The Quarterly Newsletter of OMIC-ENGINE

Q3 2020

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Editorial

Unravelling the world of Microbial Biotechnology

by Dimitis Hatzinikolaou

The general public has for long confronted microorganisms, and especially bacteria, through a rather negative perspective. This comes as no surprise, since modern Microbiology has initially been developed around the study of microorganisms that cause various diseases or contribute to food spoilage. The setting has drastically changed over the second half of the 20th century, when it was realized that the vast majority of microbial species are harmless to humans, and the central role of microorganisms in the formation and functionality of every terrestrial and marine ecosystem has been verified. Today we believe that every natural organic molecule in the biosphere participates in at least one metabolic pathway within a microbial cell. This, yet to be disproved postulate, represents the cornerstone of Microbial Biotechnology (MB), i.e. the rational exploitation of the huge metabolic potential within microbial cells for the production of chemicals, fuels and services. In that respect, Microbial Biotechnology along with Enzyme Biotechnology represent the two pillars of **White (or Industrial) Biotechnology**.

Historically though, Microbial Biotechnology has been applied in large-scale food production long before the realization of its “microbial” reasoning. For many centuries, wine, cheese, yogurt, and other fermented foods were produced through spontaneous fermentation of naturally occurring microorganisms or the use of carry-over microbial seeds from the previous batch of production. With time, Microbial Biotechnology has capitalized from the technological advances in molecular biology, next-generation sequencing, bioinformatics, high-throughput screening, and large-scale culturing systems, and managed to evolve from just a provider of alternative innovative products and processes, to a real game-changer in the efforts for **sustainable growth** and the fight against **climate change**. Today, the current application targets of modern MB include improved vaccines and better disease-diagnostic tools [1], improved microbial agents for biological control of plant and animal pests [2], modified plant and animal pathogens for reduced virulence, novel industrial catalysts and fermentation organisms [3,4] and new microbial agents for bioremediation of contaminated soil and water [5].

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The **Enzyme and Microbial Biotechnology Unit (EMBU)** at the **Department of Biology at NKUA** is involved through various funded projects in several Microbial Biotechnology areas. One of the main research areas is the bioprospecting for microorganisms and enzymes from various unique mesophilic and thermophilic Greek environments, for 2nd generation biorefinery applications. The particular geo-climatic conditions of Greece result in a multitude of diverse microenvironments characterized by a great biodiversity. Exploitation of this biodiversity has led to the development of thermophilic enzymatic biocatalytic systems for the hydrolysis of lignocellulose [6,7] in addition to the discovery and construction of wild-type and engineered microbial strains for the production of biodiesel and ethanol [8,9].

The emergence of Synthetic Biology (SB) is expected to provide additional and **more powerful tool-sets** for the construction of efficient MB biocatalysts. This is already reflected in the available SB-chassis that till today are all of microbial origin. The term "chassis" is used within the SB to denote *a microbial species that can be used as a versatile and efficient carrier and processor of any exogenous genetic information that codes for the production of specific metabolites.* EMBU, along with the Pharmaceutical Chemistry Lab (Pr. E. Mikros) represent the NKUA in OMIC-Engine team. Together with NTUA and NHRF teams, comprise the OMIC-Engine's Athens-Hub. One of the key contributions of the hub is the evaluation of thermophilic bacteria of the genus *Geobacillus,* as potential SB-chassis for performing targeted biocatalysis at elevated temperatures. A successful expansion of the array of available SB chassis to the thermophilic area is expected to significantly broaden the range of the available SB applications, through the incorporation of all benefits of high-temperature biocatalysis, such as accelerated biochemical reaction rates, minimization of microbial contamination risks, and facilitated downstream product recovery [10].

References

1. Moyle PM. Biotechnology approaches to produce potent, self-adjuvanting antigen-adjuvant fusion protein subunit vaccines. Biotechnol Adv. 2017;35: 375–389.
2. Raza W, Ling N, Zhang R, Huang Q, Xu Y, Shen Q. Success evaluation of the biological control of *Fusarium* wilts of cucumber, banana, and tomato since 2000 and future research strategies. Crit Rev Biotechnol. 2017;37: 202–212.
3. Machas M, Kurgan G, Jha AK, Flores A, Schneider A, Coyle S, et al. Emerging tools, enabling technologies, and future opportunities for the bioproduction of aromatic chemicals. J Chem Technol Biotechnol. 2019;94: 38–52.
4. Heux S, Meynial-Salles I, O'Donohue MJ, Dumon C. White biotechnology: State of the art strategies for the development of biocatalysts for biorefining. Biotechnol Adv. 2015;33: 1653–1670.
5. Vikrant K, Giri BS, Raza N, Roy K, Kim K-H, Rai BN, et al. Recent advancements in bioremediation of dye: Current status and challenges. Bioresour Technol. 2018;253: 355–367.
6. Galanopoulou AP, Moraïs S, Georgoulis A, Morag E, Bayer EA, Hatzinikolaou DG. Insights into the functionality and stability of designer cellulosomes at elevated temperatures. Appl

Microbiol Biotechnol. 2016;100: 8731–8743.
7. Kahn A, Moraïs S, Galanopoulou AP, Chung D, Sarai NS, Hengge N, et al. Creation of a functional hyperthermostable designer cellulosome. Biotechnol Biofuels. 2019;12: 44.
8. Savvides AL, Moisi K, Katsifas EA, Karagouni AD, Hatzinikolaou DG. Lipid production from indigenous Greek microalgae: a possible biodiesel source. Biotechnol Lett. 2019;41: 533–545.
9. Anasontzis GE, Zerva A, Stathopoulou PM, Haralampidis K, Diallinas G, Karagouni AD, et al. Homologous overexpression of xylanase in Fusarium oxysporum increases ethanol productivity during consolidated bioprocessing (CBP) of lignocellulosics. J Biotechnol. 2011;152: 16–23.
10. Satyanarayana T, Littlechild J, Kawarabayasi Y, editors. Thermophilic Microbes in Environmental and Industrial Biotechnology: Biotechnology of Thermophiles. Springer, Dordrecht; 2013.

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Meet iGEM Patras 2020

Students from the University of Patras use Artificial Intelligence (AI) to reduce side effects of drugs

There are two characteristics that make a medication successful: the increased effectiveness of drugs and the reduced incidence of side effects. Obviously, you do not need to be a health scientist to realize that drugs that have been widely prescribed in recent years call into question. "*That medicine doesn't work on me doctor….*" you have properly heard somebody saying after he is given a specific medicine for the first time. "*Those pills I was given last time do not do any good to me ...*" should another elderly patient tell that may have various health problems which make him take several kinds of medicine and, as a result, carry home a lot of different medicine when he visits the nearest pharmacy at the end of the day.

But why still in 2020, are there patients who complain about the drug effect that they are forced to take? Trying to answer this question, we need to focus both on the selection of the specific drug and the certain dose required, in order to help a patient with his remedy. Today, the same tactics are being followed, as they were in the last century. Before the administration of a certain drug, the factors that are taken into consideration include the personal characteristics of the patient (gender, age, weight, etc.) and also his/her history (chronic diseases, previous allergic reactions, etc.). This information combined with the personal experience of the doctor, leads to the most appropriate drug and the proposed dosage regimen.

A factor that is rarely considered and makes each person a unique case and patient is, the DNA! It is commonly accepted that depending on the genes of each patient, the required therapeutic dose can be accurately determined by minimizing side effects. The science of the above interactions is called **Pharmacogenomics** and it can explain the fact of

two patients receiving the same dose, the drug may have therapeutic effects in the former while being highly toxic to the latter.

The main obstacles that must be overcome in order to implement Pharmacogenomics in clinical practice is the lack of required training and the necessary equipment. For this reason, iGEM Patras 2020 team has developed the project Hippocrates, a **portable molecular biology laboratory**, which easily and accurately determines the required dose in patients with cardiovascular diseases, which is the leading cause of death in developed countries.

The team will be promoting their idea through the **iGEM Competition**, a global synthetic biology competition organized by the Massachusetts Institute of Technology (MIT) and conducted annually since 2003. The iGEM Patras team was established in collaboration with the Laboratory of Pharmacogenomics and Personalized Therapy of the Department of Pharmacy and the Laboratory of Technical Engineering and Oscillations of the Department of Mechanical and Aeronautical Engineering of the University of Patras. It is consisted of undergraduates, graduates of Mechanical and Aeronautical Engineers, Electrical Engineers and Computer Technology and Computer and Informatics Engineers of the University of Patras, each performing a different role in the team.

The big goal of the team from Patras, is to demonstrate the great research work of Greek Universities. Through this competition, they will try to make clear that in order to achieve your goals, nothing else is required but only an innovative idea, eager cooperation and strong motivation for work.

Join their journey by following their social media accounts on Facebook, Instagram, Twitter & LinkedIn and also by visiting their Website.

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

Archontoula Giannakopoulou

- (2013-2018) University of Ioannina, School of Health Sciences, Department of Biological Applications and Technology (Integrated Master).
- (2018-today) PhD candidate at University of Ioannina at the Biotechnology Laboratory under the supervision of Professor Haralambos Stamatis.

My research is basically focused on the development of multi-enzymatic nanobiocatalysts for cascade reactions. Multi-enzymatic cascade reactions, i.e., the combination of several enzymatic reactions in concurrent one-pot processes, have attracted recently a lot of attention since they offer considerable advantages including reduced cost and waste, shortened reaction time and increased overall production yields, offering the possibility of cell-free synthesis of complex bioproducts. The multi-enzyme co-immobilization onto various nanomaterials has recently emerged as a promising field that has already led to sophisticated nanobiocatalytic systems able to catalyze multi-step cascade processes of technological interest, including biocatalytic transformations, degradation of pollutants and development of biosensing systems.

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Briefly describe your research work.

My research is basically focused on the development of multi-enzymatic nanobiocatalytic systems for catalysis of cascade reactions of biotechnological interest. The integration of several biocatalytic transformations offer a wide range of opportunities including new paths to the synthesis of high added value products, the opportunity to mimic metabolic pathways of living cells, but also, we can develop advanced metabolic pathways that do not occur in nature. Specifically, multi-enzyme co-immobilization techniques on magnetic nanoparticles (γ-Fe_2O_3 modified with 3-(aminopropyl) triethoxysilane (APTES) were applied for the preparation of a 3-enzyme nanoconjugate comprising β-glucosidase, glucose oxidase and horseradish peroxidase. This nanobiocatalyst proved to be highly effective, presenting enhanced activity and

stability, thus, giving us the opportunity in a next step to incorporate the enzyme cellulase into the nanobiocatalytic system for the hydrolysis of cellulose in a four-step cascade reaction.

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

My collaboration with the OMIC-Engine infrastructure gave me the opportunity to gain deeper knowledge about new methods and techniques for the development and characterization of multi-enzymatic nanoassemblies that helped me to overcome efficiently any obstacles that arose during the experimental process. Also, the interaction with scientists from different research areas across Greece, helped me realize how Synthetic Biology could be applied in diverse fields, the limitations that occur in different cases and how they could be addressed. Finally, since OMIC-Engine is a multi-institutional project, it promoted the collaboration with other research teams, encouraging new projects and ideas.

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

I believe that my participation in this project has equipped me with new experience, qualities and ideas and I also acquired many valuable skills which I can exploit in future projects. The knowledge I gained about the effect of numerous parameters (substrate channeling, mass transfer limitations, architecture and compartmentalization etc.) on the overall catalytic efficiency of immobilized multi-enzymatic systems, as well as about the potential applications of such systems in various fields including biocatalytic transformations, biofuel production, synthesis of high-added value products, or the development of bioreactors will be very useful in my further studies leading to better understanding of how multiple enzymes could be combined and lead to robust biocatalysts. Furthermore, the expanded networking of the project gave me the opportunity to get in touch with researchers from different fields of expertise something that could probably lead to future collaborations not only in Synthetic Biology but also in many research areas.

Has this secondment experience matched your expectations so far?

This secondment experience has fulfilled all my expectations so far. OMIC-Engine is an infrastructure, providing the opportunity

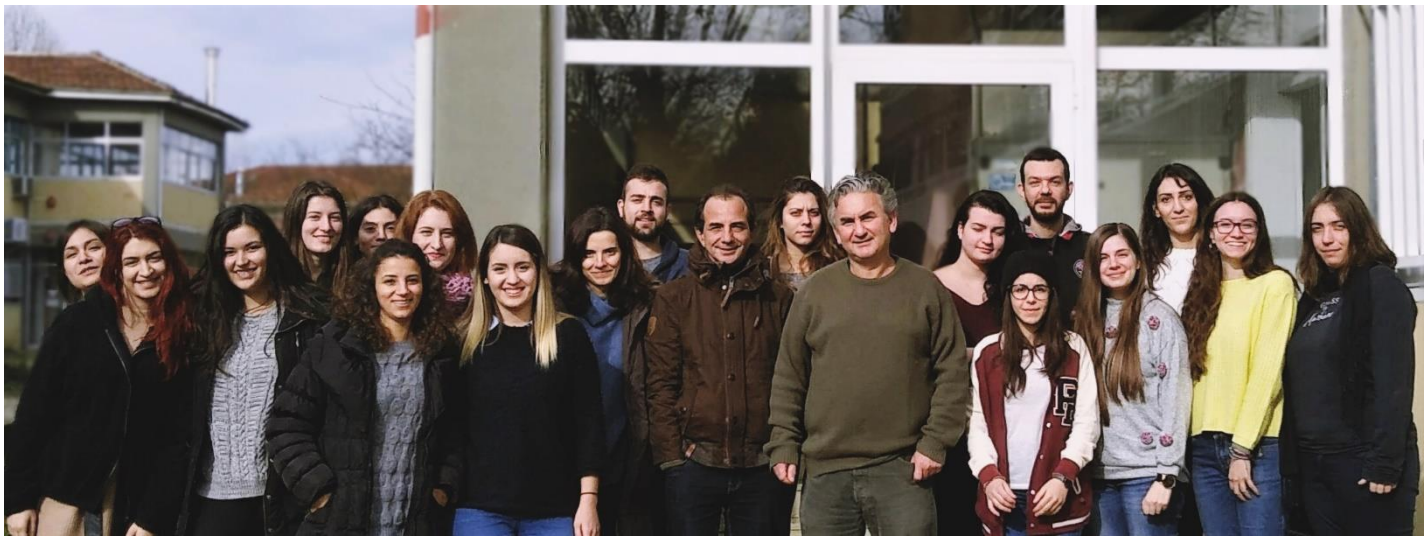
of working in an environment where I could share my experience and knowledge among people from different places of Greece and research fields that will help me to overcome any challenges in my future career. At the end of my collaboration with OMIC-Engine I gained a deeper understanding and experience about the development of multi-enzymatic assemblies incorporated in synthetic biology approaches.

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

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

Meeting the OMIC-Engine Research Groups – University of Ioannina (UOI) Node

In this section we will present each time a different hub of OMIC-Engine. This time we are presenting the research group of the Node of Ioannina participating in OMIC-Engine



Synthetic biology is an interdisciplinary branch of biology, chemistry and engineering that deals with the synthesis of complex, biologically based (or inspired) systems, which display functions that do not exist in nature. The ultimate engineering goal of synthetic biology is the **cost-competitive production** of **renewable**, **biodegradable**, and **safe materials**, new therapeutics, nutraceuticals, and bioenergy via engineered bioentities to replace current manufacturing methods. Synthetic biology can be roughly divided into two areas, ***in vivo* and *in vitro* synthetic biology**. *In vivo* synthetic biology focuses on living bioentities, which can duplicate themselves. However, some inherent constraints of living organisms (e.g., net ATP generation for cell growth and maintenance, intact cellular membrane for maintaining basic metabolism and achieving selective mass transfer and exchange) prevent them from implementing some important reactions. *In vitro* synthetic biology focuses on the construction of synthetic enzymatic pathways outside of cells to produce desired products. Moreover, cell-free systems have yet again found a niche towards the understanding of biological networks and biosynthetic pathways.

Although *in vivo* synthetic biology has been explored in a greater extent, great and rapid progress has been recently made in ***in vitro* synthetic platforms** as well. These *in vitro* synthetic platforms can be based on cell extracts, purified enzymes and multi-enzymatic systems or their combinations. Their potential applications include cell-free protein synthesis, pharmaceuticals and vaccines, nutraceuticals and bioactive products, fine chemicals, biofuels, and potentially low-cost production of bioenergy. Using such systems eliminates the laborious isolation and purification of reaction products, thus reducing costs and wastes significantly. Fewer unit operations, shorter cycle times, smaller reactor volumes, easy process control and optimization and improved space-time yields also promote atom economy, sustainability and green synthetic processes.

In this concept, **microfluidic systems** constitute a promising tool for the development of synthetic biology approaches. Such reaction systems are prepared by microfabrication techniques and take advantage of micro- or nano- fluidics to enable the use of drastically reduced volumes of reactant solutions, offering **performance of high efficiency and repeatability**. Key advantages of this technology are the rapid heat exchange and mass transfer, along with spatiotemporal reaction control under laminar flow, which cannot be achieved by conventional batch systems. When it comes to *in vitro* biotransformations, microreactor systems seem ideal for the minimization of biocatalyst volumes used while catalytic efficiencies are maximized. Immobilized enzyme or whole cells microreactors are highly preferred as microfluidic technology advances, both from a commercial and biochemical point of view. The use of **immobilized enzymes** or whole cells results in reduced operational costs and increased overall enzyme utilization. Furthermore, immobilization enhances enzyme stability at different process conditions, enzymes are commonly longer resistant to denaturation, more stable during storage, while enzyme and product separation is enabled with the possibility of further enzyme reuse. Immobilizing enzymes in the interior of a microfluidic reactor may create a highly protective environment for the biocatalyst to be repeatedly utilized for biotransformation reactions, without significant reduction of catalytic activity.

The **Laboratory of Biotechnology** (Biotech Lab- https://biotechlab.bat.uoi.gr/) **of the University of Ioannina** as a peripheral pole offers specialized support to OMIC-Engine providing biocatalytic tools, that can be used *in vitro* or in combination with *in vivo* processes, for the development of efficient biocatalytic routes for the utilization of agro-industrial byproducts and the production of high added-value products.

Bioreactors system (Bioengineering) - 2 L, 3,5 L and 15 L capacity

The engagement of Biotech Lab to OMIC-Engine rests on four main pillars: 1) a **bioreactor unit** (microbial fermenters, photobioreactors, enzyme reactors) for the *in vivo* production of novel products in various production systems (bacteria, yeast, microalgae as well as *in vitro* enzymatic processes) for semi-pilot projects with a potential scalability to respond to future industrial demands 2) **Spectrometer analysis Unit** (Circular Dichroism, FT-IR and Fluorescence spectrometers, 3) **a wet lab** for enzyme production, development of immobilized multi-enzyme systems for cascade reactions, enzyme and whole-cell continuous-flow microreactors 4) **experienced scientific personnel** consisted by postdoctoral researchers and PhD candidates having received a post-graduate education in the fields of Biotechnology, Biology, Chemistry and Material Science and Engineering. High added-value bioproducts qualitative and quantitative analysis, structural and biochemical characterization of enzymes and proteins are conducted in the Biotech Lab using HPLC, IC and GC Chromatography, UV-Vis., CD, FTIR and Fluorescence spectroscopy, Mass-Spectrometry etc.

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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

- **INteractions of Veterinary antibiotics with soil microorganisms: exploiting microbial degradation to avert Environmental contamination and ResisTance dispersal (INVERT)**

Veterinary antibiotics (VA) control microbial infections in livestock farming. VA are not particularly metabolized in vivo in animals and they are excreted in urine and feces. These are used as manures facilitating the dispersion of VA residues to agricultural soils. This practice entails risks for the environment and public health since it (a) imposes pressure on the soil microbial community for selection of antibiotic resistant traits and (b) facilitates the translocation of VAs to natural water resources and plants. However, recent studies showed that the continuous soil exposure to certain VA groups selects not only for antibiotic resistance but also for VA-degrading microbes which utilize VA as C and/or N sources. INVERT aims to **shed light in the complex interactions of VA with soil microorganisms, INVERT (project acronym) the outcome of the environmental pressure imposed by VAs on the soil microbial community from negative (selection for resistance) to beneficial (selection for energy-gain biodegradation of VA) and exploit growth-linked biodegradation to reduce environmental exposure to VA**. The project was selected for funding in the frame of the HFRI call (ELIDEK) for the support of postdoc fellows for the period 2020-2023. The PI will be Dr. Sotirios Vasileiadis, member of the Lab of Plant and Environmental Biotechnology, Department of Biochemistry and Biotechnology, University of Thessaly. Prof. Karpouzas will be the PI's advisor, while scientists from HAO-DEMETER (Dr. Zdragas and Dr Sotiraki), INRAE (Dr F Martin-Laurent), Agricultural University of Athens (Prof. Ehaliotis) and Aristotle University of Thessaloniki (Prof. Menkissoglou-Spiroudi) will collaborate in the project.

New event coming up!

OMIC-Engine goes digital! We are launching a new series of webinars and podcasts aiming to showcase the world of SynBio in Greece as well as worldwide. Our first webinar called "OMIC-Engine meets iGEM" will be held on Tuesday 10th of November at 16:00pm EEST. This webinar aims to take a look at this new world through the perspective of the upcoming Synthetic Biologists. We will discover the research projects of iGEM Athens, Thessaly, and Patras, the Greek teams that are taking part in this year's Virtual Giant Jamboree happening from November 14 till November 22. There, we will have the opportunity to discuss with the teams about their work and contribution to the world of Synthetic Biology.

For more details follow **#synbioGR** and stay tuned through our social media and website.

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Co-financed by Greece and the European Union

OMIC-Engine is implemented under the Action "Reinforcement of the Research and Innovation Infrastructure" funded by the Operational Programme "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014-2020) and co-financed by Greece and the European Union (EU Regional Development Fund)