



Algae for Aquaculture and Beauty

Newsletter 6 – December 2018

Consortium



Project Coordinator
CNRS, CERMAV
France



Fitoplancton Marino, S.L.
Spain



Agricultural University Of
Athens
Greece



Instituto Andaluz De
Investigacion y Formacion
Agraria Pesquera
Alimentaria
Spain



Apivita SA
Greece



Centro De Ciencias Do Mar
Do Algarve
Portugal



Lifesequencing, SL
Spain

The project

Microalgae were always an exciting target for Aquaculture, Cosmetology and Biotechnology, as they represent a largely untapped reservoir of novel and valuable bioactive compounds.

The ALGAE4A-B (Algae For Aquaculture and Beauty) project seeks to exploit microalgae diversity, as a source for state-of-the-art high-added-value biomolecules in aquaculture and cosmetics.

ALGAE4A-B aspires to foster both European capacity building and the strategic objectives of EU Blue Growth and Marine Biotechnology to harness the untapped potential of European seas and coasts for training and sustainable growth.

Microalgae Biomass Production

The diversification of microalgae biomass production towards two independent applications will give the microalgae industry access to alternative markets in an uncertain, highly competitive and fast changing commercial environment.

Basic and applied research

The project combines both basic and applied multidisciplinary research in the fields of -omics technologies, biochemistry and applied biotechnology in order to:

- Develop and optimize low-input and application-based microalgae culture systems
- Develop “-omic” resources for both microalgae and fishes
- Develop downstream processing of high added value products from microalgae, with an emphasis on polysaccharides, proteins, enzymes and antioxidants
- Develop, formulate and evaluate in vitro a new range of cosmetic and nutraceutical products for aquaculture

Key figures

972 000 € EC funding
7 partners
4 years (2016-2019)

More information on www.algae4ab.eu

Summer school 2 – 17-21 September 2018 - Andalusian Institute of Agricultural Research and Training (IFAPA) – El Puerto de Santa Maria, Spain

“Advances in fish aquaculture and microalgae biotechnology”

Monday 17th September 2018

09:30-10	Welcome and Registration	
Morning session		
10:00-10:45	Algae4A-B Project Overview	Member of project
10:45-11:30	Genomic approaches and breeding in fish aquaculture	M. Manchado
11:30-12:00	Coffee break	
12:00-12:45	La I+D en la empresa	Ricardo Zerolo
12:45-13:30	Fish tagging techniques for longitudinal approaches	M. Manchado
13:30-15:30	Lunch break	
Afternoon session		
15:30-17:30	Practical session. Fish handling and tagging	Mol Biol Lab. Toruño
17:30-18:00	Closing remarks	Manuel Manchado

Workshop organization:
Dr. Manuel Manchado
IFAPA

Workshop venue:
Centro IFAPA El Toruño
Carretera N. IV Km. 654°
Camino de Tiro Pichón,
11500 El Puerto de Sta María
Spain

Tuesday 18th September 2018

Morning session		
10:00-10:45	Algae Biotech: making sense using NGS approaches	Juan Martínez
10:45-11:30	Insights into microalgae evolution	Carlos Infante
11:30-12:00	Coffee break	
12:00-12:45	Microalgae nutraceuticals	Jose P. Cañavate
12:45-13:30	Microalgae as a source of bioactive compounds to improve the fish health in aquaculture	C. Carballo
13:30-15:30	Lunch break	
Afternoon session		
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15:30-17:30	Practical session: Evaluation of microalgae and embryos extracts (1)	Mol Biol Lab. Toruño
17:30-18:00	Closing remarks	Manuel Manchado



Talks are available on www.algae4ab.eu



More information on www.algae4ab.eu

Algae4A-B at “Science is wonder-ful” during the European Researchers' Night



Dr. **Sophia Letsiou**, Manager of Biochemistry Lab in **APIVITA** was invited by the EU Marie Skłodowska Curie Committee to present APIVITA research in Horizon 2020 Algae4A-B project on the open event “**Science is wonder-ful**” during the European Researchers’ Night held in Brussels on 29th September 2018. In this event funded annually by the **Marie Skłodowska-Curie actions** (MSCA) under the Horizon 2020 programme, European researchers present their work so as to make the work of scientists familiar to audiences all over Europe. Students, teenagers and grown-ups from Brussels, the Netherlands and UK were present in this event and discussed with Dr Letsiou the research of Algae4A-B in APIVITA.

Algae4A-B at INNOVAZUL2018 - <https://www.innovazul.es>



Dr **Manuel Machado**, Head of Molecular Biology Lab in IFAPA and the staff of APIVITA **Vasiliki Ioanna Boka**, **Sofia Papaspyrou** and **Elena Spanidi** participated in the 1st International meeting on knowledge and blue growth, **INNOVAZUL2018**. This meeting aimed at driving private & public competitiveness towards a circular economic model and a sustainable development. IFAPA had a stand at InnovAzul 2018 that was an **essential meeting point for technology transfer**. The members of the project participated in the event and were part of the IFAPA’s stand to show the main activities of the project that included some posters with results. Also, Manuel Machado participated in a **round table about the future of aquaculture in Europe** highlighting some activities done in Algae4AB as part of the main challenges for Southern Europe aquaculture.

Algae4A-B Implemented secondments

Research and Innovation Staff Exchange (RISE) projects fund short-term exchanges (“secondments”) for staff to develop careers combining scientific excellence with exposure to other countries and sectors. RISE enables more interaction between academia and non-academic organisations within Europe and worldwide.

CCMAR – APIVITA

Ana Patricia Mateus – 2 weeks

The objective of the secondment was to evaluate the potential changes in the expression of genes regulating human skin healing, cell migration and repair in the presence of human CRTAC1 after cell damage. The secondment continued the work initiated by João Cardoso at APIVITA in a previous secondment. The overall aim of the secondments was to establish a reliable and reproducible experimental damage procedure for a confluent monolayer of human dermal fibroblasts.

APIVITA – CNRS

Konstantinos Gardikis – 3 months

The aim of the secondment was to transfer isolation techniques of polysaccharides and more especially micro-algal beta-glucan. Analysis methods such as sugar composition were at the core of the secondment.

IFAPA - APIVITA

Israel Guerrero Cózar – 3 months

The main objective of this secondment was the evaluation of biological effects of microalgal extracts and yeast β -glucans using two types of encapsulates (CD and HRF) on human dermal normal fibroblast (HDNF). Exposure trials under standard conditions as well as inflammation and oxidative stress challenges were carried out. Biological effects were tested using the cell proliferation test (MMT) and transcriptomic responses (qPCR).

LIFESEQ – AUA

Daniel Carrillo Bautista – 1 month

The objective of the secondment was to analyze transcriptomic data from *Tetraselmis chuii* grown under different light conditions. In order to see differences in gene expression levels for this eukaryotic microorganism without reference genome we assembled RNA (cDNA) reads, obtained counts and performed statistical tests for the evaluation of gene expression.

More information on www.algae4ab.eu

AUA – FITMAR

Emmanouil Flemetakis – 3 months



During this secondment period, analysis of gene stability under different culture conditions in the microalgae species *Tetraselmis chuii* was accomplished. A set of 18 candidate housekeeping genes (HKGs) was evaluated

in samples collected in outdoor photobioreactors and indoor flasks. RNA samples were isolated and cDNAs were further constructed for further real-time PCR amplification. Ct values were recorded and analysed using two different bioinformatic tools, geNorm and NormFinder. NormFinder analysis of the combined data revealed *TchcdkA* as the most stable gene with a stability value of 0.26. In this rank, *TchRPS10* was the second most stable gene (0.27), followed by *TchACT* (0.27) and *TchEF1a* (0.31). Altogether, data revealed *TchEF1a* and *TchRPS10* as a suitable couple of reference genes to be used in gene expression normalization. These results represent a novel contribution for the potential application of molecular biology tools to control, by measuring the expression of a set of selected genes, the appropriate culture performance and productivity of selected compounds by *T. chuii* in outdoor production systems.

CCMAR – APIVITA

Rita Costa – 2 weeks

The secondment aimed to continue the evaluation of the effect of CRTAC, a highly conserved protein (both in sequence and structure) across animals, bacteria and algae on the proliferation and recovery of human skin. The main purpose of the secondment was to perform molecular analysis, by real-time quantitative PCR, of target genes in human samples obtained from *in vitro* scratch assays performed by João Cardoso in a previous secondment to the APIVITA Bio Lab.

FITMAR – AUA

S. Torres – C. Unamunzaga – 1 month

The objective of the secondment of Sonia Torres and Carlos Unamunzaga in AUA, under WP3, was the preparation and initial evaluation of *Tetraselmis chuii* cell-free extracts, from microalgae biomass collected from the FITMAR outdoor large-scale production photobioreactors (PBRs) during different sampling times. For this purpose, *T. chuii* biomass was collected from three PBRs, during three consecutive days, both early in the morning and at midday. Cell-free extracts were prepared and used to characterize and evaluate the potential bioactivities and protective effects against oxidative stress, in order to evaluate differences on the antioxidant capacity of the *T. chuii* biomass due to the accumulation of photosynthetically produced O₂ in PBRs.

LIFESEQ – CCMAR

Eric Climent Sanz – 1 month

Eric conducted bioinformatics analysis on new samples obtained in the context of the experiments with Senegalese sole larvae in WP5. In this WP, the route of application of microalgae-based compounds was assessed to determine their role as a pre-biotic; specifically, an oral intubation procedure was performed. The microbiome from the ocular skin and the middle intestine was evaluated.

APIVITA – IFAPA

Sofia Papaspyrou – 2 months

This secondment aimed at the assessment of the biological effect of two new extracts of microalgae (A-345-1 and A-56-1) supplied by FITMAR. For the evaluation, benthic fish were used. At a first stage, seabream embryos were handled as a previous step to the trials and the protease test (azocasein) was carried out to characterize the samples. For the bio-assay, fish were treated with the above extracts, the experimental conditions were optimized, and the mortality was monitored. Then, molecular analysis and gene expression studies were carried out using larvae. Eight genes involved in antioxidant defenses, growth and the innate immune system were quantified. Results revealed an increased expression for myogenic factors and reduction in apolipoprotein in larvae treated with extract A-56-1 indicating this extract may be a good candidate to promote growth. These data are considered important for further evaluation of the use of these microalgae extracts in industries like cosmetics, food etc.

APIVITA – CCMAR

Sophia Letsiou – 3 months

The aim of this study was the *in vitro* toxicity assessments of extracts or compounds as well as the development of fish epithelial model so as to build up a comparative model for human skin according to the project deliverables.

FITMAR – AUA

Carlos Unamunzaga – 2 months

The objective of the secondment at the AUA has been the development and evaluation of a purification method for protease activity from *Nannochloropsis gaditana* extracts based on aqueous-two phase system (ATPS). Stopped spectrophotometric measurements were used for protease activity determination in microalgae extracts using as substrate azo-gelatine and azo-casein. The method could be used in future studies for the purification of other novel proteases from other microalgae species.

FITMAR – AUA

Carlos Infante – 2 months

The aim of the secondment was to receive training on the analysis and data mining of RNAseq data obtained by AUA on the microalgae *Tetraselmis chuii* grown under different light regimes. During this secondment we used the recently established draft transcriptome in order to identify genes coding for key enzymes involved in starch and lipid biosynthesis in *T. chuii*. These sequences were used for designing gene-specific primer pairs for the determination of the respective transcript levels using the RT-qPCR based platform under development within the project. At the same time, training was received in the analysis, evaluation and interpretation of raw RNAseq datasets using cutting-edge bioinformatics tools at the laboratory of Molecular Biology of Agricultural University of Athens.

LIFESEQ – AUA

Juan Martinez – 1 month

The aim of the secondment was to analyse the RNAseq data obtained from human primary skin fibroblasts treated with microalgae derived exopolysaccharide samples during the secondments of AUA scientists in LIFESEQ, using state-of-the-art bioinformatic tools. At the same time, AUA scientists received training on the correct use of the respective bioinformatic tools.

More information on www.algae4ab.eu

Algae4A-B Implemented secondments

Research and Innovation Staff Exchange (RISE) projects fund short-term exchanges (“secondments”) for staff to develop careers combining scientific excellence with exposure to other countries and sectors. RISE enables more interaction between academia and non-academic organisations within Europe and worldwide.

APIVITA – IFAPA

Eleni Spanidi – 2 months

The secondment was aimed at the study and identification of microalgae using molecular markers as well as to evaluate the genomic responses in sole fed with two diets specially prepared for the project containing two encapsulates (HRF and beta-cyclodextrins) with microalgal extracts from *Phaeodactylum tricorutum* or yeast beta-glucans. The 18SrDNA markers was used as a target molecule for microalgae identification. PCR amplification followed by sequencing was carried out in target samples as a training for raw material traceability. For the diet evaluation, 50 dph fish was used and growth was monitored for two weeks. Biometric techniques were applied to determine the length gain and gene expression analyses were carried out to assess bioactivity.

APIVITA – IFAPA

Vasiliki Ioanna Boka – 2 months

The main objective of this secondment was the evaluation of biological effects of microalgal extracts (A-345-1 and A-56-1) prepared by FITMAR using as target fish post-larvae. Training in embryos and larvae handling was carried out as an initial step before carrying out the bioassays of these new microalgal extracts. A trial was designed using post-larvae and transferring small amounts of extract through live preys. The assay was optimized and mortality was monitored. Moreover, larvae were fixed for molecular analyses to quantify expression levels of a set of 8 genes related to innate immune response, growth and oxidative stress. Results indicated that a high mortality of extract A-345-1 that clearly differentiated from the control using the panel of genes evaluated. However, the extract A-56-1 appeared to act as an immunostimulant. The results obtained are the first step to identify the biological function and commercial applications of these new microalgae extracts

LIFESEQ – CCMAR

Juan Martinez – 1 month

The main objective of the secondment was to conduct analysis into the new samples obtained in the context of the experiments in WP5. In the experiments of WP5 the potential route of application of microalgae-based compounds in *Senegalese sole* was assessed. The objective was to assess their potential role as a pre-biotic and to study administration by oral intubation. The skin from the ocular side and the middle intestine were collected into RNAlater in a laminar flow cabinet and used for microbiome analysis. The objective of the present secondment was to study the microbiome of the skin and intestine using 16S rRNA. A scientific manuscript based on the results of the pilot study used to optimize transcriptome studies with the sole and to perform microbiome analysis was prepared.

FITMAR – AUA

Lalia Mantecon – 2 months

During the previous year of the project a major biomass production and evaluation experiment was performed in FITMAR, in order to evaluate the effects on *T. chuii* biomass quality of light intensity and thus O2 accumulation in large scale outdoor PBRs. Thus, in FITMAR production facilities, three 2000-L photobioreactors were sampled during three consecutive days, both early in the morning (just before dawn) and at midday (with maximum light intensity), for -omics analyses. In this line of work, the objective of the secondment of Lalia Mantecón in AUA, under WP1, was the determination of *T. chuii* metabolic properties under these conditions, using the available metabolomic platforms available in AUA. Previously developed and optimized protocols were used in order to obtain global metabolomic profile of the biomass. The results will provide FITMAR with novel and valuable information about the metabolic properties of the biomass and will allow the design and optimisation of production and harvest routines as a tool for the production of customized and application-optimized *T. chuii* biomass.

Algae4A-B project results dissemination

Events:

Oceans Meeting - 7th-8th September 2017 – Lisbon, Portugal

31st ESCPB Congress - 9-12th of September 2018 - Porto, Portugal

Publications:

Understanding pseudo-albinism in sole (Solea senegalensis): a transcriptomic and metagenomic approach

Patricia I.S. Pinto, Cláudia Guerreiro, Rita Costa, Juan F. Martínez-Blanch, Carlos Carballo, Francisco Codoñer, Manuel Machado, Deborah M Power – **Scientific reports** – Submitted

Global transcriptomic and metabolomic adaptation of Nannochloropsis gaditana grown under different light regimes

Maria Patelou, Carlos Infante, Flavien Dardelle, Dörte Randewig, Evangelia D. Kouri, Michael K. Udvardi, Eleni Tsiplakou, Lalia Mantecón and Emmanouil Flietakis – Submitted

Outreach activities

Epigenetic reprogramming using microalgal extracts to modify the larval tolerance of sole larvae.

Ana Manuela Crespo, Carlos Carballo, Lalia Mantecón, Manuel Machado.

<http://www.mispecies.com/nav/actualidad/noticias/noticia-detalle/Investigadores-usan-extractos-de-microalgas-para-modular-la-plasticidad-larvaria-de-lenguado-y-la-tolerancia-de-alevines-a-la-temperatura/#.XCEjMy3MzOR>

More information on www.algae4ab.eu



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