The REMEDIA-Life project and the cultivation of macroalgae as bioremediators: exploitation of their biomass for biotechnological purposes

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Introduction

The REMEDIA Life Project (REmediation of Marine Environment and Development of Innovative Aquaculture: exploitation of edible/not edible biomass) funded by the European Program LIFE-ENV, with Grant Agreement number LIFE16ENV/IT/000343 foresees, for the first time in Europe, the use of an integrated multi-trophic aquaculture system (IMTA), which in addition to fish breeding includes the rearing/cultivation of a new set of bioremediator organisms such as polychaetes, porifera, mussels and macroalgae. In particular, macroalgae are included in the set of bioremediator organisms because some species are able to reduce the nitrogen and phosphorus load produced by fish farming. The project aims to demonstrate that the bioremediation technologies developed can be successfully applied to an industrial mariculture plant in an environment confined, with positive effects in the specific sector and with the aim of the biotechnological exploitation and the zero-kilometer marketing of the biomass produced. In view of a circular economy, the biomass of macroalgae produced can be used for the extraction of active ingredients with antibacterial action. Recent studies have shown that algae are the source of a wide and diversified range of secondary metabolites with antibacterial, antifungal, antitumor and anti-inflammatory properties (Saha et al., 2018) as part of the growing development of biotechnology. Therefore, natural products from seaweeds could play an alternative role for drug discovery. The cultivation of macroalgae in IMTA constitutes an added value because the antimicrobial compounds produced by some of them and released in the surrounding environment are salt tolerant and therefore useful in the control of fish or shellfish pathogens in aquaculture, in which the conditions of high concentration of the salts could reduce the efficacy of conventional antibiotics. The present work reports the presence of an antibacterial and antioxidant activity in the lipid extract of some macroalgae present along the Apulian coasts (Italy, Ionian Sea) selected for the plant cultivation.

Bioremediators: the solution

Annelida, Polychaetes
Flter-feeder tubicolous worms

Porifera, Sponges
The sponges are very effective filter-feeders

Mussels
The most famous filter feeders

Macroalgae
Vegetals acting on phosphorous and nitrogen salts

Meterials and Methods

Three seaweed species were collected in the Mar Piccolo of Taranto: a semi-enclosed Transitional Water System located to the north of Taranto (Southern Italy, Ionian Sea, Mediterranean Sea) belonging to the LTER network (IT22 - Mar Piccolo of Taranto). They are: *Chaetomorpha linum* (Chlorophyta), *Cystoseira barbata* (Ochrophyta), *Gracilaria dura* (Rhodophyta). Seaweeds were collected during the season of maximum growth of each species, from three sampling stations sharing the same environmental features. For unattached species (i.e. *Chaetomorpha linum* and *Gracilaria dura*), each year three replicates consisting of about 500 g of fresh material were harvested by a rake at a

maximum depth of 50 cm; for lower depths SCUBA diving was necessary. For attached species (i.e. *Cystoseira barbata*), 3 thalli were collected by SCUBA diving each year for each station. All the collected material was then taken to the laboratory to be processed. for the evaluation of the antibacterial and antioxidant activity. In the case of *Chaetomorpha linum*, about 20 kg of seaweed were collected and transferred to the aquaculture farm where they were transplanted and cultivated in nets in the IMTA system (Figure 1)

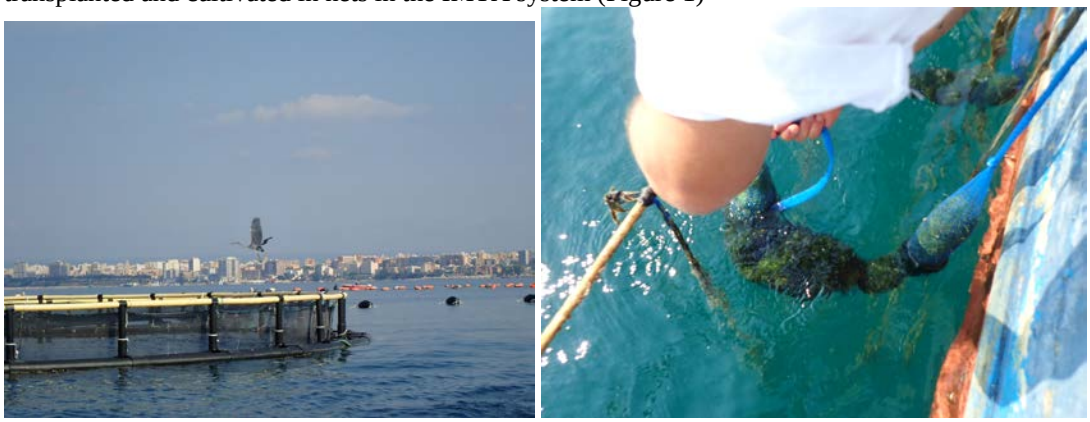

Figure 1: The realized IMTA system with algae as bioremediators

Antimicrobial activity was evaluated using the Kirby Bauer method (Bauer *et al*., 1966) on several bacteria strains including *Vibrio* species. Sterile paper discs, 7 mm in diameter were impregnated with 10, 20, 30, 40, 60, 80, and 100 µL of each extract and left to air-dry at room temperature for 4 h. To verify the possibility that antibiotic activity could be affected by residues of the solvents used for extraction (MeOH/CHCl3, chloroformanhydrous >99% with ethanol as stabilizer, methanol 99.8%; both by Sigma–Aldrich), discs impregnated only with 100 µL of solvent were used as a negative control. For each assay, autoclaved marine agar plates were seeded with 100 µL of test bacteria suspension. Impregnated discs and controls were laid onto the agar surface and the plates were then incubated for 24 h at 30°C. The clear zone around the discs was evidence of antibacterial activity. The diameter of the microbial growth inhibition was taken as the diameter of the clear zone (in millimetres). Each test was prepared in triplicate for every bacterial strain.
Antioxidant activity has been detected by two methods based on two different chemical reactions in vitro: Trolox Equivalent Antioxidant Capacity (TEAC) assay and Oxygen Radical Absorbance Capacity (ORAC) assay. For ORAC the method of Davalos *et al.* (2003) was used. The TEAC assay was performed as described by Re *et al*. (1999) with minor modifications to adapt the assay to a microplate reader.

Results

Several algal lipidic extracts showed an antibacterial activity against the tested *Vibrio* species (Figure 2). Moreover the extracts exerted an antioxidant activity ranging from 13,981 ± 0,881 mmol Trolox Equivalent/g extract in the case of *Gracilaria dura* to 225,19 ± 10,981 mol Trolox Equivalent/g extract for *Cystoseira barbata*. In addition, the cultivation of *Chaetomorpha linum* in the aquaculture plant gave a monthly biomass increase of about 5% SGR.

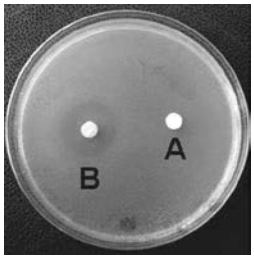

Figure 2. Disc diffusion assay. Algal lipidic extract against *Vibrio vulnificus* (A = Negative control; B = Disc impregnated with algal extract).

Discussion and conclusions

This study revealed different levels of bioactivity in the chloroform/methanol extracts from the seaweeds analysed as well as different susceptibilities of the *Vibrio* strains under investigation. It is well known that *Vibrio* species are common pathogens for farmed fish. The results of an antibacterial activity against vibrios exerted by several of the examined algal lipidic extracts are interesting considering public health hazards related to antimicrobial use in aquaculture, the development and spread of antimicrobial-resistant bacteria, the presence of antimicrobial residues in aquaculture products and in the environment, and the need to control fish and shellfish diseases due to vibriosis. Thus, the bioactive antibacterial compounds from the investigated algal species could be used for the prevention and treatment of fish diseases due to *Vibrio* species, either as an adjunct to classical antibiotics or to completely substitute them. Also

the recorded antioxidant activity is intriguing taking into account the need to find natural antioxidants useful for human health and the growing interest towards the development of strategies for algal exploitation as source of secondary metabolites. The capability of *Chaetomorpha linum* to grow well in the IMTA system with the production of a conspicuous algal biomass leads to suggest its utilization as a novel potential prospect for drug discovery and in particular as a source of bioactive compounds with antibacterial and antioxidant action. The possibility of producing a large amount of algal biomass in environmentally friendly conditions as the IMTA system with the action of bioremediators indeed offers higher chances also for a future successful industrial scale-up of the project REMEDIA Life.

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