Screening Of Sponge-Associated Fungi for Antimicrobial Activity Against Fish Pathogens

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Abstract

There is an immediate requirement for the novel antibiotics in veterinary medicine, because of the high rate evolving antibiotic resistance microorganisms and they have been treated both human and animal health. In the last few decades, the most important drug candidates have been identified from marine organisms, especially marine fungus. However, there are not enough reports about investigation of new bioactive metabolites sources. In this study, 25 fungal strains were isolated from 10 sponge samples that were collected in Kömürlü-Çanakkale and Kopmuş-Hopa (0-45 m). Isolated strains were incubated on solid rice medium. Then, their antimicrobial potential was investigated against to *Vibrio anguillarum, Yercinia ruckeri* and *Lactococcus garvieae*. According to the bioactivity screening, five active strains showed antimicrobial activity . As result of these experiments, the marine fungus can be considered as sources of new antimicrobial compounds which would be used for treatment of causing big economical losing fish diseases.

Key words: Fish diseases, natural products, sponge-associated fungus.

1. Introduction

The researchers have identified many compounds that have various chemical structure and bioactivity from various terrestrial organisms such as plants and microorganisms. However, after a period, the results of researches have been repeated itself. Because of this, marine habitats have been attracted for isolation of new bioactive metabolites. Firstly, the marine macroorganisms were investigated such as sponges, mollusks, tunicates and algae as new molecules sources. Although, the production of sufficient amounts of the bioactive compounds from marine macroorganisms was the limiting step. For example, the one tone of sponge is supplied to 1 g of active substances and it is difficult to obtain one tone sponge by marine culture, because it is extremely difficult to get enough amount of sponge biomass. For this reason, especially sponge associated microorganisms are the best alternative sources for the production of bioactive metabolites [1-3].

The biomass of sponges composes up to significant host community of microorganisms (up to % 50) [4]. They are the most important component of sponge chemical defense system. Besides, some researchers provided the producer of some metabolites which were isolated from sponges and the microorganisms can be diversifying of their secondary metabolites by adopted the different marine conditions [1-3].

The aquacultural sector is the fastest growing agricultural area all over the world. However, this growing has been caused the important health problems such as antibiotic resistance pathogens. Using of big amount of antibiotics for the treatment of fish diseases have been threatened the

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human and animal health. Besides, safety and sustainable food production must be supported with new environment friendly alternative methods. For this reason, the natural products are the most suitable alternative application [5-8].

The aim of the present study was to isolation of sponge-associated fungus and evaluation of their antimicrobial activity against to fish pathogens that are caused the healthy and economic problems in fish farming plants.

2. Materials and Method

2.1 Isolation and Cultivation of Sponge-Associated Fungus

Sponge samples were collected by scuba diving at depth from 0 – 45 on Kömürlü Port coast in Agean Sea of Turkey in June 2013. The sponge samples were identified by Semih Engin (PhD) from Department of Fisheries, İzmir Katip Çelebi University, İzmir, Turkey. Samples were transferred from sea to laboratory by 500 ml plastic bottles in ice box.
The sponge-associated fungal strains were isolated according to Kjer et. al. (2010) Isolated fungal strains were cultivated on rice medium which was prepared by autoclaving 30 g of rice and 30 ml of natural sea water in 250 ml erlenmeyer during 14 days [9].

2.2 Extraction

100 ml ethyl acetate was added thoroughly rice medium and mixed. Then, the cells and rice medium were destructed by homogenization machine. The contents were filtered by using Buchner funnel under vacuum for separation of residues and solvent. The ethyl acetate phases were washed H_2O to remove sugar and starch. Finally, they were dried under vacuum at 40˚C [9]. All extract were stored at + 4˚C until use.

2.3 Antibacterial tests

2.3.1 Disc diffusion test

Antibacterial tests were done by agar diffusion method according to National Committee for Clinical Laboratory Standards (NCCLS2000). *Vagococcus salmoninarum*, *Vibrio anguillarum* serotype O1 and *Yersinia ruckeri* serotype O1 strains which were isolated from local aquaculture plants, were incubated onto plates of Tryptone Soya Agar (TSA) (Merck, USA) overnight before the tests. Then, the strains were suspend in 5 ml 0.9 % NaCl solution and their turbidity optically comparable to that of the 0.5 McFarland standard, containing 1.5 x 10^8 bacteria/ml. The 0.5 McFarland suspension bacteria were separated onto TSA plates by using sterile cotton swabs. 100 mg crude extracts were dissolved with 1000 µl ethyl alcohol and 20 µl of each extracts were loaded on to sterile paper discs (Oxoid, 6 mm diameter). The dried paper discs that contained 2 mg crude extract were placed onto the inoculated plates. Ethyl alcohol was used as negative control and oxytetracycline (20 µg) was used as positive control. The plates with disc and pathogen were incubated overnight at 21˚C. The diameters of complete inhibition zones were

measured and all experiments were carried out ten times. Inhibition zones of 15 mm in diameter were stated as strong, from 9 to 14 mm as moderate and < 8 as weak activities [10].

3. Results

61 fungal strains were isolated from 25 sponge samples (Table 1). They were incubated in rice medium 14 days and then extracted by ethyl acetate for determination of antimicrobial activity. The results of disc diffusion assay were summarized in Table 2. According to the disc diffusion assays, 3 fungal strains exhibited antimicrobial activity against to *Vagococcus salmoninarum*, 2 fungal strain exhibited antimicrobial activity against to *Yersinia ruckeri* and 3 fungal strains antimicrobial activity against to *Vibrio anguillarum*. The antibacterial screening results were showed Table 2.

Table 1. Origin of the sponges

Sponge Code	Name	Location	Deep
1.1.	*Spirastrella cunctatrix*	Kömürlü Port	10 m
1.2.	*Petrocia ficiformis*	Kömürlü Port	10 m
1.3.	*Chondrosia reniformis*	Kömürlü Port	10 m
1.4.	*Chondrilla nucula*	Kömürlü Port	10 m
1.5.	*Acanthella acuta*	Kömürlü Port	10 m
1.6.	*Axinella damicormis*	Kömürlü Port	45 m
1.7.	*Axinella verrucosa*	Kömürlü Port	45 m
1.8.	*Crambe crambe*	Kömürlü Port	45 m
1.9.	*Phorbas sp*	Kömürlü Port	45 m
1.10.	*Petrocia ficiformis*	Kömürlü Port	45 m

Table 2. Antimicrobial activity of the investigated ethyl acetate-extracts (2 mg/disc) of fungal strains agar diffusion assay inhibition zone was measured (If inhibition zone is strong activity >15 mm, 8 – 15 mm moderate activity)

Isolate Code	*Yercinia ruckeri*	*Vibrio anguillarum*	*Vagococcus salmoninarum*
1.7.1.	***14.1±2.0***	***0***	***11,6±1,5***
1.20.1.	***9.6±1.8***	***13.9±0.9***	***0***
1.10.1.	***0***	***0***	***16,2± 1,6***
2.2.1.	***0***	***11.8±1.0***	***0***
1.16.1.	***0***	***9.0±0.5***	***20±1,4***

4. Discussion

Previous studies, different biological organisms were investigated as safer compounds to replace common antibiotics for provide sustainability in aquacuture. For that reason, the different solvent extracts of plants, sponges, seaweeds, mangrove tree and marine fungus which isolated from sediments, algae and mollusc shell, were investigated as antimicrobial agents for treatment of fish diseases [10,11]. Bansemir et al [10] isolated and elucidated two known compounds from red seaweed *Laurencia chondrioides*. But, these possessed weak antimicrobial activity and the aquaculture sector needs to much more active and characteristic extracts or compounds for treatment of fish diseases. Besides, there have not been any studies about sponge-associated fungus for treatment of fish diseases. The main objective of our study was isolation of sponge-associated fungus, screening of their antimicrobial activities against to fish pathogens and investigation as an alternative to common antibiotics in aquaculture.

The differences of inhibition zone values of strains may accordance with in their habitats, because, secondary metabolites are produced by organisms for converse itself in its environment niche [14]. The crude extracts of these fungal strains can be recommendable to isolation, purification and elucidation of bioactive secondary metabolites for treatment of fish diseases.

The researchers have isolated and identified amino acids, nucleosides, macrolides, porphyrins, terpenoids, aliphatic cyclic peroxides and sterols derives from sponge-associated fungus [15]. In addition to, marine environment conditions stimulate the production of new metabolites capacity by salinity, extreme temperature and pH, pressure [16, 17] and it can be changed from side to side. Because of this, marine fungal strains have huge potential for new natural products. Moreover, the sponge-associated fungus of Aegean Sea has not studied very much and we will isolate new bioactive compounds that possess different chemical structures. These strains would be a potential producer for effective new and different chemical structural metabolites for treatment of fish diseases.

This should explore the significance of the results of the work, not repeat them. The results should be drawn together, compared with prior work and/or theory and interpreted to present a clear step forward in scientific understanding.

Conclusions

This report is one of the prior study and showed that the sponge-associated fungus have great potential for aquaculture. Besides, the sponge-associated fungus is a new potential alternative source for purified compounds that can be used as fish feed components or urgently treatment applications. However, toxicity, stability in nature, and metabolism of fungal extract components should be investigated.

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