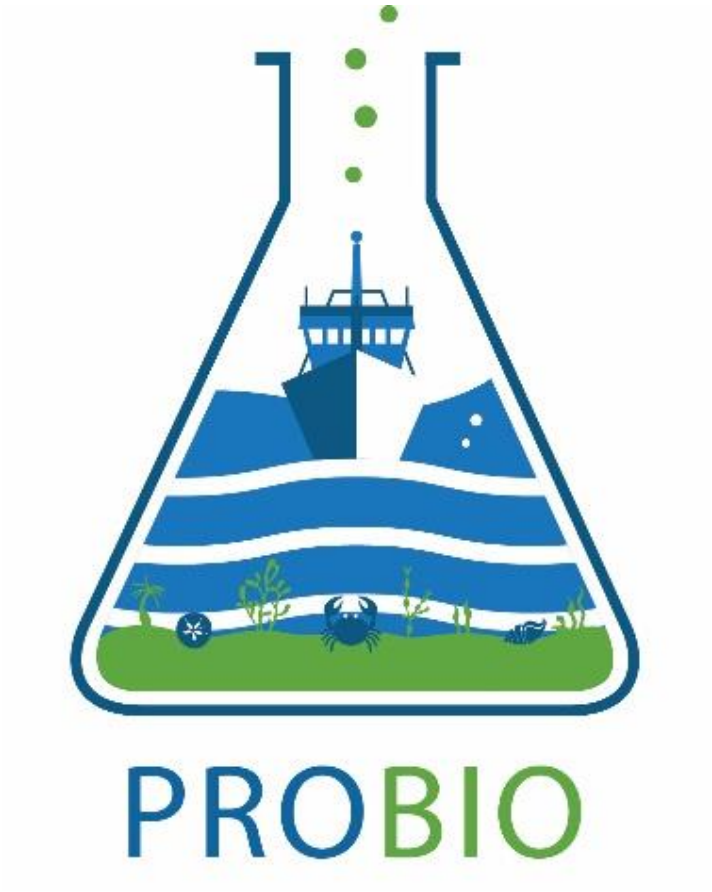

PROspection for BIOactive compounds in the North Sea

Deliverable 5.2.& 5.3 Valorisation report
Date: October 2022
Author: Blue Cluster (DBC), Flanders Marine Institute (VLIZ)

AGENTSCHAP INNOVEREN & ONDERNEMEN BLAUWE CLUSTER
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Project name	PROspection for BIOactive compounds in the North Sea
Project acronym	PROBIO
Start date of the project	01.11.2019
Duration	30 months + 6 months extension
Project type	Strategisch Basisonderzoek voor clusters (cSBO)
Funding	VLAIO – De Blauwe Cluster
Deliverable ID	D5.2. Industry-driven case studies with valorisation plan D5.3. Draft proposals of follow-up projects
Due date of deliverable	M30
Authors	Lien Loosvelt (DBC) Matthias Sandra (VLIZ) Fien De Raedemaecker (VLIZ)

Confidential report – PROBIO SAB members only

Loosvelt L., Sandra M., De Raedemaecker F. (2022). *Valorisation report*. D5.2 & D.5.3. PROspection for BIOactive compounds in the North Sea. Oostende, Belgium. 17 pp.

Acknowledgements:

Special thanks to the PROBIO-Consortium and Advisory Board for their input to the use cases, bilateral consultations and workshops.

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1. Summary

Marine environments offer a wide variety of bioresources containing potential bioactive compounds. The potential of bioactive compounds from these marine bioresources is currently hugely underexplored in our seas and oceans, and will offer a 'sea of opportunities' in several sectors involved with the research, development and production of functional additives and products. Primary and secondary metabolites in marine organisms are of particular interest because they have unique properties and a broad valorisation potential in e.g. pharma, cosmetics, agriculture, nutraceuticals, chemicals and functional bio-based materials. The **PROspection for BIOactive compounds in the North Sea (PROBIO) project** is focusing on the discovery and characterisation of new bioactive compounds derived from local North Sea species.

The results from the bio-activity screening of 50 North Sea species is the starting point for further valorisation of the PROBIO-project. The species **shortlist contains species with commercial potential** regarding marine taxonomic groups and compounds with potential biological activity, based on an assessment of the relevant scientific literature. The Strategic Advisory Board (SAB) was involved in the formulation of criteria and in the species selection procedure for the species shortlist. Criteria that were considered were the potential of cultivation, the available biomass in sufficient replicate sites.

The 50 species from the shortlist were sampled and fractionated into 99 batches, each containing 96 fractions. Each species contains on average 800 compounds. There was a **hit rate of 4%, which resulted in a set of 200 bioactive fractions** (containing one or more active compounds). Five species were chosen based on the most promising results from the antimicrobial and electrophysiological assays (*Asterias rubens*, *Echinocardium cordatum, Ophiura ophiura, Sargassum muticum* and *Ophiura albida*). The results were cross-linked with the bioprospection index cards in order to identify promising application(s) for the detected active compounds (cosmetics, pharmaceuticals, antifouling, functional ingredient and hygienic packaging). This resulted in **a set of five use cases for promising hit species** that were described in a fact sheet.

From an application perspective, the advisory board identified the use case **"antifouling solutions in offshore and onshore aquaculture facilities"** as most promising. In a brainstorm session the assumptions that need to be validated regarding problem fit, solution fit and business model fit were identified. This resulted in a **concept note for a feasibility study**. However, it became clear that there is still a large gap between the research results and the industrial applications. Therefore, a next step needs to be taken by the research partners in identifying and purifying the compounds that were found in the "hit" fractions and sub-fractions. This knowledge is important for the industry in order to be able to register new products and to develop new processing technologies/partnerships. A **concept note for a follow-up basic research project** was drafted.

In addition to the follow-up R&D projects, there is also a need to initiate other actions for valorisation of the PROBIO results. A table of action points was compiled during an interactive session with the advisory board.

2. Introduction

Little is known on the chemical composition of marine organisms from the Belgian part of the North Sea. Besides literature giving an indication of the potential for bioactive compounds, the high variability between species, locations and seasons, justifies the screening of local species that are adapted (genetically and physiologically) to the harsh conditions of the North Sea. About 2500 different species occur in the Belgian part of the North Sea (BeRMS[1]), with several taxonomic groups of interest for bioactive screening (Hu et al., 2015[2]): Porifera (33 sp.), Cnidaria (54 sp.), Mollusca (124 sp.), Tunicata (11 sp.), Echinodermata (26 sp.), Vertebrata (233 sp.), Arthropoda (566 sp.), Macroalgae (107 species). Within PROBIO, 50 species were sampled and fractionated into 99 fractions. Each species contains on average 800 compounds. There was a hit rate of 4%, which resulted in a set of 200 bioactive fractions (containing one or more active compounds). When extrapolating to the entire North Sea, this means that if all 1000 species would have been analysed, there is potentially a database of 90.000 fractions (containing 772.800 compounds) to be explored and therefore 3.600 active compounds to be discovered.

Table 1: Overview of direct and indirect market opportunities for bioactive compounds from (cultivated) marine organisms and innovation potential for Flemish companies

Sector	Challenges	Innovations
Aquaculture	Improve rentability of aquaculture and manipulate quality of end products;	- Substrates to grow new species, e.g. tunicates; - Cultivation techniques to manipulate the chemical composition of biomass; - Bioactive extracts as additive (<10%) in aquafeed to stimulate immunity, promote digestibility and control diseases; - Antifouling solutions that are non-toxic and cost efficient;
Biotechnology	Need for efficient refinery processes with maximal valorisation of side streams;	- Upscaling of purification and bioprocessing steps; - Development of refinery protocols;
Agriculture	Reduce use of antibiotics, pesticides and production of methane in order to make food chain more sustainable.	- Application of bioactive extracts as biostimulans or natural pesticide in disease control; - Application of bioactive extracts as additive (<10%) in feed to stimulate immunity, promote digestibility, control diseases;
Health	Search for more effective or breakthrough drugs and medicins with high biocompatibility;	- Synthetic reproduction of new families of bioactive compounds from marine organisms; - Natural extracts of marine organisms for neutraceuticals; - Development of innovative wound care devices;
Materials & chemicals	Need for functional additives that are not harmful when leaching into the environment;	- Development of food packaging to extend shelf life; - Biobased antifouling products for marine shipping or structures;

[1] VLIZ Belgian Marine Species Consortium (2010 onwards). Belgian Register of Marine Species. Accessed at https://www.marinespecies.org/berms on 2022-11-25

[2] Hu, Yiwen, Jiahui Chen, Guping Hu, Jianchen Yu, Xun Zhu, Yongcheng Lin, Shengping Chen, and Jie Yuan. 2015. 'Statistical research on the bioactivity of new marine natural products discovered during the 28 years from 1985 to 2012', *Marine drugs, 13*: 202-21.

The direct market potential for marine bioactive compounds lies in the development of innovative products based on the discovery of active compounds. Different markets are aimed at: aquaculture, food/feed, agriculture, cosmetics, pharmaceutical, medical & chemical. Depending on the type of application, the production of bioactive compounds might differ: 1/ natural extracts, 2/ purified compounds, 3/ synthetic analogues. In addition, discovery of bioactive compounds could indirectly lead to new markets in aquaculture and biotechnology as technology suppliers in the cultivation of new marine organisms (table 1).

3. Industry-driven case studies

The hit species found from the lab screening were cross-linked with the bioprospection index cards (Sandra and De Raedemaecker, 2022[3]) in order to identify the most likely application(s) for the detected active compounds. For some species, evidence of bioactivity was already reported in literature, while for others the discovery is quite novel. Moreover, whenever cultivation opportunities are known for a certain species, the corresponding publication was included in the bioprospection index cards. For five species (*Asterias rubens*, *Echinocardium cordatum, Ophiura ophiura, Sargassum muticum* and *Ophiura albida*) and five applications (cosmetics, pharmaceuticals, antifouling, functional ingredient and hygienic packaging), a use case was developed. The species were chosen based on the most promising results from the antimicrobial and electrophysiological assays. The applications correspond with the five pre-defined sectors which are represented by the SAB members (figure 1).

Figure 1: Five applications that were targeted in PROBIO and reflect the composition of the advisory board

[3] Sandra, M. and De Raedemaecker, F. (2022). PROspection for BIOactive compounds in the North Sea - Bioprospection Index Cards. D 1.3. Flanders Marine Institute (VLIZ): Oostende. 163 pp.[details]

3.1. Cosmetics

Skin aging is characterised by the progressive deterioration of the functional properties of the skin owing to changes in the functioning of certain organs of the body and overall fitness levels. Therefore, the growing consumer demand for products that rapidly lower the signs of aging, which include wrinkles, lines, blemishes, frown lines and pigmentation, has increased the demand for anti-wrinkle products. The global anti-aging products market size was valued at USD 40.49 billion in 2020 and is expected to expand at a compound annual growth rate (CAGR) of 5.4% from 2021 to 2028. The growing demand for plant-based alternatives has resulted in a recent shift in consumer preference for organic and natural products. Furthermore, with the growing concerns over animal welfare and the environment, a number of consumers are opting for cruelty-free products, which has increased the demand for vegan or plant-based ingredient blends in skincare products. New product launches and innovations are expected to drive the demand.

The evolution in the skin care industry has been driven by the recent interest shown by consumers in novel bioactive compounds from natural sources, instead of synthetic ingredients, and fostered the efforts to develop biologically active ingredients from natural organisms. Although plant-derived extracts are usually among the main constituents of the cosmetic formulations containing natural ingredients, with the advance of the biotechnological field observed in the marine industry, an increase in the use of marine ingredients for this purpose has been noticed over the last years. While in 2011, only 6% of the 190 anti-aging cosmetics was from marine origin, a noticeable growth was observed in 2018, where these ingredients were used in 33% of the products (Resende et al., 2021[4]). Algae were undoubtedly the most used marine ingredient in these anti-aging formulations, probably due to their biodiversity, easy cultivation, and growth modulation.

[4] Resende et al., 2021. Trends in the use of marine ingredients in anti-aging cosmetics. **Algal Research** Volume 55, May 2021,

Fact sheet use case 1 – cosmetics

- **Marine organism**: Common Starfish - *Asterias rubens*
- **Sources**:
  - By-product in fisheries
  - By-product in mussel cultivation (predator)
- **Hit compound:**
  - Unknown, in fraction F81 (propably saponins or neuropeptide)
  - Literature findings: Neuropeptides, adhesive proteins, nitric oxide, etc.
- **Functionality**: nAcHRr blocking 80-100 % - nAChR stands for nicotinic Acetylcholine Receptor, a membrane-bound peptide that respond to the neurotransmitter acetylcholine. Nicotinic receptors also respond to drugs such as the agonist nicotine. They are found in the central and peripheral nervous system, muscle, and as such play a crucial role in the cholinergic transmission in our body. Preventing the release of acetylcholine from axon endings at the neuromuscular junction or blocking the nAChR there, causes flaccid muscle paralysis and an anti-wrinkle effect.
- **Application**: Antiwrinkling, anti-aging lotion or cream
- **Novelty**: Muscle relaxant neuropeptides confirmed in literature → see Bioprospection Index Card

3.2. Pharmaceuticals

Neurodegenerative diseases are characterized by a progressive loss of neurons that leads to a broad range of disabilities, including severe cognitive decline and motor impairment, for which there are no effective therapies yet. Along with increasing global population and average lifespan, the prevalence of neurological disorders is on the rise, worldwide. The Alzheimer's Association states that around 5.4 million people in the United States were been living with Alzheimer's disease, as of 2016. This disease is the sixth leading cause of death in the United States and is more prevalent among people above 65 years of age (5.2 million out of the 5.4 million patients fall in this age bracket). The number is expected to increase during the forecast period, worsening the situation in the coming years. Thus, the prevalence of neurodegenerative disorders is on the rise, contributing to the growth of this market. The neurodegenerative diseases drugs market is estimated to reach US$ 53 billion by 2026. Currently, no neurodegenerative disease is curable, and the treatments available only manage the symptoms or halt

the progression of the disease. Therefore, there is an urgent need for new treatments for this kind of disease. The World Health Organization has predicted that **neurodegenerative diseases affecting motor function will become the second-most prevalent cause of death in the next 20 years.** New therapies can come from three main sources: synthesis, repurposing existing drugs and natural products.

Previous epidemiological and genetic studies have documented the association of NRs with the risk of **inflammatory bowel disease** (IBD). NRs play complicated roles in regulating intestinal immunity, mucosal barriers, and intestinal flora. In the gut, NRs play a broad range of intestinal functions, including nutrient absorption and transport, solute and water absorption/secretion, gut–liver communication, and gut microbiome regulation. Moreover, several members of the NR family are involved in immune regulation. As the gut immune system comprises 70%–80% of the body's immune cells, dysregulation of NR signalling may underlie the mechanisms of intestinal inflammatory diseases such as IBD. The global inflammatory bowel diseases (IBD) drug market is estimated at $6.7bn in 2017 and $7.6bn in 2023.

Fact sheet use case 2 – pharmaceuticals

- **Marine organism**: Sea potato - *Echinocardium cordatum*
- **Source**: cultivation in lab conditions have been demonstrated
- **Hit compound**: Unknown, in fraction 47
- **Literature findings**: Hedathiosulfonic acids A and B, mannan-binding lectins
- **Functionality**: Kv1.3 blocking 80 % - Kv1.3 is a potassium-selective, voltage-gated channel, also known as KCNA3, expressed as membrane-bound protein mainly in T and B lymphocytes. It is generally accepted that effective treatment of autoimmune and neuro-inflammatory diseases, such as multiple sclerosis (MS), stroke, epilepsy, Alzheimer's and Parkinson's disease, remains a challenge. Since Kv1.3 plays a crucial role in subsets of T and B lymphocytes, as well as microglial cells, it is considered a novel therapeutic target for treating these disorders.
- **Application**: Therapeutic agent to treat autoimmune and neuro-inflammatory diseases
- **Novelty**: Limited evidence in literature

3.3. Antifouling agents

Fouling is an issue that crops up in almost all areas of the blue economy where offshore structures - fixed, floating and mobile - are exposed to seawater. This requires these structures to be regularly cleaned or treated with a coating that slows or prevents biomass fouling. Fouling poses a major challenge for the growing blue economy (floating wind, solar, aquaculture, among others) because they increase the tensile force on the structures, increasing the risk of incidences and oversizing the anchoring. This is obviously associated with additional costs both in the investment and operational phases. For example, offshore oyster farming is currently not profitable due to fouling issues.

Fouling prevention or remediation solutions are crucial to make floating technology cost-effective. At present, chemical solutions are the main focus, but the industry is looking for sustainable alternatives because of the ban on toxic components by Europe. Physical solutions present difficulties (and high costs) concerning manual operations at sea or in terms of connectivity as technology cannot withstand a harsh marine environment.

Fact sheet use case 3 – antifouling agents

- **Marine organism**: Serpent brittle star - *Ophiura ophiura*
- **Source**: By-product in coastal fisheries
- **Hit compound:**
    - Unknown, in fraction F55
    - Literature findings: none
- **Functionality**: good inhibitory or killing activity against *S. aureus* - Growth inhibition of more than 50% against *S. aureus* at a dilution of 1/32, bactericidal activity against *S. aureus* at a dilution of 1/16; no activity against *Candida albicans* and *Acinetobacter baumannii*
- **Application**: Fouling restraining agent in coatings in aquatic/marine environments
- **Novelty**: no evidence in literature

3.4. Functional ingredients

The global nutraceutical market should reach $336.1 billion by 2023 from $230.9 billion in 2018 at a compound annual growth rate (CAGR) of 7.8%, from 2018 to 2023 (BBC Research 2018). The functional beverages segment of global nutraceutical market is expected to grow from over $83.1 billion in 2018 to $124.4 billion in 2023 at a CAGR of 8.4%, from 2018 to 2023. The functional food segment of global nutraceutical market is expected to grow from $75.1 billion in 2018 to $110.9 billion in 2022 at a CAGR of 8.1%, from 2018 to 2023. The U.S. market is currently focused on diversifying its products. In Europe, however, the focus is more on innovation, research and development. The European market is also looking to consolidate and organize itself. European companies are looking to dominate the global nutraceutical market, as can be seen by their synergistic acquisitions worldwide (BBC Research 2018).

Specific feed additives that enhance a number of important processes in the animal body can be considered to be plausible candidates and alternatives for the animal feed for livestock or aquaculture. These feed additives may also alter immune responsiveness or disease resistance of the farmed and cultivated species. Safeguarding the health of animals and preventing animal diseases serves to protect public health, the environment and the economy of a country (EU Food Safety Authority). These new feed additives may be included among supplements that are aimed to positively affect feed quality, health of animals as well as animal products by means of their specifically efficacious substances. Feed ingredients and additives can be classified into several groups: **sensory additives** (substances affecting food odour and palatability, and colourings), **technological additives** (antioxidants, substances decreasing mycotoxin contamination, etc.), **zootechnical additives** (immunomodulators, digestive stimulants, growth promoters of non-microbial origin, substances increasing performance or quality of animal products, etc.) and **nutritional additives** (vitamins, minerals, plant enzymes, etc.).

Sources of new bioactive compounds with specific sensory, antimicrobial, immunostimulatory, immunomodulatory and/or stress reducing characteristics will offer opportunities for the development of new innovative feed or feed ingredients/additives for cattle breeding and aquaculture. It is known that marine bioactive compounds exhibit very interesting biological activities (e.g. anti-inflammatory, immuno-stimulating and anti-microbial), which through their use in aqua-feed can provide both nutritional value as well as physiological benefits to the cultivated species.

Fact sheet use case 4 – functional ingredients

- **Marine organism**: Wireweed - *Sargassum muticum*
- **Source**: Suited for cultivation
- **Hit compound**:
    - Unknown, in fractions F7-9
    - Literature findings: Lipids, sulfated polysaccharids, Carotenoids, etc.
- **Mechanism**: Low to moderate activity against *S. aureus* - Growth inhibition of more than 50% against *S. aureus* at a dilution of 1/32, bactericidal activity against *S. aureus* at a dilution of 1/16; no activity against *Candida albicans* and *Acinetobacter baumannii*
- **Application**:
    - Antioxidant and anti-inflammatory additive
    - Functional feed/food
- **Novelty**: Multiple references in literature

3.5. Hygienic packaging

Despite the problems associated with antimicrobial resistance (and the rapid development of resistance against novel antibiotics), the current development strategy in the antimicrobial market still focuses on identifying such novel compounds that interfere with cell growth or disrupt cell integrity. While other therapeutic strategies have been (and continue to be) considered, these strategies have so far failed to make it to the market. A well-known class of molecules that can serve as an example are quorum sensing inhibitors (Brackman and Coenye 2015). These compounds interfere with the bacterial communication system, and while there is a huge body of scientific literature showing that these compounds hold great promise based on in vitro experiments, results from clinical trials are disappointing; despite decades of research, none of these compounds has made it to the market (NH US National Library of Medicine, 2019). This indicates that looking for novel compounds with a 'conventional' mode of action (i.e. interfere with cell growth or disrupt cell integrity) is still a very valid approach.

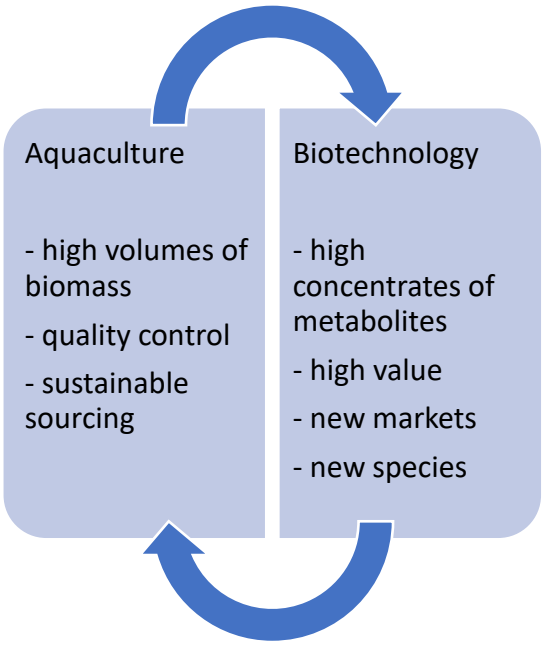
Although the food market is very competitive, the demand for safe marine biotech food products is really large. Marine biotech offers the solution for many issues that consumers currently want. Think of unique, natural and sustainable products, free from chemical additives (as pure as possible) etc. In addition, the customer now increasingly wants traceable and environmentally friendly products. This is especially applicable in the food industry, but can also be extended to clothing, new materials, bioplastics etc. A very specific market, but with exceptionally large growth is that of sports nutrition (Kristinsson 2019).

Fact sheet use case 5 – hygienic packaging

- **Marine organism**: Serpent's table brittle star - *Ophiura albida*
- **Source**: by-product in coastal fisheries
- **Hit compound**:
    - Unknown, in fraction F83
    - Literature findings: Hydroxybezene compounds
- **Mechanism**: Excellent activity against *S. aureus* - Bactericidal up to 1/64 dilution! No activity against *Candida albicans* and *Acinetobacter baumannii*
- **Application**: Antibacterial agent in food packaging or medical devices
- **Novelty**: Limited evidence in literature

4. Potential for aquaculture and biorefinery

By combining the needs from the different sectors, being product development, biotechnology and (offshore) aquaculture, it will be easier to create new value chains for marine organisms in Flanders. Due to the natural variability of products from biological origin, the quality is highly variable which is not desirable especially for high-end applications. This challenge could be addressed by cultivating biomass in farms and control the environmental conditions that have an impact on the composition and quality of the biomass. Especially for metabolites, management of farms should correspond to buyer's needs. Metabolites are often produced under specific or stress conditions, and are thus variable in time, location and genetics. This means that species selection, growing conditions and harvesting period can be steered to optimize the concentration of metabolites. As such, both the aquaculture and biotechnology sector can benefit from a close collaboration.

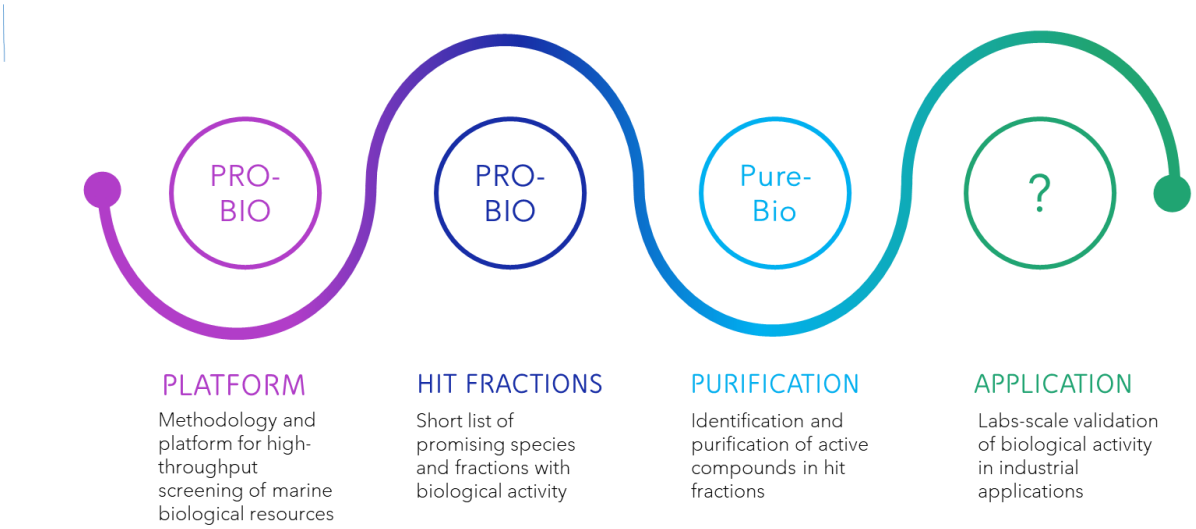


Not only the sustainable cultivation and harvesting of seaweed resources are points of attention while upscaling, but also the sustainable use and processing of seaweed biomass. The combined use of seaweed fractions for bioactive compounds, food, non-food and feed applications through biorefinery allows the most promising opportunity for efficient use of resources and an economically viable business case. In this respect, research is needed to evaluate the nutritional, functional, and safety aspects of all residue fractions of algae processing (Pycke and Faasse 2016). Specific for the production of bioactive compounds from seaweed, it is necessary to evaluate what the residual stream consists of and for what it can be used. One possibility is to use the remaining biomass for the production of biodegradable biobased plastic. Extracts of the seaweeds can be incorporated in commercial biopolymers, e.g. for edible food packaging for froze seafood (Lemos et al. BIOPROSP 2019). Seaweed extracts incorporation in polymeric films can lead to a new type of biodegradable active packaging to replace plastic formulations actually used in food industry, which also has the potential to be used in ready to cook meals.

5. Draft proposal for follow-up projects

From an application perspective, the advisory board identified use case 3, and more specifically "antifouling solutions in offshore and onshore aquaculture facilities" as most relevant for developing follow-up research. In a brainstorm session with some companies the assumptions that need to be validated regarding problem fit, solution fit and business model fit were identified. However, it became clear that there is still a large gap between the research results and the industrial applications. Therefore, a next step needs to be taken by the research partners in identifying and purifying the compounds that were found in the "hit" fractions. This knowledge is important for the industry in order to be able to register new products and to develop new processing technologies/partnerships.

In this chapter, **two follow-up projects for PROBIO are described in the form of a concept note** (including problem statement and project objectives). The first project is also the most critical research step and covers the "purification and identification of bioactive compounds from bioprospection of North Sea species (PureBIO)". This research is low TRL level and high risk and therefore considered as strategic basic research with limited involvement of the industry. The second project will allow to bring the results of the PureBIO to higher TRL levels in the form of an industry driven feasibility study regarding "novel, marine-based antifouling agents for aquaculture". This study should stimulate further industrial R&D activities in case of positive results.



5.1. Purification and identification of bioactive compounds from bioprospection of North Sea species

Problem statement

Marine environments offer a wide variety of bioresources containing potential bioactive compounds. Primary and secondary metabolites in marine organisms are of particular interest because they have unique properties and a broad valorisation potential in e.g. pharma, agriculture, nutraceuticals, chemicals and functional bio-based materials. However, the biodiscovery of new bioactive compounds has so far been limited in the North Sea region. The PureBIO project further builds on the knowledge

base of the previous PROBIO project, which screened 50 North Sea species containing potential bioactive compounds. The established high-throughput screening will be optimised to purify and identify bioactive compounds found in the most promising fractions from several marine organisms of the PROBIO project. Purified compounds from these marine species will be tested on antimicrobial activity to characterise their bioactivity. Nuclear magnetic resonance (NMR) analysis will be performed to structurally identify the purified bioactive compounds. Antimicrobial testing of the purified compounds will be tailored to potential industrial applications (e.g. antifouling). With this, the project takes a next step in the process of marine bioprospection in Flanders and the identification of valuable biobased products and resources. The broad economic valorisation potential of these purified compounds will initiate new collaborations and industry-driven follow-up research.

Project objectives

The main objective of PureBIO is to purify and identify bioactive compounds from marine bioresources, and, hence continue where PROBIO stopped. In PROBIO very promising results were obtained regarding the prospection of bioactive molecules and setting up a fluid workflow on the detection of fractions containing bioactive molecules form a set of very diverse marine organisms. This outcome is the perfect starting point to further explore the positive results with the aim of absolute identification of the bioactive compounds by their structure and chemical composition. This will open opportunities to upscale the purification of targeted bioactive compounds or even chemically synthesize them once the structure is known. For industrial or pharmaceutical applications this could be of great interest since the demand for new bioactive compounds in general is high.

In PureBIO the main objectives are:

- To harvest large quantities of fresh material, and as such also bioactive compounds, from targeted species with bioactive compounds as indicated by the antibacterial assays in the PROBIO project;
- To confirm the presence of bioactive compounds, to purify the active compound by 2D preparative chromatography, and finally to isolate the active pure compounds by high resolution analytical (orthogonal) chromatography;
- To determine the chemical structure of a bioactive compound based on nuclear magnetic resonance (NMR) and high resolution mass spectrometry (HRMS); and
- To test these bioactive compounds in conditions that mimic those found in vivo in patients (e.g. wounds) or in real-life industrial applications (e.g. anti-fouling).

Once the structure of a bioactive compound is found, targeted searches for these compounds can be performed in other unexplored biological organisms or natural sources in future research projects. Through the HRMS analysis the accurate mass, chemical formula, fragmentation data and retention time will have be determined, allowing the research community to screen targeted for these compounds and even find related compounds (chemical classes) with potential bioactive characteristics from many other natural resources. It will also enable targeted literature searches based on the known identity and allow upscaling of the purification of the target compound (e.g. for industrial applications) through developing optimized targeted methods, which is not possible until the identity of a compound is known.

5.2. Novel, marine-based antifouling agents for aquaculture

Problem statement

Fouling is an issue that crops up in almost all areas of the blue economy where offshore structures - fixed, floating and mobile - come into contact with seawater. This requires these structures to be regularly cleaned or treated with a coating that slows or prevents biomass fouling. Fouling poses a major challenge for the growing blue economy (floating wind, solar, aquaculture, among others) because they increase the tensile force on the structures, increasing the risk of incidences and oversizing the anchoring. This is obviously associated with additional costs both in the investment and operational phases. For example, offshore oyster farming in the Belgian part of the North Sea is currently not profitable due to fouling issues.

Fouling prevention or remediation solutions are crucial to make floating technology cost-effective. At present, chemical solutions are the main focus, but the industry is looking for sustainable alternatives because of the ban on toxic components by European Commission. Physical solutions present difficulties (and high costs) regarding manual operations at sea or in terms of connectivity as technologies cannot withstand a harsh marine environment and a lot of maintenance is required.

Marine organisms are an unexploited source to seek for innovative biobased solutions to fouling on (floating) offshore structures with the precondition that they have no negative impact on the marine environment. Especially in aquaculture, alternative antibacterial/biofilm solutions are sought for (i) current antibiotic products by means of novel compounds for health & environment (e.g. immunostimulants, desinfectors, …), (ii) selective restraining of macrofouling (e.g. lobsters, polyps) on substrates as alternative for high pressure cleaning and (iii) ecological fouling restraining products (not toxic for marine environment) as alternative to cupper based products – which will be banned in a few years.

In the PROBIO project, a significant antibacterial activity was detected in Serpent brittle star (*Ophiura ophiura*), more specifically in fraction 55. This is a species that is often collected as by-product in coastal fisheries and nearshore mussel farming. Lab scale tests confirmed its functionality: good inhibitory or killing activity against *S. aureus* - Growth inhibition of more than 50% against *S. aureus* at a dilution of 1/32, bactericidal activity against *S. aureus* at a dilution of 1/16; no activity against *Candida albicans* and *Acinetobacter baumannii*. There is no evidence found in literature that reports on similar activities, which makes this discovery novel.

Project objectives

This project will study the feasibility of developing a novel, eco-friendly coating solution to prevent fouling (micro/macro) of aquaculture systems (onshore/offshore) using novel compounds from bioprospection of marine organisms. Activities will include desktop studies and lab-scale testing to validate a set of assumptions concerning the problem, solution and market.

Problem fit validation:

- activity against Vibrio species with plate testing (without organisms)
- microfouling restraining properties using with antibiofilm tests
- selective restraining properties against microspecies (e.g. lobster)
- relationship between micro- & macrofouling

Solution fit validation:

- activity period of coating versus frequency of manual cleaning through on site testing in offshore aquafarm
- application form (water soluble) in binders and evaluate ease of implementation
- toxicity for the marine environment based on in vitro toxicity tests (tank, substrate)
- no interference with probiotics (e.g. bacillus species) based on in vivo shrimp disease testing

Business model validation:

- upscaling of the production process and sustainability of production
- availability of resources affecting the feasibility of production pathways (biological versus synthetic)
- functionality versus purity of the active compound
- regulatory compliance and registration of the product
- cost effectiveness and competitiveness of the product (as determined by the longevity of the product)

Each of these assumptions needs to be validated through desktop or lab-scale experiments. Based on the results the experiments, it can be decided whether or not it is both technological, economical, and ecological feasible/desirable to invest in a follow-up research project to develop a production process for this novel compound. This project should elaborate in-depth on technological solutions for the harvesting, extraction, purification of antifouling agents and processing and application in aquaculture systems. Industrial partners along the entire value chain will be involved.

6. Valorisation plan

In addition to the follow-up R&D project as mentioned in chapter 5, there is also a need to initiate other actions for valorisation of the PROBIO results. The table below gives an overview of the input gathered during an interactive session with the advisory board.

	SHORT TERM	LONG TERM
Market & business drivers (WHY?) • *Major environmental trends* • *Opportunities* • *Threats* • *…*	• IP protection: difference between protection of use and resource; usually company secrets • Customer-driven approach: identify applications (e.g. antifouling offshore wind farms) • Need for storytelling (showing concrete and real results, examples of successful projects) • Promotion of project in newsletter of clusters to activate interest of companies in follow up projects	• Pharmaceuticals • Wound healing • Nagoya protocol (cost driver as % of profit) applicable for both synthetic and natural compounds and academic and industrial • Cost of pure extract versus extracts
Product & service features (WHAT?) • *Major objectives* • *Alternative courses of action* • *Sales, profits, product development* • *…*	• Targeting added value of products (food combined with other value e.g. health) • Aquafeed applications “as a whole” e.g. shrimp • Cleaning business (e.g. detergent) • Food production sites (e.g. dairy): application of coating on inox to avoid fouling in piping	• Services for the aquaculture sector e.g. Companies for screening functionalities of products (e.g. waste as a value) • Sustainable de-worming compound for offshore culturing environment (salmon/ sea bass / seabream in cages) • Feed additives with marine extracts for improving robustness, registration of group compounds • Offshore applications e.g. coatings/glues
Technology & capability needs (HOW?) • *Major policies and resources* • *Marketing, products, finance* • *Employees, prices, technology* • *…*	• Enforcing screening platform • Identification of pure compounds • Translation of scientific results to industrial applications • Specific indutrial questions as incentive to develop specific models (e.g. marine environment)	• Accessibility of science: making it understandable through communication and integration • Valorisation of side and waste streams • In vitro testing and at company level (e.g. effect on microbiome)