GROWING SEAWEEDS FOR POLYSACCHARIDES EXTRACTS AS POTENTIAL IMMUNOSTIMULANTS IN FISH AQUACULTURE

E.-j. Malta*, B. Partida, M. Macías, J. Cabello, I. Folgueira, M.M. Agraso Martínez

Centro Tecnológico de Acuicultura de Andalucía (CTAQUA), Muelle Comercial S/N, 11500, El Puerto de Santa María, Spain
Contact person: e.malta@ctaqua.es

Introduction

Research on the use of seaweeds in aquaculture feeds has been growing exponentially over the last few years. We have been building a literature database over the last few years. First publications date from the 1980s, with a total of only eight references in the past century, 19 in the first decade of this century and no less than 116 in the period 2011-2020. Moreover, 99 of these are from the last five years. Of this total of 143 references, 85 are studies on the use of seaweeds in fish aquaculture, indicating that this a topic of high scientific interest. The majority of the studies focus on the use of whole seaweed meal, mainly as a source of protein and carbohydrates substituting basically wheat, soy and to a lesser extent, fish meal, in artificial diets for aquaculture. Only in the last five years, studies started concentrating on the use of seaweed extracts as potential functional feed components, where the sulphated polysaccharides appear to be of particular interest (e.g. Coste et al., 2015).

At CTAQUA, we started the IMMUNO&ALGAE project in January 2019 with the aim to further explore the use of seaweeds in fish aquaculture as a source of functional feed compounds, in particular that can have immunostimulating effects. Objectives of the project are to optimize and upscale the cultivation of three pre-selected seaweed species from the southwest of Spain, to optimize the extraction of sulphated polysaccharides and to evaluate their use as potential immunostimulating compounds in finfish aquaculture. Seaweeds were selected based on their commonness in the area, prior experience with cultivation and their apparent potential for upscaling cultivation. In addition, the ambition was to test at least one species of each of the major seaweed groups. This led to the selection of the chlorohyte alga *Ulva ohnoi*, the rhodophyte *Gracilaria gracilis* and the phaeophyte *Dictyota dichotoma*.

The first phase of the project was dedicated to the optimization of the cultivation, to experiment with different cultivation conditions and to obtain biomass, either from culture or from harvest of wild populations, to obtain a sufficient amount of polysaccharide extract for inclusion in experimental diets. This phase is nearing completion and we here present the first results of these efforts and the next steps planned in the project.

Material and Methods

Ulva ohnoi was isolated from an earthen pond extensive aquaculture system in the SW of Spain in 2017 and maintained in culture at CTAQUA since then. The majority of the biomass (90%) used for polysaccharide extraction was grown at CTAQUA, 10% is from harvest from wild populations. Its taxonomic status has been confirmed by DNA sequence analysis. *Gracilaria gracilis* was isolated from the same earthen pond and brought to CTAQUA for cultivation trials. Additional biomass was harvested at the end of 2020 and beginning of 2021 (delay due to pandemic) for extraction. *Dictyota dichotoma* biomass was harvested from the La Caleta beach, city of Cádiz. Unfortunately, no sufficient biomass could be harvested for nutritional trials, also due to the lower percentage of polysaccharides in the algae and the potential confusion with the exotic seaweed *Rugulopteryx okumurae* that recently invaded the area.

As a first step towards cultivation, algal fronds were grown in filtered (1 µm) natural seawater under constant temperature, light and nutritional conditions in an attempt to obtain unialgal cultures. To increase the amount of clean biomass, cultivation volumes were increased stepwise from 250 mL to 5 L bottles. In the next step, algae were grown indoor in photobioreactors under LED lighting at controlled temperature, following a specific nutrient addition regime. Finally, for larger scale cultivation, outdoor tanks were deployed and cultivation was followed through an annual cycle with weekly harvests and weekly nutrient addition.

Polysaccharide extraction was carried out on dried algal biomass by the research group "Photobiology and Biotechnology of Aquatic Organisms" of the University of Málaga (Spain) using repetitive cycles of hot water extraction followed by selective precipitation, purification, flocculation and dialysis. Finally, dry extracts were obtained by lyophilization (see Abdala Díaz et al. 2019 for details).

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Results and Discussion

Clean cultivation for *U. ohnoi* was obtained at the first attempt and clean, fast-growing fronds were obtained. For *G. gracilis*, despite various attempts, including cultivation of the youngest growth tips only, cleaning with diatom sand, etc., completely unialgal cultures could not be obtained. Nevertheless, epiphyte growth could be maintained at low levels using a specific pulsed nutrient addition regime. For *D. dichotoma*, no successful cultures could be obtained and more studies are required for this species.

For both *U. ohnoi* and *G. gracilis*, steady growth was obtained in photobioreactors with growth rates ranging between 10 – 13 % d^{-1} and stable weekly biomass yields, although occasional collapses occurred in *U. ohnoi*. In a separate project, the potential relation with the algae microbiome is studied. *U. ohnoi* could be successfully cultivated in outdoor tanks all year long, with maximum biomass yields in spring (May – June) and minimum in winter (Jan – Feb). The annual cycle is still not completed for *G. gracilis*, provisional results also indicate that spring is the most favourable period, whereas in late summer growth rates decreased to close to zero, most likely due to high temperatures (≥ 30 ° degrees daily maximum temperature).

As a next step for the optimization of growth in photobioreactors and maximization of useful compounds, experimental trials are being carried out using diffent LED colours.

Polysaccharides could be successfully extracted from all algae, with the highest yields for *G. gracilis*, followed by *U. ohnoi* and *D. dichotoma*. Carbohydrate contents were considerably lower for the latter. Extracts are currently being tested for antibacterial activity. Subsequently, their potential immunostimulating effect will be tested *in vitro*, using cell line cultures and *in vivo* in a nutritional trial with sea bass. The most promising polysaccharide will finally be tested at different addition levels in a second nutritional trial, followed by a challenge study.

References

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