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Leader: Helena Peres

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Nature based solutions for estuarine areas recovery – targeting priority and emergent pollutants
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Non-invasive tools to assess fish welfare and disease susceptibility under farming conditions
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Bio-warfare: role of animal venoms to assist the molecular treatment against COVID, cancer and other diseases
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Assessing pre-exploitation baseline numbers and population dynamics of the European sardine *Sardina pilchardus* Walbaum, 1792 using palaeogenomics
Leader: Paula Campos

Reference INPhINIT/CIIMAR_2023_08
Natural Products discovery from African cyanobacteria
Leader: Pedro Leão

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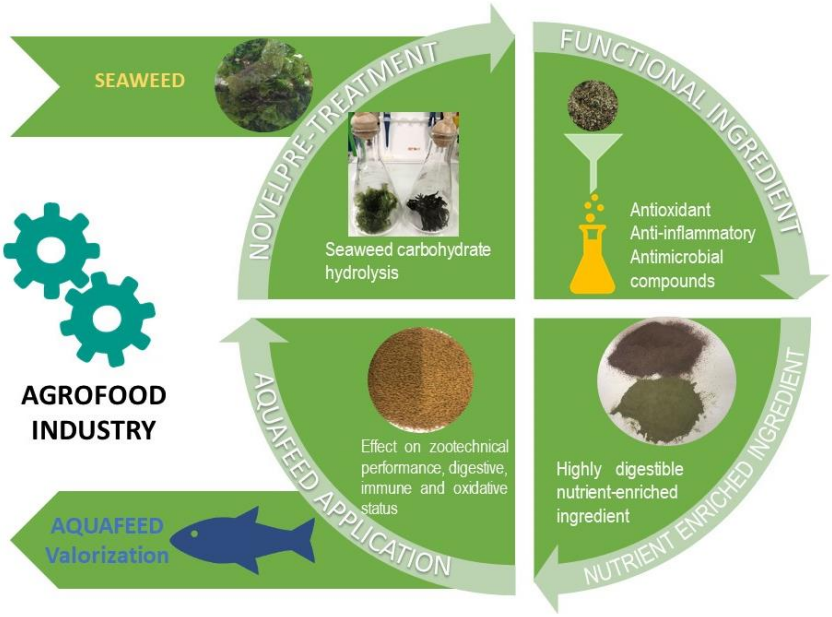
Cyanobacteria extracellular polymeric substances for sustainable and multifunctional active food packaging.
Leader: Nereida Cordeiro

Reference INPhINIT/CIIMAR_2023_10
NeoBiotics: Novel grape functional extracts to prevent antibiotic use in aquaculture
Leader: Cláudia Serra

Reference INPhINIT/CIIMAR_2023_01



Unlocking seaweed biotechnological potential, a Blue economy responsibility

Description

Seaweeds are essential Blue Economy bioresources due to their pivotal role in ocean decarbonization, contributing to the fixation of one-third of anthropogenic CO_2. Besides this ecosystem service, seaweeds are an excellent source of vitamins, minerals, and n3-highly unsaturated fatty acids (n3-HUFA; 50-80% of seaweed lipids) and functional components (oligosaccharides and secondary metabolites) with well-documented prebiotic, antioxidant, and immune-related benefits.

Despite the potential of seaweeds as a nutrient-rich feedstuff and source of bioactive compounds for aquafeeds, their high content of indigestible polysaccharides limits their use. Dietary inclusion of >10% seaweeds in aquafeeds usually decreases nutrient digestibility and induces intestine damage, impairing intestinal function and health. Pre-treatments, namely enzymatic hydrolysis, will be needed to reduce seaweed polysaccharides' adverse effects. However, available commercial enzymes cannot hydrolyze seaweed's lignocellulosic matrix as it is different from that of terrestrial plants. Therefore, the development of specific enzymes for seaweed carbohydrate hydrolysis is pivotal for increasing seaweed biomass availability and its potential as a feed ingredient.

Recently, the project team developed a specific toolbox of enzymes capable of degrading seaweed polysaccharides. Using expression plasmids containing these seaweed enzymes, this proposal aims to produce and apply enzymatic cocktails to ferment seaweeds, aiming to produce two categories of

valuable and novel feed ingredients 1) fermented seaweed as highly digestible nutrient-enriched ingredients and 2) bioactive-enriched seaweed-extracts as functional ingredient. In vitro and in vivo approaches will guarantee the adequacy of these novel biotechnology-enhanced products to promote fish zootechnical performance, health, and fillet quality, by applying the circular bioeconomy concept and enhancing aquaculture sustainability and food security.

Job position description

Driven by the need to unlock the potential of seaweeds, a critical bioresource for the Blue Economy, this project aims to develop and use a toolbox of enzymes (CAZymes) specially designed for the degradation of seaweed polysaccharides. Seaweed fermentation with CAZymes will upgrade seaweeds into novel and valuable ingredients and nutraceuticals with direct application in the aquafeed industry. This project will involve 3 steps:

1) Products development:
i. Plasmids containing enzymes optimized for degrading seaweed polysaccharides by the project team will be used to produce CAZymes cocktails.
ii. Seaweed fermentation conditions with CAZymes cocktails will be optimized to maximize the production of two product categories: 1) highly digestible seaweed (CAZYmes-Seaweed), and 2) BioActive Seaweed Extracts (BASE) with functional proprieties.
2) Product characterization by in vitro assessment:
i. Antioxidant, anti-inflammatory, and antimicrobial activity
3) Potential of CAZymes-Seaweed and BASE incorporation in aquafeeds, as an alternative ingredient and functional ingredient, respectively, by in vivo assessing:
i. Growth performance and digestive function;
iii. Whole-body composition and fillet quality;
iv. Immune and oxidative status;
v. Microbiota diversity.
The in vivo proof of concept will be done using gilthead sea bream as model species, as it isone of the most relevant marine aquaculture species in the EU.

This proposal copes with SDG2 by promoting fish production; SDG8 by stimulating employment opportunities in aquaculture and seaweed bio-refinery; SDG12 by seaweed valorization; SDG14 by increasing aquaculture and seaweed production efficiency, reducing environmental impacts, and aquafeeds dependence on fisheries resources.

This proposal is within the framework of the recently approved FCT project, ref. 2022.06587.PTDC, "MB4aqua- Macroalgae biorefinery: a novel approach to produce sustainable feedstuffs and functional additives towards low carbon footprint aquafeeds"

Group Leader

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Reference INPhINIT/CIIMAR_2023_02

Nature based solutions for estuarine areas recovery – targeting priority and emergentpollutants

Description

Nature Based Solutions (NBS) are defined as “living solutions” inspired by nature to address various societal challenges in a resource-efficient and adaptable manner, delivering simultaneously economic, social, and environmental benefits. NBS can improve water quality, reducing the release of organic matter and nutrients into water bodies. However, information on NBS capability, namely estuarine salt marshes, to reduce common and emergent pollutants and improve water quality is scarce. This project aims to evaluate how NBS can reduce environmental pollution, achieving sustainable development together with restoring biodiversity in line with the current European green deal. The ecological functions and ecosystem services that these NBs provide as solutions to reduce anthropogenic pressures on marine and freshwater ecosystems will be also assessed, contributing for the “zero-pollution” European target. The project will have a major impact on enhancing the use of sustainable NBSfor coastal and estuarine management plans. The application of NBS will become increasingly imperative in the coming years due to the economic costs of conventional water treatment andthe need to recover ecosystems health.

Our team couple fundamental ecology and ecosystems function research with biotechnology tools for ecosystems recovery, exploring the diverse services and societal benefits that marine and estuarine ecosystems provide, namely through native microorganisms and plant- microorganisms associations for environmental cleaning/recovery by NBS. Marisa Almeida has expertise in bio and phytoremediation, using it as biotechnology tools for recovery and remediation of aquatic

environments contaminated with different pollutants; Sandra Ramos hasexpertise in estuarine ecology, with special emphasis on human activities impacts, including marine ecosystem services and new environmental assessment approaches; Ana Paula Mucha has expertise in bioremediation processes for ecosystems recover.

Job position description

A holistic assessment of a NBS will be carried out, covering the continuum from the riverine untilthe coastal sections of estuarine environments. The NBS selected will be an estuarine saltmarshin the Lima river. The Lima River Estuary (41.41ºN; 08.48ºW (WGS84)) is the end member of an international watershed located in NW Portugal, an urban-industrialized water body with a largesalt marsh area (267ha). This saltmarsh system through which river water impacted by wastewater passes on will be surveyed. The performance of the saltmarsh system for reducing aquatic pollutants will be evaluated by assessing inputs from the river basis locaed upstream of the saltmarsh (which includes local contamination sources as small strings that drain into the estuary) and monitoring water quality going through the NBS. Different families of pollutants (e.g., common water quality parameters (nutrients, organic matter, pathogens), metals, endocrine disruptors, pharmaceuticals) will be select and determined in water, sediments and salt marsh plants collected seasonally. Data will allow to evaluate salt marsh role, a natural NBS,in water quality enhancement.
Research will also include the study of ecological functions, and biotic and abiotic processes involved in the removal of contaminants in natural NBS, namely in estuarine salt marshes, identifying the role of sediments, plants and microorganisms in these processes. Such knowledge will be used to assess the ecosystem service bioremediation provided by the Lima estuary salt marsh, following the ecosystem-service cascade approach.
The knowledge regarding their role will allow to identify potential key players in the enhancement of salt marsh performances. Finally, a framework will be develop integrating all the knowledge acquired to support and help in the decision making of conservation and management plans of estuarine environments.

Group Leader

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Reference INPhINIT/CIIMAR_2023_03

Non-invasive tools to assess fish welfare and disease susceptibility under farming conditions

Description

The PhD student will be deeply involved in the project IGNITION. This project was recently funded by the European Union with reference 101084651 and under the call HORIZON-CL6-2022-FARM2FORK-01-06 into the topic Biosecurity, hygiene, disease prevention and animal welfare in aquaculture. The IGNITION consortium will be led by the Animal Health and Aquaculture team from CIIMAR with Dr. Benjamin Costas as PI. The consortium is composed of 17 institutions from 8 different European countries. The project intends to go beyond the state of the art through several specific objectives such as: animal welfare by going deeper into molecular, physiological and behavioral responses of farmed animals; Biomarker discovery by identifying biomarkers related to stress and health in response to environmental and biological challenges; functional feeds by developing new supplemented feeds with natural bioactive compounds focusing on nutritional immunomodulation to improve the response to pathogens or stress in fish; Vaccination by developing new fish immunization approaches and antigen delivery systems; Genomic tools to improve animal welfare by unveiling the genetic component of stress response in fish and shellfish for selection of new phenotypes into breeding programs and finally using non-invasive biosensors and machine learning to predict disease outbreaks in animal farming.
The Animal Health and Aquaculture team is formed by more than 30 people and it is a highly dynamic and proactive group with focus on the study of the immune response in fish, vaccination, immunomodulation through fish feeds, non-invasive tools to assess welfare and health, and also on the study of the host-pathogen interaction to minimize the impact of diseases in aquaculture by identifying novel biomarkers of disease resistance or pedigrees with higher resistance to pathogens.

Job position description

The student will be involved in WP2 and WP5 of the project dealing with animal welfare assessment, novel non-invasive tools and machine learning approaches. Although aquaculture represents the fastest-growing food production sector globally, its sustainability is at stake due to the predicted effects of climate change that are not only a future but also a present reality. Adapting to the predicted changes in the short-term while taking mitigation measures in the long-term could be the only way towards sustaining the sector's production. Therefore, new knowledge on how farmed animals cope with current aquaculture practices is of outmost importance to improve management and sustainability of the sector. The identification of solid biomarker signatures for animal health and welfare will support the development of innovative prediction tools and non-invasive biosensors as a

scientific breakthrough in a precision veterinary medicine context, such as animal disease susceptibility, diagnosis, and treatment response for smart farming.
The student will gain deeper insights into the welfare of farmed animals under a scenario of climate change and current farming practices, in line with the main gaps addressed by the recent SCAR-Fish and SCAR-AHW studies regarding animal welfare. Efforts will be directed to both the effects of acute and chronic stress. Fish undergoing infections episodes will also be considered.
The main objectives include: i) discover and select non-invasive biomarkers in Atlantic salmon and European seabass which will be assessed for their suitability to be integrated into a sensor device to detect disease and monitor the health of farmed animals; ii) to develop non-invasive electrochemical sensors for quantitative analysis of selected biomarkers in farm products, using single and multiplex sensor platforms; and iii) to validate biomarker protocols and sensors for prototypes validation at industrial scale.

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Reference INPhINIT/CIIMAR_2023_04

Bio-warfare: role of animal venoms to assist the molecular treatment against COVID, cancer and other diseases

Description

Venom animals can produce potent cocktails of bioactive compounds – venom, which evolved over millions of years of natural selection for predation/defense. Venomics research, showed that toxin compounds discovery represents an appealing source across applied and translational fields, including industry, by offering incredible opportunities for biomedical drugs development, potentially assisting in the treatment against COVID-19, cancer and other diseases, derived from marine sponges (e.g., marine terpenoids), cone snails (e.g., neurotoxic conopeptides), etc.
The huge diversity of SARS-CoV viruses, including their cross-infection of multiple species, requires their detail genomic diversity characterization, likely to illuminate its molecular pathogenic potential and suitable inhibitory matches with animal venoms.
Unsustainable harvesting of wildlife has a direct impact on the propagation of emerging diseases (e.g. HIV, SARS-CoV-1, Ebola), and wildlife trade surveys are crucial for the surveillance of future diseases outbreaks, as in our funded project, (i) AFRICoV-Genomic surveillance of SARS-CoV-2(-like) viruses. Moreover, our other funded projects, (ii) EU-EVEN European Venom Network, (iii) Ocean3R - Reduce pressures, restore and regenerate the NW-Portuguese ocean, and (iv) UNORTE – ATLANTIDA: Platform for the monitoring of the North Atlantic Ocean, are allowing us to translate animal venoms to their putative relevance in biomedicine. Finally, our genomics data allowed us to infer the possible role of wild species (e.g. pangolins) in the COVID-19 outbreak (e.g. https://doi.org/10.1111/conl.12754), the role of genomic changes for SARS-CoV-2 pathogenicity (e.g. DOI: 10.1016/j.virusres.2021.198472), lung cancer progression (e.g. https://doi.org/10.1016/j.ygeno.2020.08.020), and how marine venom snails could assist treatment against COVID-19 (e.g., neurotoxic conopeptides; our own work: https://doi.org/10.3390/md20020105), etc.

Job position description

Here, we will develop a deep learning model using state-of-the-art genomic data analyses to understand SARS-CoV-2 diversification, assessing signatures of adaptive evolution and simulate the ongoing selection pressure, providing us a better understanding of the virulent pathogenicity of the virus and potentially allowing the identification of promising natural drugs derived from animal venom biomolecules.
The genomic work to be developed will focus on cutting edge Genomic and Bioinformatics analyses, relevant for Biotechnological application and performed at the genome level of the SARS-CoV-2 and its

functional relation, with biological interactions with natural molecules such as those found in venom animals.
Detailed surveys based on whole genome/proteome searches, synteny, gene network interactions, phylogenetics, recombination, selection, and large-scale data retrieval (deep learning with relevant omics data) and assays (e.g. recombinant and cell-based assays, including normal and cancer cell lines).
Relevance for biotechnological potential of the findings will be highly appreciated and developed along the course of the study.
Detailed methodologies could be further evaluated in our recent publications on modern venomics, virus, cancer, antibiotics, gene networking, functional genomics, biotechnological relevance, etc (e.g. https://doi.org/10.1016/j.cub.2019.10.066, https://doi.org/10.3390/md20020105, https://doi.org/10.1093/gigascience/giac048, DOI: 10.1016/j.virusres.2021.198472, https://doi.org/10.1016/j.ygeno.2020.08.020, https://doi.org/10.3390/antibiotics9110757, https://doi.org/10.1016/j.ygeno.2020.03.015, https://doi.org/10.1093/gbe/evz111, https://doi.org/10.1073/pnas.1819778116).

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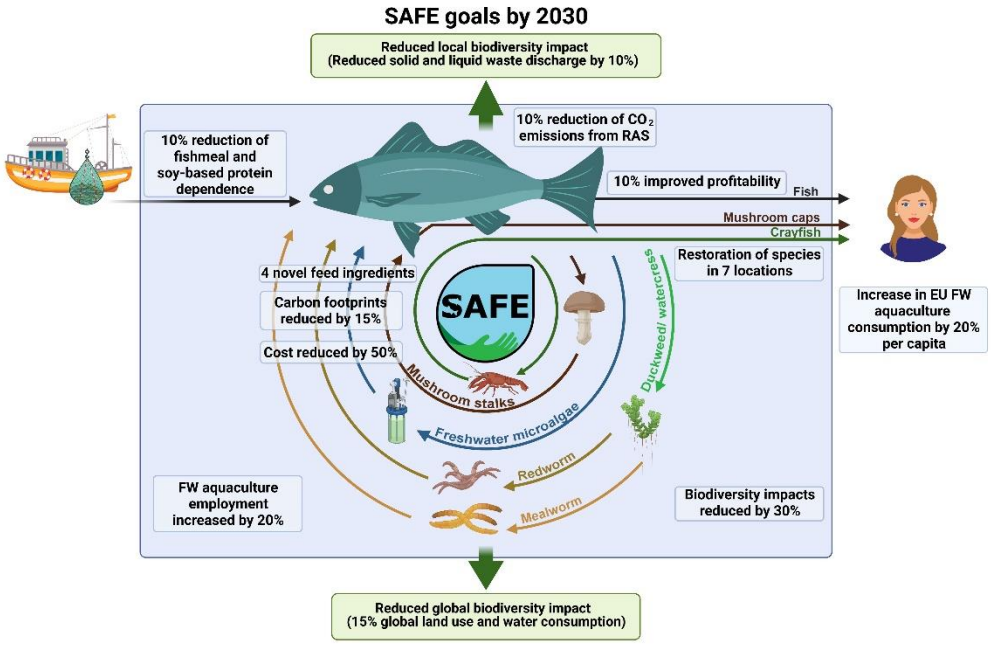
Reference INPhINIT/CIIMAR_2023_05



I4F - Intelligent Fish Farming For Future

Description

Aquatic food is one of the most important food resources to meet the growing global protein demands. Besides contributing to food security, it provides valuable sources of micronutrients, not readily available in other foodstuffs. Growth in aquatic food supply,
i.e. the “Blue economy”, can only come from aquaculture, as fisheries are fully exploited. The negative impact of mariculture on benthic systems sets pressure on moving aquaculture to freshwaters. However, there are constraints to the growth of freshwater aquaculture (FW) sector: 1) Provision of sustainable feed at competitive
prices, 2) Impact of aquaculture on the environment, locally and through its supply chains, 3) Falling profit margins for low impact systems and recirculating aquaculture systems (RAS), and 4) Wastes management. Consequently, FW aquaculture is in decline within the EU. This contrasts sharply with rapid rise in FW aquaculture production in the rest of the world. According to the SCAR-Fish study, FW aquaculture not only needs to become more profitable but also must adopt sustainable management practices to lessen environmental impacts, such as more efficient waste stream management, lower carbon footprints, reduced dependence on wild-catch fisheries and to ultimately improve customer perceptions.
This PhD project will be carried under the SAFE project (ID: 101084549), recently approved by the Horizon Europe programme. The consortium involves 12 institutions from 7 European countries, with a total budget of 5 million euros. The SAFE aim to reduce the environmental impact and improve the

viability of the FW aquaculture by applying circular economy approach to the valorization of solid and liquid wastes from recirculating aquaculture systems (RAS) and integrated multi-trophic aquaculture (IMTA) systems. The economic viability of FW aquaculture will be improved through advances in management systems and the integration of waste streams from aquaculture systems into the aquaculture feed chain.

Job position description

The candidate will carry out his work under the Workpackages 3 and 4 of SAFE project.The candidate main research activities will be: 1) In vitro bioactive properties of the novel ingredients produced in WP2 of SAFE project; 2) Fish feed formulation and production using the novel ingredients; 3) Validate the novel feeds on fish physiological status, gut integrity, and product quality. The candidate will prepare the scientific publications continuously through the research period and will participate in selected International conferences to present the scientific results. Briefly, in 1) the novel ingredients will be analyzed for their antioxidant and antimicrobial properties as total phenolic (TPC) and flavonoid (TFC) contents, free radical scavenging activity (DPPH) and lipid peroxidation inhibitory capacity (TBARS) using spectrophotometric techniques. Antimicrobial efficacy will be tested against reference strains of fish pathogenic microorganisms. Prebiotic properties will be tested for the growth effects on the probiotic cultures. In **2)** novel feeds will be formulated with increasing inclusion levels of the novel ingredients through linear least-cost models using typical aquaculture ingredients. The dietary inclusion rates of the ingredients will be determined based upon their bioactivity profile and nutritional content. In **3**), the novel feeds will be tested in fish trials using standardized biomarker methods, including haematology and plasma clinical chemistry and immune analyses. In addition, physiological stress responses will be determined, as well as the activities of enzymes involved in antioxidative protection, metabolic function, and immunological defence. The gut structural integrity and immune modulation in fish will be assessed by conventional histology and by molecular biology techniques. Digestive capacity will be assessed by the quantification of secreted pancreatic enzymes (amylase, lipase, trypsin, and chymotrypsin).

Group Leader

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Reference INPhINIT/CIIMAR_2023_06

MICROARCTIC - Decipher Climate Change Impact on the Arctic Ocean Plankton Microbiome

Description

The Arctic Ocean is currently facing the consequences of the longterm warming trend, largely caused by anthropogenic forcing. Numerous studies have exemplified the implications of these remarkable environmental changes on the hydrography and physical properties of the Arctic water masses with consequent impacts on Earth's climate system. Recently the terms “Borealization” and “Atlantification” have frequently been used to express the shift from an Arctic towards a more temperate marine ecosystem state. These shifts result from an increased connectivity between Atlantic and Arctic waters facilitated by climate warming. However, our understanding of how Arctic biota will respond to this Atlantification is still limited. Microbiomes represent the majority of the total live biomass and diversity in every ocean and are responsible for mediating critical Ocean stabilizing mechanisms by sustaining global primary production and the basis of the food webs. Thus, it is essential to understand the ongoing changes on the plankton microbiome at multi diversity and functionality levels. In the MICROARCTIC PhD project we propose to greatly improve the knowledge on the biological implications caused by a warmer Arctic Ocean by extending microplankton and contextualized environmental observations in the Svalbard archipelago, a region with a pronounced waring signal in the Arctic. The scientific activities of MICROARCTIC will be fully integrated in ongoing international Arctic monitoring programs coordinated by CIIMAR (Portugal), Norwegian Polar Institute (Norway) and Ocean Frontier Institute (Canada) and on multidisciplinary scientific projects (e.g. CONNEC2OCEANS, N-MICROARTIC, TINYARCTIC). The project will use environmental observations, experiments, and big data analysis in an transnational and interdisciplinary effort to produce reliable scientific data to decipher how the

genetic diversity of microplankton communities and their functions are likely to change in a key transition Arcticregion and which feedbacks might be expected from this changing microbiome.

Job position description

To address the goals of this PhD project the distribution, diversity and functional attributes of microplankton communities across a long -term data sets being generated in the Arctic Marginal Ice Zone by NPI and CIIMAR will be investigated. The candidate will participate in future Arctic summer expeditions to collect new samples for genomic (16S and 18S), metagenomic and transcritomic analysis. Previously developed bioinformatic pipelines by OFI and CIIMAR will be also applied to the complex datasets generated during this project. These analyses will generate an enormous amount of information at different taxonomic and functional complexity levels that will be the input, together with contextualized environmental data, for downstream data analysis and visualization. All the analytic approaches together will give an in deep information about how the highly diverse microplankton communities will respond to the main drivers of climate change capture in our long term Arctic data sets. The power of the technologies proposed to be used in this study will provide new knowledge that combine community structure and its molecular metabolic pathways, taking us to the next level of understanding how the complex biological network reacts to key global warming stressors. Furthermore, experiments will be performed, on board the research vessels, during future Arctic expeditions. Microcosm experiments will be set up enabling stable, manipulative experiments to provide the necessary information to characterize and determine the consequences of increasing North Atlantic and Polar Water masses connectivity in microbiome surveillance and shifts on its metabolic functions.
The development of this PhD program will fill an urgent gap in microplankton monitoring by increasing future spatial and temporal sample resolution, especially critical in remote aquatic ecosystems like North Atlantic and Arctic regions.

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Reference INPhINIT/CIIMAR_2023_07

Assessing pre-exploitation baseline numbers and population dynamics of the European sardine *Sardina pilchardus* Walbaum, 1792 using palaeogenomics

Description

Overfishing has been a major problem for several marine species, reducing their effective population sizes to remnants of their pristine levels. However due to the lack of accurate fishery catch data we still have a poor understanding of how these impacted their evolutionary path. One of the most impacted species was the European sardine (*Sardina pilchardus*), an important pelagic fish resource, with enormous economic value especially in Southern Europe and Morocco, where it is the main target of purse-seine fleets and represents a major source of income for local populations. The species is distributed from the southern Celtic Sea and the North Sea to Mauritania and Senegal, being also abundant in the Mediterranean. In a recent population genomic study, we have characterized 12 sardine populations encompassing the entire species range. Our results clearly show that the species' genetic diversity can be partitioned in at least three genetic clusters. One including individuals from Azores and Madeira, the second encompassing Iberian populations, and the third gathering the Mediterranean and Canary Islands, with individuals from Iberia showing some degree of admixture. Here we will use ancient DNA techniques tolook at samples that pre-date periods of intensive fishing and compare those results tothe ones obtained from current populations (post exploitation). To do this we have collected specimens from different Roman archaeological sites within the species distribution range (e.g. Adro Vello, Tahaddart, and Lobos). We will extract DNA and sequence full genomes from these samples allowing us to compare historical pre- exploitation populations to current ones. Doing this will enable us to get a more accurate perspective of the effective population size and genetic diversity levels of thespecies prior to overfishing, invaluable information for stock delimitation and management and definition of fishing effort in the different Food and Agricultural Organization (FAO) fishing areas.

Job position description

We seek an enthusiastic, motivated individual with a background in genomics, molecular biology, or a related discipline. Experience in the use of genomic tools developed for low depth shotgun sequencing

data is an advantage (e.g. Angsd, NGSAdmix). The student will be based at CIIMAR, University of Porto and their work will mainly involve bioinformatic analyses of ancient genomic data recovered from archaeological sardine populations in order to uncover pre-exploitation genetic diversity levels of sardine populations. We hypothesize that genetic diversity was lost over time in response to the unsustainable fishing practices of the last century, so assessing how much diversity was lost in the past millennium and establishing a baseline for this species before commercial fishing started will give us a more accurateperspective of pristine populations, invaluable information for stock delimitation and management and definition of fishing effort. The student will also have the opportunity to work in other projects going on at the lab, namely the genomics of whale hunting in Iberia; local marine resource exploitation in the Canary Islands and the population demise of the Caribbean monk seal. The research will be conducted in an international, highly diverse research environment, involving collaborators and partners from various continents and scientific backgrounds.

Group Leader

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Reference INPhINIT/CIIMAR_2023_08

Natural Products discovery from African cyanobacteria

Description

Cyanobacteria produce a plethora of bioactive small molecules, often referred to as natural products. Over 1000 cyanobacterial natural products have been reported, including some that have reached clinical trials and the clinic for their potent biologicalactivity properties. Still, analysis of genome data for cultured isolates of the phylum Cyanobacteria indicates that known natural products account for less than 20% of theirgenetic potential for the production of such molecules. Adding to this, the biological and chemical diversity of cyanobacteria is also understudied. Therefore, there is a huge potential in these organisms for natural products discovery.

Accessing novel cyanobacterial diversity presents as an opportunity to discover structurally novel small molecules that might be endowed with potent bioactivity. A privileged strategy to access such novel diversity involves the sampling of understudied geographical locations. The African continent has been poorly studied in this context. Inthis project, we propose to sample African inland and insular locations, namely Morocco and Cape Verde, in the context of the ongoing EU-funded project Emertox, led by co- supervisor Vitor Vasconcelos. This will lead to samples that can be used for direct chemical exploration or for obtaining cyanobacterial isolates that can be grown in largescale in the laboratory for the same purpose. State-of-the-art metabolomics and dereplication methodologies developed in the supervisor's (Pedro Leão) and co-supervisor's (William Gerwick) laboratories will accelerate discovery of new natural products. The structures of the compounds will be elucidated by advanced NMR and Mass Spectrometry methodologies. Their biosynthesis will be studied using bioinformatics and protein biochemistry methods, which may lead to the discovery of unusual enzymatic transformations. The biological activity of the isolated novel molecules will be evaluated through access to CIIMAR's biodiscovery platform.

Job position description

The PhD student will perform SCUBA diving to collect cyanobacterial samples (mats/tufts) from African insular and continental locations. The student will also be involved in the isolation of cyanobacteria from such samples. In addition, biomass of these cyanobacteria (either directly collected, or lab-grown from obtained isolated) willbe subjected to extraction and fractionation procedures. The student will perform modern analytical techniques, namely feature-based molecular networking, SMART NMR and precursor feeding, to accelerate discovery from these biomasses. Isolation ofnovel molecules will be carried out through and bioassay-guided or mass-guided approaches involving sequential chromatographic steps. This will lead to pure compounds that will have their structures elucidated by the student using a combination of modern NMR and mass spectrometric methodologies.

Furthermore, the student willhave access to the biodiscovery platform at CIIMAR in order to screen for pharmacologically-relevant biological activity among isolated compounds. The student will also be trained in bioinformatics and molecular biology/protein biochemistry techniques to study the biosynthesis of the new compounds.
The student will perform research activities at CIIMAR (U. Porto, Portugal) and at the Gerwick lab in Scripps Oceanography (UCSD, USA).

Group Leader

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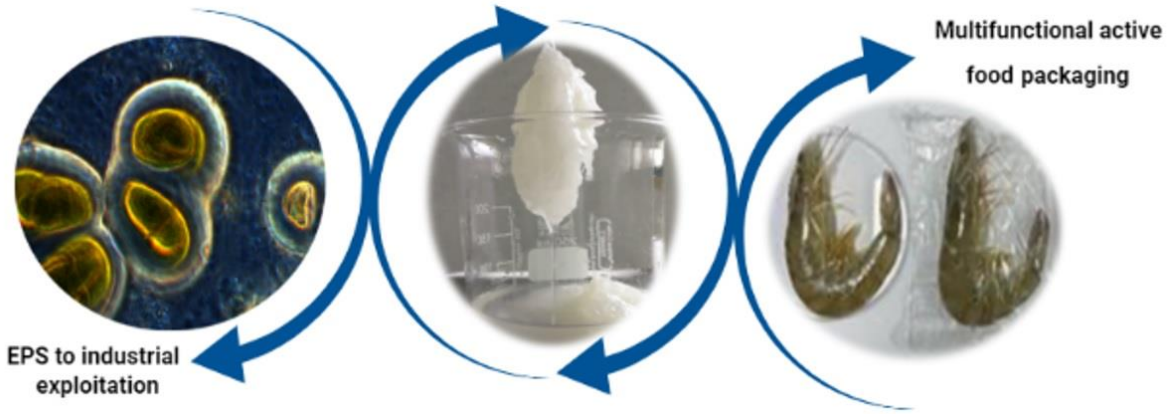
Reference INPhINIT/CIIMAR_2023_09



Cyanobacteria extracellular polymeric substances for sustainable and multifunctional active food packaging

Description

International demand for polymers continues to climb as the world population increases. Nearly all polymers are formed from fossil resources. Amidst the increasing worry regarding harmful environmental damage and exhaustible fossil resources, there is significant interest in using natural resources for polymeric materials. Various sustainable polymers extracted from macroalgae (agar, carrageenan and alginate) and bacteria (dextran and xanthan gum) have been exploited. However, these biopolymers are extracted following chemical or enzymatic transformations and by hydrolysis, polymerization, or condensation. These traditional methods are time-consuming, expensive and require large sample volumes, chemical agents and numerous manipulation steps. They can also negatively impact the functional and structural properties of these polymers. In recent years, the potential uses of microalgae/cyanobacteria in industrial applications have received increased attention, not only because of their biomass but also because of the existence of a large quantity of polymers in a cell-free medium available from biomass cultivation, known as extracellular polymeric substances (EPSs).

Food packaging materials based on natural biopolymers have attracted considerable interest. The use of renewable, environmentally benign, and inexpensive natural resourcesto prepare biopolymer-based packaging films has increased in food packaging applications. Natural biopolymers hold substantial prospects as food packaging materials due to the integrated advantages of excellent film-forming properties, biodegradability and appropriate mechanical properties.

The present research project intends to develop: *(i)* industrial operation strategies to enhance the production of EPSs (release -RPSs and/or capsular - CPSs) produced by unexploited cyanobacteria; *(ii)* new and attractive industrial methodologies for the EPSs isolation; *(iii)* sustainable and multifunctional active food packaging.

The PhD student will integrate the REBECA-CCT project-(code MAC2/1.1b/26) financed by the Interreg MAC Cooperation Program. The REBECCA-CCT project seeks synergies and boosts an emerging industrial sector based on the biotechnology of microalgae and cyanobacteria in the Macaronesia geographical area.

Job position description

The candidate will be joining a very active research team based in Madeira Island (www.lb3mab.com), involved in the research of the use of microalga/cyanobacteria as a solution to the three greatest challenges of our time: environmental pollution, climate change and sustainable food.
The candidate is expected to have a solid background in biochemistry and should have previous scientific experience in microalga/cyanobacteria culture, EPSs research and statistical data treatment.

The candidate will have the opportunity to interact with several international investigationteams and gain in-depth knowledge on the isolation and characterization of EPSs and biomaterials manufacturing to apply in food packing.

Group Leader

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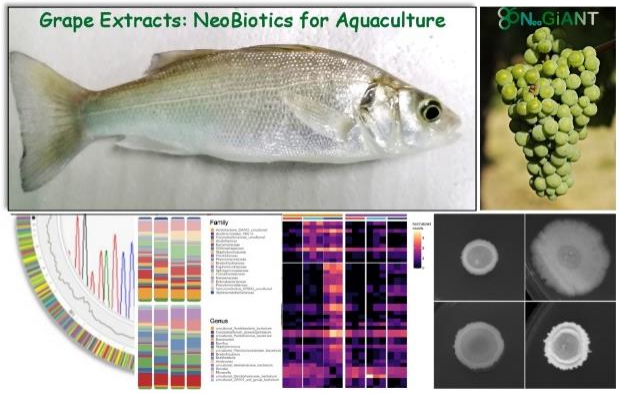

NeoBiotics: Novel grape functional extracts to prevent antibiotic use in aquaculture

Description

Bacterial diseases are a major constraint to aquaculture. Besides massive animal losses, bacterial diseases are associated with misuse of antibiotics, a serious threat to public health. Ina post-antibiotic era, where the decreasing efficacy of antimicrobials is a global problem, it is urgent to find alternatives that assure integrated health care for humans, animals, and the environment (One Health).

Functional extracts, due to their arsenal of phytochemicals, in particular phenolic compounds, have known potent natural antimicrobial and antioxidant activities, are recognized as immunomodulators, reshape the gut microbiota towards beneficial health effects and, in fish, have also been associated to improved growth, feed intake and intestinal histomorphology. Functional extracts are thus promising novel substitutes for antibiotics-NEOBIOTICS. Although the beneficial effects of functional extracts have been demonstrated, their exact mode of action, toxicity, immunomodulation & disease prevention potential, are product-specific and need to be defined.

Functional extracts from grapes were found to have an exceptional antioxidant activity, a potent antimicrobial activity against aquaculture pathogens, and are the basis of the recentlyapproved H2020 NeoGiANT project (https://www.neogiant.eu) of which the Supervisors are Team Members, and that will financially support this PhD project.

The PhD will be conducted at NUTRIMU–Nutrition&Immunobiology group of CIIMAR, under the Group Leader of Cláudia Serra, expert on fish gut microbiota, novel probiotics & vaccines for aquaculture. Co-Supervisor Ana Couto is expert on functional ingredients to improve fish health&welfare. Co-supervisor Aires Oliva-Teles is Full Professor at Porto University and NUTRIMU coordinator, expert in fish nutrition & physiology. CIIMAR infrastructures and the H2020 NeoGiANT project financial support, assure the competencies, facilities, and resources necessary to successfully execute this PhD.

Job position description

NEOBIOTICS will investigate grape extracts potential as substitute for conventional antibiotics in aquaculture, by testing different formulations in aquaculture fish. European seabass and turbot will be used as animal models, due to their economic importance in Europe. Results are expected to be extrapolated and cross species boundaries, being applied to diseases caused bydifferent pathogens in different fish. One or more extracts will be established as novel alternatives to antibiotics-NEOBIOTICS, contributing to improve aquaculture practices by reducing usage of conventional antibiotics. The collaborative network with animal nutrition&health industries, together with the team experience in turning science into IP (patents,licensing agreements) anticipates that at least one commercial product will derive from this project.

The specific objectives are:

1. Establish the maximum tolerable dosage of NEOBIOTICS to use in fish
2. Incorporate NEOBIOTICS in fish feed and determine its *in vivo* oral efficacy
3. Establish NEOBIOTICS immunomodulatory, antioxidant, and antimicrobial effects
4. Characterize fish gut microbiota modulation by NEOBIOTICS and correlate it with NEOBIOTICS immunomodulatory, antioxidant, and antimicrobial effects
5. Evaluate NEOBIOTICS efficacy in disease PREVENTION and TREATMENT bychallenging fish with bacterial pathogens before or after NEOBIOTICS administration

The PhD candidate will become familiar with Biochemistry, Molecular Biology, Microbiology, and Animal Science techniques, including DNA&RNA extraction; PCR&RT-PCR; bacterial strains manipulation; fish-diets formulation & manufacturing; experimental fish(larvae, embryos & juveniles) maintenance & rearing; fish immunization & challenge studies; sampling of animals, blood & tissues for analysis; proximal analysis of ingredients, diets, feces & other biological materials; blood biochemistry analysis; enzymatic activity analysis; fish gut microbiota analysis;data processing & statistical analysis.

Group Leader

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