Virtual Meeting 12th of June 2020 – 11.00 – 13.00

"MARINE BIOBASED PRODUCTS: CHALLENGE FOR A SUSTAINABLE INNOVATION BASED ON THE THREE P's (PLANET, PEOPLE, PROFIT)

ABSTRACT BOOK

P1. Sustainable utilization of coastal by biodiversity for biobased product: the case study of salt work of Trapani

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Halophytes, that include more than 2500 species worldwide, are known to tolerate and grow in high salt concentration environments.
Marine plants, in response to stress factors, secrete secondary metabolites which are accumulated in the cells and used as a deterrent against grazers, epiphytes, epibionts and other competitors, but which are also active in disease prevention and protection from oxidative stress.
Hypersaline environments have been employed for human usages since ancient times, while nowadays, their biotechnological potential is being explored.
The aim of this study was the characterization of the antioxidant power of polyphenol extracts obtained from the halophyte *Halocnemum strobilaceum*, that grows in the solar saltworks of western Sicily (Italy), and on the sustainable production of *Calendula* sp. and *Mesembryanthemum nodiflorum* potentially useful as source of important molecules for biotechnological applications.
The antioxidant properties of *H. strobilaceum*, *Calendula* sp. and *M. nodiflorum* as sources of polyphenols, were assessed comparing traditional extraction with an eco-friendly technique (supercritical fluid extraction (SFE)) to evaluate which extract showed a higher amount of antioxidants. The obtained extracts showed a high antioxidant activity with a protective effect comparable to that induced by a synthetic antioxidant.
Based on the outcome, this resources might be considered in future as a possible source of income for saltworks sector in cosmetic or nutraceutical applications.

P2. Screening and characterization of carotenoids production from the wild and mutated marine yeast *Rhodotorula mucilaginosa* RmTun15

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Among the various sources of carotenoids, yeasts of the genus *Rhodotorula* have been reported as carotenogenic yeasts or pink yeasts because of their production of carotenoids. They are single-celled, their morphology has been described as soft, smooth and moist, and sometimes the presence of mucoid provides a high growth rate. The aim of this study was to extract and quantify different pigment of carotenoid from the wild and mutated *Rhodotorula mucilaginosa* RmTun15. The marine yeast *Rhodotorula mucilaginosa* RmTun15 (GenBank Acc. N°MF327252), isolated from coastal shallow water was investigated to determine its potential production of carotenoid. After a random mutagenesis using the Ethylmethane Sulfonate as a mutated agent, 4 mutated selected strains were as well studied to select the potential strain for pigment production: MR-1 (GenBank Acc. N°MN700656), MR-2 (GenBank Acc. N°MN535021), MR-3 (GenBank Acc. N° MN526028) MR-4 (GenBank Acc. N°MN506031) and RmTun15 (GenBank Acc. N°MF327252). The carotenoid fraction was extracted, purified and quantified according to the Davoli et al., (2002 et 2004) methods. The antioxidant activity was determinate following the method described by Brand-Williams et al., (1995).
In this present study, the carotenoids contents increased significantly to an average of 2.66 ± 0.05% -fresh biomass for the control strain and MR-1 to reach 5.68 ± 0.04% for MR-2. Such levels decreased to 4.20 ± 0.07% and 1.57 ± 0.04% for MR-3 and MR-4 respectively. Chromatographic analysis showed that β-carotene, γ-carotene, Torulene and torularhodin are the carotenoids identified in the studied species with Torulene as the main pigment. However, mutated strains showed significantly lower (46.43 ± 1.65%) Torulene content than in wild strain (57.54 ± 2.17%), while Torularhodin level remained constant in all studied strains (44.25 ± 1.22%). As carotenoids, Torularhodin and Torulene have strong antioxidant properties and are potential ingredients of health benefit.

Keywords: Rhodotorula, carotenoids, Torulene, torularhodin, characterization

P3. Marine waste recycling for biobased product

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The expansion of fish processing is creating increasing quantities of offal and other by products, which constitute up to 70% of fish used in industrial processing.
In the past, fish by-products were often thrown away as waste, used directly as feed for aquaculture, livestock, pets or animals, or used in silage and fertilizers. However, other uses of fish by-products have been gaining attention over the past decades, as they can represent a significant source of nutrition and can be used more efficiently as a result of improved processing technologies. In some countries, the use of fish by-products has developed into an important industry. Fish by-products can serve a wide range of purposes. Heads, frames and fillet cut-offs and skin can be used directly as food or processed into cakes, snacks (crispy snacks, nuggets, biscuits, pies), gelatine, sauces and other products for human consumption. By-products are also used in the production of feed, biodiesel and biogas, dietetic products, pharmaceuticals (including oils), natural pigments, cosmetics and in other industrial processes**.** Fish waste is one of the waste management fields that is gaining more and more ground. Among the most important current uses for treated fish waste are the isolation of collagen and antioxidants for cosmetics, biogas/biodiesel, fertilizers, dietary applications, food packaging and enzymatic isolation. Research and the business community should work closely together to increase the use of fish waste as a raw material for a new product and to improve competitiveness between companies and environmental sustainability, implementing the circular economy.

P4. The effect of diet mixing on the nutritional quality of oyster (*Crassostrea gigas*) during live storage: Macroalga *Entheromorpha intestinals* and crab shell powder as a natural diet

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Developing a low-cost feed for oyster during live storage including the depuration stage would reduce the operating costs of bivalve post harvesting. During this preliminary study, we tried to set up a low-cost diet based on a mixture (50% / 50%) of macro-algae (*Entheromorpha intestinalis*) and crab shell powder (*Portunus segnis*). The particle size of the powder was less than 45 µm. Oysters were kept alive for 15 days in tanks filled with filtered seawater at controlled temperature of 14 ± 2 ° C. The water was renewed daily. Each tank contained 5 oysters in 10 liters of water. The oysters were divided into 2 lots with n = 10 oysters for each lot. The first batch was considered as the control batch and the oysters were not fed; the second batch was fed twice a day with the dietetic mixture (0.12 g / L / day). The effect of the diet on the nutritional quality (proteins, lipids, carbohydrates, moisture, ash and fatty acids content) of oysters' meat after sacrifice was studied in comparison with the starved specimens considered a control lot as it is the common practice used during bivalve post harvesting.
After 15 days of storage, the oysters were sacrificed and the biochemical analyzes were carried out in B3Aqua accredited laboratory (ISO 17025 -2017). Results showed a non- significant difference between the initial composition lot and 15-days live stored and fed oysters, however a substantial increase of carbohydrate, lipid and protein (36.61%, 24.30%, 18.90% DM respectively) were observed when compared to starved oysters.
Keyword: oyster (*Crassostrea gigas*), Macroalgae (*E.intestinalis*), crab shell powder, feeding, Nutritional quality.

P5. Use of food industry discards as ingredients in echinoculture sustainable feeds

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Food waste is an important issue worldwide, resulting in environmental impacts and leading to economic loss due to their management costs. However, vegetal and animal discards from the food industry have the potential to be recycled as feed ingredients due to their nutrient content. In this context, the present study aims to evaluate the suitability of a new feed based on the food industry discards for the sea urchin *Paracentrotus lividus*. Two experimental formulations were obtained using different proportions of outermost endive (*Cichorium endivia*) leaves and anchovy (*Engraulis encrasicolus*) industrial discards in different proportions, plus a low amount of agar. First, an evaluation of proximate composition and fatty acids content of both formulations was conducted, and then the effect of the feed provision on the gonad growth was evaluated in a short-term feeding trial. Both formulations showed a proper nutritional value and fatty acid profiles, which play a key role in the development and growth of gonads, and hence resulting suitable for sea urchin nutritive requirements. Moreover, an increase in gonad somatic index was recorded throughout the feeding trial. Despite further analysis is needed to assess the long term performance of the feed, in terms of gonad yield and quality, these encouraging results indicate that food industry discards provide suitable alternative ingredients for the production of sustainable feeds for sea urchin aquaculture.

P6. Marine biobased product from microalgae: Production of high value-added nutraceuticals in a multitrophic aquaculture system within a closed-circuit marine hatchery (NUTRAQUA)

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Research into new microalgae processing technologies, development of industrial cultivation and their applications into the industry field is a clear focus in marine biotechnologies.
Microalgae are largely used in many fields of application: food industry, nutraceutical and health field, aquaculture facilities and terrestrial farms as functional food and wastewater treatment.
It is within this context that the project NUTRAQUA "Production of high value-added nutraceuticals in a multitrophic aquaculture system within a closed-circuit marine hatchery is placed, in collaboration with the Laboratoire des Sciences de l'environment Marin (LEMAR) Université de Bretagne Occidentale, whose main objectives are the extraction and the characterization of bioactive compounds obtained with a green extraction method from marine species cultivated in integrated multi-trophic aquaculture systems (IMTA), to evaluate their effects in cell culture for pharmaceuticals and cosmetics applications and simultaneously, to subtract nutrients from the wastewater facility reducing the effect of the environmental impact of aquaculture productive activity.
In order to develop marine biotechnologies, to give access to sustainably produced and renewable biomass and to apply bioremediation and innovative industrial processes, the new aspects of the project are represented by the identification of natural extractive marine resources, by the application of different methodologies and the individuation of the optimal and green extraction process, and by the use of the energy flowing from the closed-circuit marine hatchery to feed a biorefinery for the production of nutraceuticals of high commercial value and to reduce the nutrients produced by the aquaculture system.

P7. Alternative to plastic for fish packaging. Chitin and chitosan from shrimp by products for the production of edible coatings.

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As the production and processing of crustaceans have increased, efficient use of their shells has become important to maximize financial return and address waste disposal problems. Chitosan is obtained by alkaline deacetylation of chitin extracted mainly from the exoskeletons of crabs and shrimps. Chitosan can be used in food preservation because of its antimicrobial and antioxidant properties that can help maintain product quality and extend shelf life, beside it is a biodegradable packaging. The chitin extraction from shrimp (*Pandalus borealis*) by-products was optimized by treating shrimp shells by chemical and biological deproteinization followed by demineralization. Moisture, ash and protein contents as well as the degree of acetylation (DA%) were determined in chitin samples. Based on protein content, results showed that the chemical extraction yielded a chitin with a better quality than that obtained following biological extraction which produced chitin with higher protein residue. Moreover, the DA% was significantly different between the various treatments carried out as for the chemical and the biological extractions. A chitosan-based film was prepared with good textural and mechanical properties, when chitosan was solubilised in a solution of commercial vinegar and mixed with glycerol.

P8. The federating project CatalyMar: marine fungi and biotechnology

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BIODIMAR is a technological platform of the University of Western Brittany, located in Brest (France) and part of the Laboratory of Marine Environmental Sciences (LEMAR) UMR6539. It belongs to the functional exploration axis of the Biogeouest network of technological core facilities in life sciences and the environment. BIODIMAR's primary vocation is the development of natural bioactive substances of marine origin in the fields of health, cosmetology, nutraceuticals and biotechnology. This year, BIODIMAR initiated the federating project CatalyMar around an underexplored marine biomass: marine fungi. Marine fungi are microscopic eukaryotic organisms found from the foreshore to the deep sea, in a pelagic or benthic form and associated or not with other organisms. Thanks to their large diversity, they represent an interesting potential source of new original biomolecules. In this context, the federative project CatalyMar gathers 9 core- facilities of the Biogenouest's network. This collaboration aims to stimulate reflections and experimentations around the bioprospecting of marine fungi, expecting the discovery of new molecules for biotechnological applications.

P9. Biobased marine oils from aquaculture by-product: the case study of seabass (*Dicentrarchus labrax* L)

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The importance of the n-3 series polyunsaturated fatty acids (PUFA), such as EPA and DHA (eicosapentaenoic and docosaexaenoic acid) in human and animal nutrition and in wellbeing and disease prevention is well recognized. The interest for n-3 PUFA and the decline in natural marine resources stimulated research to "find out" new sources alternative to fisheries, such as by-products originated from fish transformation industry and fisheries by-catch.
Total by-products (TBP) obtained by filleting farmed and wild European sea bass (*Dicentrarchus labrax*) were analyzed to evaluate if, on the basis of the percentage yield, total lipid content and fatty acid composition, they can be considered a resource of PUFA. Results show that TBP from intensively farmed fish (IFF) contain higher total lipid content and have a higher level of n-3 PUFA rich in EPA and DHA, compared to extensively farmed fish (EFF) and to wild fish (WF) ($p<0.05$). This difference may suggest a way of promotion of TBP from IFF sea bass through the n-3 PUFA recovery by extraction.

Acknowledgements
This study was supported by grants from Italian Ministry of Education, University and Research, University of Palermo: cod. ORPA07ARYE, cod. ORPA07P53E.

P10. BioVecQ - Biotechnologie marine vecteur d'innovation & quality: a strategic project on marine biobased products

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The strategic project "BioVecQ" coordinated by Institut National des Sciences & Technologies de la Mer (INSTM) has been financed within the ENI CBC Italy-Tunisia programme 2007-2013. The Marine Biology Institute of Trapani has been part of the project together with other research entities and productive representatives in Tunisia and Sicily.
The general aim of this project was to develop new biotechnological and analytical processes for the sustainable development of the aquatic and halio-food sector in the Tunisian and Sicilian regions. One of the specific objectives was to improve the management of innovation and technological production of SMEs in the fisheries and marine biobased sectors.
The project has been built according to 4 main activities which started with an analysis of the halio-food/bioproducts sector, harmonization of techniques for fish quality determination, development of new procedures for the processing of aquatic products, extraction and production of bioproducts and the transfer of results among the different actors of the sector.
The research activity has led to interesting discoveries and innovations in the sector of Marine Biobased products with nutraceutical, pharmaceuticals and cosmeceuticals applications.

P11. Autochthonous sicilian microalgae in salt and temperature stress: growth and biochemical induction

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Microalgae are a feedstock of increasing interest because of its applicability in different fields. Recent research focused on the potential of indigenous species of microalgae to be exploited in regional applications; this may bring economic advantages because they are already adapted to the stresses of their environment, and thus potentially more resistant to contaminants. In this study we aimed at expanding the Sicilian regional-based industry by growing indigenous algal isolates. We exploited the biodiversity of the Mediterranean Sea and obtained monoclonal algal strains from Sicilian littoral. In this first part of the study, three strains were cultivated in stress conditions in order to assess the possibility to employ them in the Sicilian algal industry. They were cultivated in Low Temperature-Low Salt (LT-LS), High-Temperature-Low Salt (HT-LS) and Low Temperature-High Salt (LT-HS). We observed that Sicilian microalgae growth better at higher temperature while the tolerance to high-salt stress is species-specific. Moreover, the biochemical composition appears to be influenced by temperature and salt- stress. Further research will be conducted in order to assess the production of bioactives in stress conditions. This will help the developing of a Sicilian regional-based microalgal industry.

P12. Immunostimulating effect of *Gracilaria gracilis* powder in zebrafish (*Danio rerio*)

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The research group of the University of Messina has unique environments and ecosystems available, such as the Strait of Messina and the two lakes connected to it, Faro and Ganzirri. Therefore, it has a great biodiversity at its disposal which is studied in order to exploit interesting biomolecules for various commercial uses. Among these, several species of macroalgae lend themselves functionally such as *Gracilaria gracilis*, widely used as a source of agar.
In an attempt to use these extracts in veterinary medicine, however, preliminary studies have shown that in zebrafish (*Danio rerio)* it stimulates the immune system, this result could be useful in aquaculture farms.

P13. Marine biobased product from microalgae: lipid and antioxidant from *Phaeodactylum tricornutum*

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Different studies have shown that microalgae, when subjected to energy stress conditions that modify their metabolic patterns, such as nitrogen deprivation, are able to improve lipid biosynthesis.
In this experiment we evaluated how changes in culture's environmental conditions can affect the quali-quantitative modulation of lipids and antioxidants production in the marine diatom *Phaeodactylum tricornutum*.
The result related to the production of lipids shows that *P. tricornutum* maintained under stress conditions, through nitrogen starvation, produces a higher quantitative of lipids then when cultivated under standard conditions. The standard culture shows a higher content of polyphenols and carotenoids, resulting in a higher antioxidant activity, as highlighted by the DPPH free radical inhibition values and the reducing power test.
Data obtained indicate that it is possible to modulate the production of lipids and antioxidants in *P. tricornutum* by varying environmental conditions, so as to obtain, even on a large scale, bioactive compounds for industrial applications.

Acknowledgements
This work is supported by the project INTEGRAQUA "Intervento pilota per la coltivazione di microalghe integrata ad un impianto di acquacoltura marina" POFEAMP2014-20_SICILIA

P14. Survival and oxidative stress of *Spicara smaris* after being caught by traditional fishing nets

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The picarel (*Spicara smaris*) is a popular food with a long tradition in the Balearic Islands. Fishing is carried out using traditional fishing nets and the capture is self-regulated to maintain a balance between the demand and the fishing obtained. For this reason, when fishing is too abundant, excess catch is returned to the sea. The objective was to evaluate the effect of this practice, analysing the degree of oxidative stress and whether the survival of the specimens is possible after the fish have been caught. The results obtained have evidenced a survival of 100% of the fish caught and kept in a controlled environment after one week, and with a recovery of behaviour after the third day, presenting the normal pattern of coloration and stable and non-agitated behaviour. In a parallel experiment, blood samples from *S. smaris* specimens were obtained at the time of capture, day 1, at day 3, and a final sample at day 7. Blood samples were centrifuged to obtain erythrocytes. The activities of the antioxidant enzymes – catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase - in erythrocytes and the levels of malondialdehyde were progressively decreasing from the sample on day 1 to the sample on day 7. Although the action of fishing generates considerable stress on the fish, they are able to recover and should not significantly affect the life cycle of the fish. The traditional release of excess capture is useful for this species, allowing the catch to be adjusted with market demand.

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