Salvati [1], Valerio Gigantino [1], Tuula A. Nyman [3], Luciano Milanesi [4], Roberta Tarallo [1]

[1] Laboratory of Molecular Medicine and Genomics, University of Salerno, Baronissi (Italy); [2] Genomix4Life, Baronissi (Italy); [3]Institute of Clinical Medicine, Rikshospitalet, Oslo (Norway); [4] Institute of Biomedical Technologies, National Research Council, Segregate (Italy)

Available Online: 2 October 2017

Abstract

The Estrogen Receptor beta (ERβ) is a member of the nuclear receptor superfamily of transcriptional regulators endowed with oncosuppressive activities, antagonizing estrogen-induced carcinogenesis and inhibiting growth and oncogenic functions in luminal-like breast cancers (BCs), where its expression correlates with a better prognosis of the disease. By systematically applying interaction proteomics coupled to mass spectrometry (MS) to characterize factors acting in concert with ERβ for regulation of BC cell activity [1], among >300 interacting proteins we identified Argonaute 2 (AGO2) as a novel partner of this receptor in human BC cells. ERβ-AGO2 association was confirmed 'in vitro' and 'in vivo' both in the nucleus and cytoplasm, and was found to be RNA-mediated. AGO2 is an RNA-binding protein acting as a key effector of RNA-silencing pathways, due to its direct involvement in microRNA maturation and activity, that are both controlled also by ERβ [2], able to modulate chromatin remodeling, gene transcription and RNA splicing. Functional genomics was applied to investigate the biological roles of the ERβ-AGO2 complex in luminal-like BC cells expressing human ERβ. ChIP-Seq analyses demonstrated co-association of AGO2 with ERβ in a large number of chromatin binding sites of the receptor, and total and nascent RNA-Seq in ERβ+ vs ERβ- cells, and before and after AGO2 knock-down in ERβ+ cells, revealed a widespread involvement of this factor in the well known effects of ERβ on gene transcription rate and RNA splicing [3]. Many genes directly targeted by the ERβ-AGO2 complex are involved, among others, in growth-inhibitory and oncosuppressive pathways, indicating that AGO2 plays an active part in the antioncogenic activity of ERβ. Moreover, RIP-Seq demonstrated involvement of the receptor in RISC loading via interaction with AGO2. These results demonstrate that AGO2 is a pleiotropic functional partner of ERβ in BC cells, indicating that both factors are endowed with multiple roles for control of BC cell functions.

Acknowledgments: Work supported by: AIRC (Grant IG-17426), Italian Ministry of Health (Grant GR-2011-02347781), CNR (Flagship Project InterOmics), Univ. Salerno (Grant FARB 2015-16) and EU (Elixir-Ita HPC@CINECA).

References:

1] G. Nassa, R. Tarallo, C. Ambrosino, A. Bamundo, L. Ferraro, et al., Proteomics 11 (2011) 172-179.
2] G. Nassa, R. Tarallo, G. Giurato, M.R. De Filippo, et al., Mol Cell Proteomics 13 (2014) 1076-1090.
3] D.N. Dago, C. Scafoglio, A. Rinaldi, D. Memoli, G. Giurato, et al., BMC Genomics 16 (2015) 367-349.

Correspondence: Email - aweisz@unisa.it