The Quarterly Newsletter of OMIC-ENGINE

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In this issue

Editorial

Contribution of NMR analysis in the National Infrastructure OMIC-Engine

by
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Nowadays, the rising global population, the aging and the more health-conscious societies shape the demands for agro-food industry and science, urging them to provide a healthy, nutritious, wholesome and adequate food supply in the coming decades. The rapid evolution of advanced equipment, potent fertilizers, plant growth treatments and pesticides raise the agro-food science to an opportunity. However, a global ecosystem under intense pressure asks for an agro-food industry that significantly reduces its environmental impact[1]. At this point, synthetic biology appears to be a potential solution and the synergistic action of different scientific fields (Chemistry, Molecular Biology, Biotechnology, Bioinformatics, etc.) should contribute significantly in this purpose. In the context of the accomplishment of these goals, the National Infrastructure OMIC-ENGINE was established on 2018 and already coordinates eight Greek Universities and Research Centers.

In particular, synthetic biology focuses on the engineering of multigene complex constructs influencing pathways and maybe whole genomes[2].The main goal of "OMIC-ENGINE" is to monitor the genetic-engineered organism's (e.g. plant, insect and microorganism) new genotype-phenotype relationship via its metabolome. Metabolomics is the newest of "omic" sciences and aims at the characterization, quantification of the low-molecular-mass endogenous metabolites and the study of metabolic pathways in which they are involved. Each phenotype is the result of specific metabolic alterations that consist its metabolic fingerprint and NMR-based metabolomics is an exceptional analytical method towards the metabolome investigation. Actually, NMR-metabolomics offers the potential for a holistic approach to many fields such as nutrition, natural products, quality control and product evaluation for geographical origin prediction(PDO), with an ultimate purpose to contribute to the creation of high value products for the

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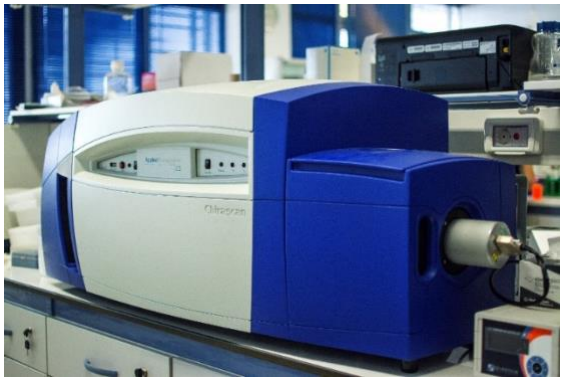
Greek agro-food sector[3].The great advantage of the non-destructive NMR spectroscopy for metabolomics is the minimal sample preparation and the short data acquisition time which allow the analysis of many samples in a short time span[4].

The Protein Architecture and Biomolecular NMR laboratory is the UPAT Hub of the OMIC-ENGINE infrastructure providing NMR analysis of high sensitivity and repeatability. Its engagement to OMIC-ENGINE rests on four main pillars: 1) a Bruker 700 MHz Avance III HD magnet/spectrometer equipped with a triple resonance TCI CryoProbe, 2) aChrirascan™ Circular Dichroism Spectrometer, 3) a wet lab for sample preparation and protein production and 4) experienced scientific personnel consisted by postdoctoral researchers, PhD candidates and individuals having received a post-graduate education in the fields of Pharmacy, Biology, Chemistry and Physics. Agri-food qualitative and quantitative analysis, physicochemical and biochemical characterization of natural products are conducted in the UPAT Hub using Chromatography, UV vis, CD, NMR, Mass-Spectrometry etc.

A



B

Figure 1:A. Bruker 700 MHz Avance III HD NMR spectrometer and **B.**Applied Photophysics Chrirascan™ Circular Dichroism (CD) spectrometer

In the context of Seed-projects, NMR spectroscopy is already applied to the metabolic profiling of:

- Sea-buckthorn (*Hippophae L.*) from different samples (fresh berries, osmotically treated berries, seeds, juice)
- raisin extracts and
- olive extracts from different producers in Greece.

Besides this, we also perform Targeted & Untargeted Metabolomics analysis (non-destructive) of biological samples:

- cell extracts (e.g. cell extracts and cell medium of HepG2 cells)
- biofluids (plasma, serum, urine, saliva, cerebrospinal fluid, etc.)
- tissue extracts

Hence, NMR analysis is a powerful tool that can contribute to the development of high value products for the Greek Agro-Food sector, which is the main aspire of the National Infrastructure "OMIC-ENGINE".

Georgios A. Spyroulias is a Professor of Chemistry at the Pharmacy School, University of Patras and Research Coordinator of Patras OMIC-Engine Hub.

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Seed project: "Black Aspergilli" in vineyards of conventional and organic farming: Investigating the role of epiphytic microbiome using Next Generation Sequencing.

By: Stefanos Testempasis (Ph.D. candidate)
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Grapevines are considered to be one of the most important crops worldwide as well as in Greece. Grape's Black rot disease caused by several mycotoxigenic species of Black *Aspergilli* section Nigri, is one of the most important pre- as well as, post-harvest diseases of grapevines, while contaminated grape products and derivatives with *Aspergillus* mycotoxins may have an important impact on consumers health. We initially observed that Black Aspergilli frequency was higher in the samples originating from conventionally cultivated vineyards. We tested the hypothesis that the observed reduced frequencies of Black aspergilli in the organic vineyards are associated with a higher microbiome diversity or the presence of specific members of the carpospheric community that affect the diversity and proliferation of Aspergilus. Hence in this seed project we will determine the carpospheric bacterial and fungal microbiome on grapes collected from conventional and organic growing systems that appear to be infested by *Aspergillus* spp via amplicon sequencing. In this work, supported by OMIC-Engine seed funding, I had the opportunity to pursue one part of my Ph.D. thesis and to become thoroughly familiar with various techniques and the preparation steps to performing Next Generation Amplicon Sequencing and Bioinformatic analysis. The results of the amplicon analysis will be an asset to understand the Black aspergilli incidence and population structure between the two grapevine growing systems.

This project is a collaboration between the Laboratory of Plant Pathology, Faculty of Agriculture, Forestry and Natural Environmental Sciences, Aristotle University of Thessaloniki and the Laboratory of Plant and Environmental Biotechnology, Department of Biochemistry and Biotechnology, University of Thessaly.

I am grateful for this opportunity. Thank you, Omic-Engine.

Contact Us

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Interviewing Researchers of OMIC-Engine

In this section we will present you the researchers of the OMIC-Engine Research Infrastructure

Evdokia (Evi) Garagouni holds a degree in Pharmacy from the University of Patras, she was awarded an MSc in Pharmaceutical Sciences and Technology, focusing on the specialization "Drug Design and Discovery", from the University of Patras and has since worked as a pre-doctoral researcher for the OMIC-Engine Patras Hub at the laboratory of Professor Georgios Spyroulias. Her research is focused on the metabolic discrimination of Sea-buckthorn (*Hippophae L.*) from different samples (fresh berries, osmotically treated berries, seeds, juice) using NMR spectroscopy.

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Question: *Evi, Describe briefly your research work.*

My research is focused on the analysis, characterization and quantification of the low-molecular-mass endogenous metabolites in plant extracts by NMR spectroscopy. Specifically NMR spectroscopy and multivariate data analysis were applied to the metabolic profiling and discrimination of Sea-buckthorn (*Hippophae L.*) from different samples (fresh berries, osmotically treated berries, seeds, juice). Sea-buckthorn contains a high level of active ingredients, such as fatty acids and vitamins. Metabolomic analysis of *Hippophae* has recently attracted considerable attention because of nutritional importance and the increasing interest in the physiological role of the monounsaturated fatty acids.

Question: *Which opportunities did your secondment offer you in terms of training, networking and personal growth?*

My participation in OMIC-Engine has offered me the opportunity to deal with the field of plant metabolomics, which is very challenging because of the complexity of the diverse metabolic characteristics and abundances of molecules. The nature of the samples has led to the development of new methods and techniques, in order to overcome the current limitations, which involved the processing and analysis of the samples. Also, the OMIC-Engine infrastructure provided to me new opportunities to work with other scientists in a stimulating

scientific environment, exchange information and ideas and find solutions to problems using modern scientific approaches and equipment. Furthermore, we established new collaborative links with partner universities across the Greece, which makes projects more diverse.

Question: *What do you think will be the impact in your future career?*

The knowledge that I gain significant experience through the understanding of plant (*Sea-buckthorn*) physiology and biology from the view of small chemical molecules that reflect the end point of biological activities, will be very important in stimulating further studies leading to better crop improvement of yield and quality. Also, I believe the experiences I have had during my time as a participant in this project, have equipped me with many valuable skills and expanded my professional network. In addition, I had the opportunity to acquire new skills, qualities and methods that I can implement in my future career.

Question: *Has this secondment experience matched your expectations so far?*

All my expectations, in regard to this, have been fulfilled. Omic -Engine is an infrastructure, which supports projects related to the development of high value products for the Greek Agro-Food sector and offers a good working environment where researchers from different places of Greece have the privilege of working together and sharing their different experiences.

Question: *Three words that sum up your experience within the OMIC-Engine infrastructure.*

Inspiration, interaction and communication can be used to characterize my experience within the OMIC-Engine infrastructure.

Meeting the OMIC-Engine Research Groups - University of Thessaly Hub

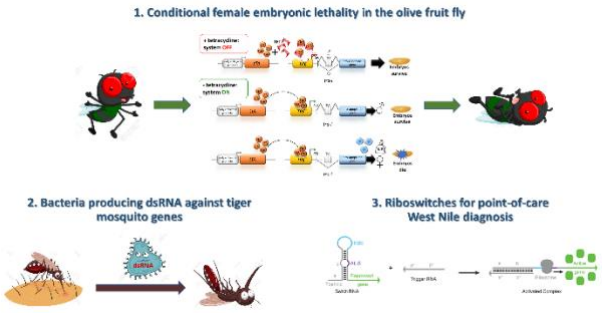
In this section we will present each time a different hub of OMIC-Engine. The first one presented will be the research groups of the Hub of Thessaly participating in OMIC-Engine

University of Thessaly and Department of Biochemistry and Biotechnology is the coordinating hub of the OMIC-Engine Research Infrastructure (RI) and is in Larissa at the region of Thessaly.

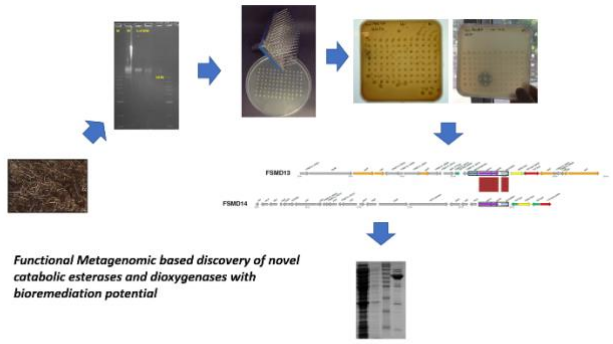
Four Research Laboratories (Genetics Comparative and Evolutionary Biology, Structural and Functional Biochemistry, Molecular Biology and Genomics and Plant & Environmental Biotechnology) with six Principal Investigators Prof. K. Mathiopoulos, Prof. Z. Mamuris, Prof. D. Karpouzas, Prof. D.D. Leonidas, Assoc. Prof. K. Moutou and Assoc. Prof. K. Papadopoulou participate at the OMIC-Engine infrastructure.

Research performed within OMIC-Engine falls within the main research activities of the four participating laboratories and can be outlined as follows.

The major research direction of the laboratory of Molecular Biology & Genomics is related to the molecular, genetic and genomic analysis of insect pests of both agricultural (olive fruit fly and Mediterranean fruit fly) and medical importance (tiger mosquitoes). The goal is to control the pests by applying environmentally friendly and insecticide-free solutions, particularly using synthetic biology. More specifically, in the framework of OMIC-Engine, three projects are undertaken. Firstly, engineered olive fruit flies are being developed, that possess conditional and selective embryonic lethality of the female sex. In a rearing facility, such a strain can lead to the production of male-only flies. Subsequently, these male flies can be sterilized and released into the environment; once mated with wild female flies sterile eggs will be produced, thus leading to population reduction. Secondly, microorganisms (bacteria or yeasts) are engineered in order to produce dsRNA against vital genes of the tiger mosquito that can be used to control these mosquitoes in their breeding sites. Lastly, a synthetic riboswitch for point-of-care detection of West Nile virus is being developed. West Nile virus is a mosquito-transmitted virus that due to costly diagnosis remains highly underdiagnosed, but it may affect the nervous system and lead to death.

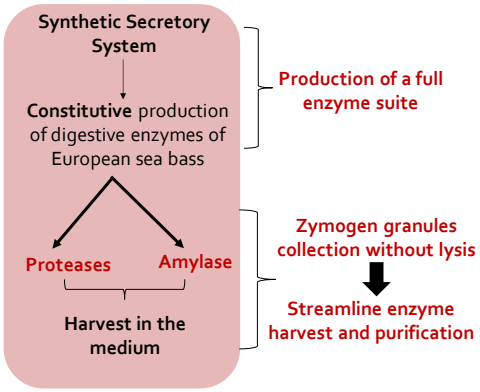


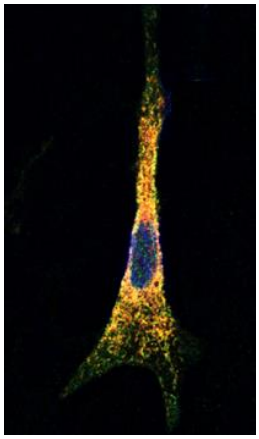
The Laboratory of Plant and Environmental Biotechnology provides services in the analysis of the microbiome in variable matrices and tissues (plant, soil, water etc.) offering solutions in problems of the agro-food sector (i.e. plant and human pathogens detection and quantification). In the frame of OMIC-Engine the group of Environmental Biotechnology works: (i) on the discovery, characterization and optimization of novel catabolic enzymes for the detoxification of priority environmental pollutants (pesticides, veterinary drugs) using functional metagenomics and (ii) on the use of synthetic microbial ecology approaches to optimize wild-type and synthetic microbial communities for environmental clean-up.



Research on the Laboratory of Structural and Functional Biochemistry is mainly focused on the structure-function relationship of macromolecules with emphasis on drug discovery. In the framework of OMIC-Engine the Laboratory works on (i) the production of a new synthetic protein for plant protection by using elements of human calmodulin and *Lotus japonicus* SHAGGY like kinase (in collaboration with Assis. Prof G. Papadopoulos and Assis. Prof. V.T. Skamnaki), (ii) construction of stable eukaryotic cell lines that express a chimeric protein of the estrogen receptor together with GFP with mitochondrial targeting for the assessment of plant derived compounds (coordinated by Assis. Prof A.M.G Psarra) and (iii) establishment of circadian miRNA expression protocols in mammalian cells (coordinated by Assis. Prof N. Balatsos). The laboratory offers services on the protein bacterial expression and purification by automated FPLC methods.

The Laboratory of Genetics, Comparative and Evolutionary Biology develops projects and services relevant to animal biodiversity and production. In the context of OMIC-Engine, its team works towards a) the development of a cell system that harbors synthetic cassette(s) for the production of a series of major digestive proteases and amylase of the European sea bass, b) the development and validation of a fully secretory system for harvesting the suite of the digestive enzymes.





The University of Thessaly Hub has recently acquired and offer for open access to the Hellenic academic community: (i) a Laser Scanning microscope (LSM 800 ZEISS) equipped with 4 laser 405, 488, 561, 640, two PMT detectors and one Airy scan detector with three mode of actions (Confocal mode, Virtual Pinhole mode, Super Resolution mode, (ii) a Bench Top Ultracentrifuge Beckman Coulter with max speed of up to 150,000 rpm with a Fixed Angel rotor (8 x 8 mL) and a Near Vertical rotor (8 x 3.9 mL) for separation of subcellular particles, separation of virus particles, pelleting RNA through a CsCl gradient, Isopycnic separation of plasmid DNA, Stable Isotope Probing applications, and (iii) a liquid handling unit for the preparation of reactions in 96 and 384-well plates for high-throughput diagnostic PCR/q-PCR and high throughput amplicon sequencing and metagenomic libraries screening.

In the framework of OMIC-Engine, the University of Thessaly Hub is currently employing six Post-Doctoral Scientists and five research technicians.

New Projects coming in the OMIC-Engine Network

In this section we will update you on research activities and new project coming in the research network of the OMIC-Engine Research Infrastructure

- **RESEARCH-CREATE-INNOVATE call, Coordinator: Associate Professor Katerina Moutou, University of Thessaly, Department of Biochemistry and Biotechnology, Laboratory of Genetics, Comparative and Evolutionary Biology. *kmoutou@bio.uth.gr***

Fish_SuperFoods is set to produce innovative fish superfoods with a positive impact on the health, development and nutritional value of farmed fish, such as seabass and seabream. These new improved fish foods will be enriched with bioactive peptides and oligosaccharides derived from the blue-green algae spirulina (*Arthrospira platensis*), as well as with modified fish food lipids, characterized by increased stability against oxidation. These ingredients added as fish food additives are expected to enhance the survival and improve additional biotic indices (condition factor, growth rate etc) and biochemical/haematological parameters of the cultured fish. Within the framework of the proposed project, enzymes will be used in order to hydrolyze spirulina proteins and carbohydrates in order to generate bioactive peptides and oligosaccharides. Also, in order to enhance the oxidative stability of fish feed oils, natural antioxidants, such as ascorbic acid and hydroxytyrosol, will be enzymatically conjugated to fish food lipids. The resulting bioactive ingredients will be incorporated into fish food pellets and their efficiency as feed for first breeding in seabass and seabream diet will be tested. Important health and fitness parameters, such survival, growth rate, morphology, and mobility of fish juveniles supplied with the newly generated functional fish foods will be evaluated and compared to conventional commercially available ones.

Successful realization of the proposed research is expected to contribute greatly to the development of easily digestible foods of high nutritional value and oxidation stability. These novel fish superfoods will

promote the growth, health, fitness and nutritional value of cultured fish and will contribute significantly to enhancing the efficiency and commercial success of tomorrow's fisheries.

- **Coordinator: Associate Professor Katerina Moutou, University of Thessaly, Department of Biochemistry and Biotechnology, Laboratory of Genetics, Comparative and Evolutionary Biology.** ***kmoutou@bio.uth.gr***

FishPhytoFeed. Fish farming relies on the extensive use of feeds with high protein content (40-60%) that supply the energy and nutrients required for fish growth. Feeds undergo digestion before these nutrients become bio-available. The sum of farming practices results in the synthesis and deposition of proteins in the white muscle that makes the actual product of fish farming, the fillet. The growing trend for fishmeal substitution with alternative ingredients of plant origin has introduced unprecedented problems in the overall quality of fish feeds that derive from the presence of antinutritional factors and phytoestrogens as well as the impaired digestion and bioavailability of nutrients. Thus, the use of alternative ingredients in fish feeds calls for the development of adequate methodology for the control of their quality. The current methodologies are of analytical character and concern the determination of the concentration of specific compounds that might be implicated in the biological effects of plant ingredients, lacking however the capacity to actually determine the dimension of their biological effects.

FishPhytoFeed will recruit methodologies of classical enzymology to determine the presence of KTI and BBI protease inhibitors, the pH-stat method to determine the digestibility of dietary proteins in vitro using digestive enzymes of European sea bass and gilthead sea bream, the Microtox Assay System© for the determination of general cytotoxicity, the ER-CALUX assay and YES assay© for the determination of estrogenic activity of plant ingredients, and primary myogenic cell cultures of gilthead sea bream for the determination of their myostatic activity.

The ultimate goal is the development of an advanced integrated toolkit for the in vitro high throughput screening of ingredients based on their biological activities and effects. No use of live fish will be required and the toolkit determinations will be combined in a synthetic quality factor that will reflect the effects of ingredients on key biological processes that consist fish growth.

OMIC-Engine Open Positions

Here we will keep you updated about open positions that are available in the different hubs of OMIC-Engine

A position for a postdoc fellow in Biotechnology - Synthetic Biology - Biochemistry. The position is available for 12 months with the possibility of further extension and it is based in the Laboratory of Plant and Environmental Biotechnology, Department of Biochemistry and Biotechnology, Larissa, Greece. The person employed will be part of the Research Infrastructure OMIC-Engine. He or she will work on (a) the screening of metagenomic libraries for the identification and characterization of novel enzymes with advanced catabolic properties against emerging pollutants and (b) the evaluation of novel synthetic microbial communities for the effective detoxification of persistent organic pollutants. We expect the successful candidate to have a first degree in Biosciences, and a PhD in Molecular Biology, Biotechnology or Biochemistry. Experience in heterologous expression of proteins or metagenomic library construction and bioinformatics will be desirable.

The successful candidate will be contracted with the University of Thessaly through scholarship. The monthly net salary will be 1330 euro.

For more information please contact Prof. Dimitrios G. Karpouzas,
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