



ALGAECOM NEWSLETTER

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EDITORIAL

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New People in the

ALGAECOM project

Three new postdoctoral researchers have been recruited by the ALGAECOM project. To the next lines the new postdocs present themselves giving informations regarding their background, their role in the project and their expectations.



Dörte Randewig

"I have been recruited by ALGAECOM project since 1st of May 2013, contracted by the Agricultural University of Athens (AUA), Greece. Before I started my PhD research at the Institute of Tree Physiology in Freiburg, I studied Biology at the Department of Experimental Ecology and Ecosystem Biology at the University of Bielefeld. During my studies my interest in research was focused on plant biology and plant physiology, especially in relation to global change drivers and to factors of environmental issues, such as global warming or greenhouse gases. In my PhD research I had the opportunity to work on such a topic by investigating plant responses to SO₂ at the physiological and molecular level of woody (*i.e.*, *P. x canescens*) and herbaceous (*i.e.*, *A. thaliana*) plants.

The main focus of my work in the AlgaeCom project is the metabolomic and transcriptomic analysis of microalgae under different culture conditions. A very important aspect in the study of systems biology of microalgae organisms is the focus on their metabolome. Here, the performance of modern metabolomic studies are aimed to identify and quantify the metabolites which are of biotechnological importance such as amino acids, lipids, vitamins, natural products *etc.* and finally to get an insight in the biochemical and physiological state of the cell and the organism. With the establishment of metabolomic and transcriptomic resources we will be able to provide a valuable tool for the identification of novel metabolites and metabolic pathways for the selected microalgae. Moreover, these resources will provide a kind of label and will be a very efficient mean in the application of biotechnology for microalgae production technique and their products.

I am convinced that this post in the AlgaeCom project will be a great opportunity for myself. I do expect wide opportunities for developing my professional career *e.g.*, to further develop my scientific profile by acquiring new knowledge, new skills in organization and project management as well as more practical expertise in other plant physiological methods. Working and deepening my knowledge by using state-of-the-art "–omic" technologies is a further positive side of this post. The collaboration in a multidisciplinary network with other researchers will provide me new perspectives and deeper insights in other interesting research fields."



Flavien Dardelle

"I have been recruited into the ALGAECOM project the 1st of July of 2013. I dispose of a 22 months contract in the company of Fitoplancton Marino, S.L., located in Spain.

I originate from France where I have had a solid basic education in carbohydrate biochemistry at the University of Rouen. After different lab trainings, I orientated my know-how and my knowledge to the field of polysaccharide extractions and analysis in plants. My PhD, realized on the pollen cell model in the lab GLY-CO-MEV (Glycobiology – Plant Extracellular Matrix, Rouen), brought me an additional knowledge in physiology and microscopy, while reinforcing my knowledge in carbohydrate analysis. Following this enriched experience, I continued with a post-doctoral position (in the same lab), participating in the determination of the *N*-glycosylation pathway and its relative structures associated in the *Chlamydomonas reinhardtii* microalgae model, in collaboration with the University of Munster (Germany) and the University of Madrid (Spain).

My aim into the AlgaeCom project is to collect, to analyze and to obtain a library of different extracts of polysaccharides coming from selected microalgae. The diversity of the used microalgae also represents a diversity of carbohydrates that could be useful and provide any interest in cosmeceuticals. So, the physical as the biochemical properties of the polysaccharides could be investigated. However, far from substituting to the work of our French partner (CNRS, Grenoble) specialized in the detailed carbohydrate analysis, my goal will be also to extract carbohydrates at a factory's scale. In parallel, with the hypothesis that gene expression in microalgae is correlated to the polysaccharide biosynthesis and so, to the quantification of carbohydrates, we will attempt to identify molecular markers, using the molecular biology platform set up at the Fitoplancton Marino facility. Altogether, I expect from my research, to extract new carbohydrates of interest with a high value as well as to provide new evidences or basic information that could enrich our knowledge in microalgae."





Sophia Letsiou

"Hello everyone,

I have been recruited by ALGAECOM project since 1st of December 2013, under a 22 months contract with APIVITA SA. I hold a PhD in Biomedical Sciences with emphasis on Biochemistry from the Harokopio University in Athens, a MSc in Analytical Chemistry and a BSc in Chemistry from the University of Crete. Specifically, during my PhD studies I worked on proteomics field, trying to determine selenoproteins in human plasma and to understand selenium biochemistry. At the meantime, I was trained on Biostatistics and Chemometrics in the University of Copenhagen. The three last years, I worked as a postdoctoral researcher at the University Medical Center in Utrecht (UMCU). My project was a collaboration among UMCU, the Netherlands Metabolomics Center (NMC) and Merck Sharp & Dohme, MSD with a focus on biomarker diagnostic research in the field of metabolomics. In particular, this research combines data analysis with state-of-the-art mass spectrometry instrumentation and biostatistics. The disease of interest was endometriosis. In the ALGAECOM project, I am going to explore the first part of -omics cascade (genomics-transcriptomics) so I will work on a RT-qPCR platform for the transcription analysis of skin related genes as well as on the assessment of the effect of microalgae derived products on the transcription of skin related genes using cell lines and in vitro skin model. This will be achieved in collaboration with our partners from the Agricultural University of Athens (AUA). Furthermore, another part of my project will be the formulation and the development of new algae-based cosmetic products that will take place in APIVITA's R&D department. Last but not least, I will be involved in the set up of a Biochemistry laboratory at APIVITA giving another important dimension to the research that takes place in APIVITA.

AlgaeCom is a promising project and I do expect to broaden my knowledge on -omics area to collaborate with experts in different fields as well as to transfer knowledge from the academia to industry."



Partner's New Laboratory

FITOPLANKTON MARINO, S.L.: Set-up of a new molecular biology laboratory

During the last year, a new molecular biology laboratory has been set-up at the company Fitoplancton Marino, S.L. It has been equipped with all the necessary devices for the basic molecular biology techniques, including systems for cell disruption and nucleic acid quantification in the pre-PCR area, as well as thermocyclers for standard and real-time PCR in a separated post-PCR area. Throughout this year, procedures for RNA isolation have been optimized for different strains of microalgae commonly cultured in the company. In parallel, genetic information available at database GenBank for some of the species has been used to design primers for a wide range of genes involved in main metabolic pathways. A real-time qPCR platform has been developed to study differences in the expression levels of key genes under different culture conditions. All the information generated using molecular biology tools will be used in the quality control of cultures, and will help to improve productivity of bioactive compounds with commercial interest through optimization of culture protocols. As a proof of concept, expression profiles of a range of genes involved in the biosynthesis of the omega-3 fatty acid eicosapentaenoic acid (EPA) have been determined in *Nannochloropsis gaditana* (strain B3) for different culture systems, both in outdoor (photobioreactors for production) and indoor conditions. Additionally, influence of some physical parameters of water known to have a significant impact on EPA content in *Nannochloropsis gaditana* have

been evaluated in order to find putative molecular markers for EPA productivity. Benefits derived from the application of biotechnological tools to industrial production systems will be surely seen in the next years.



Picture of the pre-PCR laboratory



Picture of the post-PCR laboratory

1st AlgaeCom workshop

From the 24th to 28th of June, the 1st AlgaeCom workshop on “Microalgae Bioactive Ingredients in Cosmetics” was held at the new APIVITA plant. This 1st scientific workshop was held directly after implementing the new infrastructure of APIVITA (inaugurated in February 2013) and was therefore, a good opportunity to get to know each other. Next to the AlgaeCom project partners, a number of graduate and post-graduate students from the Agricultural University of Athens (AUA) as well as members of APIVITA staff, scientists and employees from the marketing/sales departments, attended the workshop.

The workshop started with a welcome talk by APIVITA’s vice president, Mr. Nikolaos Koutsianas, followed by an introduction to GLP and GMP regulations for cosmetics by the plant director Mr. Karafylakis.

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The next day, the head of the R&D department, Mr. Giota Dragani presented an overview of the company, about its history, philosophy and evolution through the years. The first scientific talk by Dr. Konstantinos Gardikis, was about extraction techniques used in laboratory and industrial scale. Special emphasis was focused on the techniques implemented by APIVITA in order to produce microalgae extracts out of freeze-dried microalgae material. Moreover, preliminary results concerning the microalgae extracts were presented and discussed during the presentation. Furthermore, an overview of the ongoing research programs of APIVITA was given. The next talk on “EU regulation” by Ms. Sophia Papaspirou was about regulatory aspects of the use of algae and microalgae in cosmetic products. Special focus was given on the Registration, Evaluation and Authorization of Chemicals, Classification, Labelling as well as Packaging. The introduction in cosmetic regulatory issues was very informative by giving an overview of a complex framework of production and formulation techniques for microalgae to be used in cosmetic industry. The final talks of this session was given by Ms. Mariliz Gika and Ms. Danai Georgiou entitled as “Algae and microalgae in cosmetics (Total extracts, proteins, sugars)”. A detailed presentation of the mechanism of action of proteins and polysaccharides on the human skin and their use in cosmetics was addressed. The commercial applications of these biomolecules derived from algae or microalgae were further discussed including in-vitro efficacy studies and clinical tests.

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The next day, practical exercises were scheduled. An excursion took place through the botanical gardens and the beehives of APIVITA – that provide the greatest part of APIVITA’s raw materials, such as medicinal and aromatic plants and bee products like honey, propolis and royal jelly. In the afternoon, the workshop attendees visited the laboratory of APIVITA. During an extended laboratory presentation, R&D technicians presented the physicochemical background of special formulation processes. Three formulations were developed in the new APIVITA R&D labs: a tightening gel, a tonic shampoo and an after sun cream, all of them incorporating either algae or microalgae extracts.



Excursion to the Beehives of APIVITA



Laboratory presentations by R&D technicians.

At the 27th of June, the workshop was placed at the AUA. The topic of the morning sessions was on the metabolomics theory and its application on plants and algae profile analysis. Dr. Dörte Randewig, post doctoral researcher of AlgaeCom project at the AUA, gave a presentation entitled as “Introduction in Metabolomics” by addressing different Metabolomics technologies along with a workflow from data analyses to visualization. Ms. Maria Patelou and Ms. Chrysanthi Kalloniati, both PhD students of the Lab of Molecular Biology of the AUA, were presenting their work on the Metabolomics study in the Microalgae *Chlorella vulgaris* and the legume plant *Medicago truncatula*, respectively. Their talk was especially focused on GC-MS analysis, on its application in plant samples and on how GC-MS analysis can reveal biochemical regulations in plants. Afterwards a dinner of the AlgaeCom partners took place. Scientific and administrative aspects of the project were discussed .

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In the closing session at APIVITA’s new plant, it was concluded that the presentations and demonstrations covered all aspects of important procedures and steps that takes place in the cosmetic industry.

2nd AlgaeCom workshop

The 2nd workshop of the ALGAECOM project (IAPP 286354) took place in El Puerto de Santa María (Cádiz, Spain) between the 9th and the 11th of September 2013, under the title “Enhancing microalgae production for cosmetic industry. Growing systems, harvesting and processing”. It was organized by Fitoplancton Marino, S.L. (FITMAR), one of the SME partners involved in the project. The General Manager of FITMAR, Carlos Unamunzaga, opened the session by giving a general overview about the history and the philosophy of FITMAR Company. The commitment in the AlgaeCom project as well as their recent integration in Hisparroz S.A., which is linked to Ebro Foods S.A. (the first group in the food industry in Spain) giving the company a promising opportunity combining research and industry, and, moreover, a great chance of improving and developing as a product company. In this respect, two researchers were recruited by FITMAR, and were especially assigned in the field of molecular biology (therefore a new laboratory has been recently set up by the company) and biochemistry of polysaccharides in

microalgae. Within the workshop sessions, both researchers inspired by talking about the great needs to be carried out in their research fields *e.g.*, by introducing the complex world of polysaccharides in microalgae (Dr. Flavien Dardelle), and how different molecular biology tools can be applied to monitor and enhance production of bioactive compounds of interest in these organisms (Dr. Carlos Infante). FITMAR invited also guest speakers introducing important aspects on the manifold research fields in Algae science. Dr. Pedro Cañavate (IFAPA Centro *El Toruño*, Junta de Andalucía, Spain) gave a general overview of the lipid world in microalgae, with their distinctive features. Dr. Olivier Coste (IFAPA Centro *El Toruño*) focused in his presentation on the range of bioactive compounds that can be found in seaweeds, with their potential applications in the field of aquaculture and other industries. Dr. Manuel Manchado (IFAPA Centro *El Toruño*) made a summary of the project AGUAGENET in which biotechnology, including Next Generation Sequencing tools, can be applied to improve aquaculture of different species. As a closing remark, the project coordinator Dr. Emmanouil Flemetakis (AUA, Greece) presented main preliminary results on microalgae production for the obtaining of high added value cosmeceuticals. For this purpose, extracts of three different microalgae species were grown under several stress conditions in the FITMAR facility. The Workshop also included a visit to the FITMAR facilities, where all the participants got a detailed insight in the specialised microalgae production systems, which is in high commitment to the specific aims and goals of the AlgaeCom project.

Workshop presentation by Dr. Carlos Infante on “Gene expression analysis in microalgae as a molecular tool to enhance productivity of selected bioactive compounds.”



Midterm Meeting

The ALGAECOM mid-term meeting was subsequently held after the 2nd workshop on the 12th of September. Representatives of all the partners, involved in the AlgaeCom project, presented their current state of research.

- Dr. Dörte Randewig (AUA, Greece) presented the novel results on the metabolomics analysis of three different strains of Microalgae with special focus on Amino acids, Antioxidants, Fatty acids and Stable Isotope profiling.
- Dr. Konstantinos Gardikis (APIVITA, Greece) presented preliminary results of the extractions of the same strains of microalgae in butylenes glycol, concerning physico-chemical properties, stability over time, antioxidant properties, and total polyphenol content. Furthermore the preliminary results of the nano-encapsulation of microalgae ingredients into β -hydroxypropylcyclodextrin were presented.
- Dr. William Helbert (CNRS, France) gave a summary on the progress in the implementation of a purification protocol of microalgae polysaccharides. Moreover, a detailed structure analysis of purified β -glucan and glucuronomannan of *Phaeodactylum sp.* were undertaken. Aiming at producing new series of oligosaccharides, a collection of marine bacterial strains isolated from Rotifer and Artemia were grown in the presence of microalgae.
- Dr. Carlos Infante (FITMAR, Spain) presented the new molecular biology laboratory that has been set up by FITMAR, in which different protocols were optimized for microalgae disruption, RNA isolation, standard PCR, and quantitative real-time PCR. Using these tools, the expression of a range of selected genes involved in PUFA biosynthesis has been analyzed under different culture regimes and conditions in the microalgae *Nannochloropsis gaditana*. A group of potential genes involved in oxidative stress response was also selected in this species in order to quantify their expression as a quality control of outdoor and indoor culture systems developed in the company.

DID YOU KNOW

Since 1979 APIVITA has developed a unique philosophy to develop every product by combining three basic elements: Nature, Effectiveness and Holistic Wellbeing. In APIVITA, we have developed over 5,000 formulas based on natural raw materials throughout the past 32 years of operation. This has guided us to acquire extensive know-how in the development of only the best natural cosmetics in the industry.

Algae is one of the ingredients that is used in APIVITA products since algae act as a protective shield for skin's micro capillary integrity and against both anti-aging and photo-aging caused by UV radiation. Currently, three algae derived products are available. A face cream for sensitive skin with SPF50+ that contains *Ascophyllum nodosum* and *Asparagopsis armata* as basic ingredients. *Ascophyllum nodosum* is a brown seaweed, also known as Norwegian Kelp that grows in cold, crystal clear water withstanding both freezing in winter and full exposure to the sun in summer. It contains over 60 known minerals and elements more than 12 vitamins, carbohydrates as well as a full range of amino acids. It also contains secondary components that are made up of polysaccharides. *Asparagopsis armata* is a red alga commonly known as Harpoon weed. It was first recorded in Algeria in 1923. It is believed that its inadvertent introduction to mainland Europe in 1925 was associated along with the deliberate import of oysters. It has been demonstrated that the most abundant water soluble polysaccharides found in *Asparagopsis armata* are sulphated galactans that help prevent the appearance of signs of aging especially when the skin is exposed to external stresses such UV, atmospheric pollutants and other deleterious agents. Another algae derived product is a body contouring oil. That product contains *Fucus vesiculosus*, as basic ingredient, which is found on the coasts of the North Sea, the western Baltic Sea, and the Atlantic and Pacific Oceans. Traditionally used in the diets of people residing bicoastal areas. This seaweed is commonly used in anticellulite products since activates fat lipolysis and has firming, detoxifying as well as decongestant properties. The last product is a firming and restoring rich face cream (Queen Bee) where Pepha-Tight is contained along with honey and royal jelly. Pepha-Tight is a highly purified biotechnologically produced extract from the microalgae *Nannochloropsis oculata*, combined with a well balanced fraction of polysaccharides. It functions as a skin tightener. It has been shown to protect human fibroblasts from oxidative stress, simultaneously increasing the formation of collagen-I thereby promoting a long term tightening effect through the strengthening of the skin's connective tissue.

The exploitation of the Greek microalgae diversity for the development of novel high added value cosmeceuticals is in APIVITA interest since we believe that every detail matters when create and develop each product. With this philosophy APIVITA promises and delivers effective cosmetic products that provide solutions to skin needs and problems.



Body Contouring



Face Cream for Sensitive Skin SPF



Queen Bee Face cream