SCREENING OF BIOACTIVE NATURAL PRODUCTS FROM MARINE MICROORGANISMS, OMAN COASTAL WATERS, POSSIBLE TO COMMERCIAL PRODUCTION IN FUTURE

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Abstract - The first marine-derived antibiotic compound was isolated from a sponge in the 1950s. This was considered the starting point for the search for "drugs from the sea". Since then, more than 16,000 new anti-microbial, anti-viral, anti-cancer and anti-inflammatory compounds from marine organisms have been discovered. Only a few studies have been done and a limited number of natural products bioactive compounds have been isolated from Arabian and Oman seas. So, this research has initiated and focused on marine natural bioactive products from marine microorganisms in Sur coastal waters, Sultanate of Oman. Summary of the project was, to isolate the marine bacteria from different fish guts and tested against selected animal pathogens, Aeromonas salmonicida, Staphylococcus auroea, Aeromonas vronii, Vibrio vulnificus and Vibrio parahemolyticus, in which 6 different fishes (Trachurus, indicus, Lepidsmismultitaeniata, Thryssawhiteheadi, Scarusfalcipinnis, Pempheriskuriamuria and Tripterodonorbis) were used for the study. Total of 320 bacteria were isolated, out of these, 27 strains shown antibacterial activity, two strains showed the prominent activity and 5 strains found moderate activity. Concluding that in future, further more research could be taken on this field and commercialize in the Oman, once achieving this, it could be helpful to make the entrepreneurship for people of the Oman.

Keywords - Marine Natural Products, Oman coastal area, Commercialization

I. INTRODUCTION

High potential of marine bacteria to produce novel biological active molecules is one convincing reason to further examine microbial communities from different host species and geographic locations. These bacteria produce active compounds in order to adapt to the environment in which it survives. Many surface-attached bacteria produce antibiotic molecules that restrain the attachment, growth or survival of rival species in order to expand an advantage in highly aggressive environments [1]. Fish receive bacteria in the digestive tract from the aquatic environment through water and food that are populated with bacteria. Being rich in nutrient, the environment of the digestive tract of fish confers favorable conditions for the microorganisms. The importance of intestinal bacteria in the nutrition and well–being of their hosts has been established for homoeothermic species, such as birds and mammals. However, there is limited information for fish, the poikilothermic vertebrates [2]. Gut micro flora plays an important role in the digestive process, growth and disease of the host, however some bacteria which possess the ability to tolerate the low pH in gastric juices resist the action of bile acids, lysozyme secreted in intestines, immune responses and adheres to the mucus or enteric wall surface could persist for a relatively long time and eventually make intestinal micro flora specific to each host animal. It is clear that bacterial species presents in the gut can influence the health and robustness of the host [3].

The influence of the gut flora on the host is clearly of great interest in aquaculture, particularly where poor productivity and/or stock losses are widespread. Within marine and other aquatic animals, the colonization of the digestive system by micro-organisms is influenced by a number of both host and non-host related factors [4]. On the other hand, bacteria producing antibacterial substances were isolated from marine fish intestines thereby suggesting that these bacteria may inhibit the establishment of invading bacteria in the fish intestine. However, it remains to be elucidated whether freshwater fish harbor the antibiotic-producers as well as marine fish [5]. The antimicrobial molecules produced by marine bacteria can be divided into two major groups: secondary metabolites and antimicrobial proteins. Secondary metabolites are chemical derivatives that are not involved in primary metabolism but can increase the suitability of the host [6]. In present study, an attempt has been made to investigate the antagonistic activity from fish gut microflora. There are different types of fish guts were used for this study.

II. MATERIALS AND METHODS

2.1. Study site

Fish samples were collected from the different sites of Sur coastal waters, Arabian sea, Sultanate of Oman. It is located at around (22°34′0″N59°31′44″E) (Fig.1)

2.2. Sample Collection

There are six commonly available marine fishes were chosen for this study. The live fishes, Trachurus, indicus, Lepidsmismultitaeniata, Thryssawhiteheadi, Scarusfalcipinnis, Pempheriskuriamuria and Tripterodonorbis(Fig.2-7) were collected from sur

coastal waters during February to May months. Samples were immediately transferred to laboratory and identified the species. The fishes were washed with sterile sea water and rinse with the sterile distilled water and kept inside the laminar flow for the dissection.



2.3. Isolation of marine bacteria associated with fish gut

The fishes were anaesthetized in an ice bath for 5 minutes and each individual surface sterilized by immersion for 30 seconds in 70% ethanol. The gut was aseptically dissected from the animal's musculature and swabbed using sterile cotton swab in 9 ml sterile sea water, and then do same procedure for all the fishes and follow the serial dilution method for the sampling. Simultaneously, prepared the Zobell Marine Agar 2216 (ZMA). There are two concentrations, like 0.5 and 1 ml of each samples were poured on the petri dishes and put the ZMA over on the all sample, were spread slowly and kept in incubator and set for Temperature at 27^0C. After 24-32 hours. After the incubation total no number of the bacteria were counted using colony counter.

2.4. Antagonistic Activity of marine bacteria

The screening of antagonistic substances carried out following the standard methods by Spragg et al and Prem Anand 2011 (7 and 8). In which, the isolated marine bacteria strains were streaked onto Trypton Soya Agar + 1 % NaCl (TSA) and allow them for incubation at room temperature for 24-32 hours. The test strains of the animal pathogens, Aeromonas salmonicida, Staphylococcus auroea, Aeromonas vronii, Vibrio vulnificus and Vibrio parahemolyticus (The pathogens were obtained from fish quality control center, Ministry of Agriculture and Fisheries Wealth, Muscat, Sultanate of Oman) were streaked perpendicular to the marine strains onto the agar media and incubated for an overnight. The inhibitory activity was indicated by inhibited growth of pathogenic bacteria on the agar as compared to the control plate showing healthy growth of the test strains. The potential strains are keeping for further study.



Fig.2.Trachurus indicus



Fig.3.*Lepidsmismultitaeniata*



Fig.4.*Thryssawhiteheadi*



Fig.5*. Scarusfalcipinnis*



Fig. 6.*Pempheriskuriamuria*



Fig. 7.*Tripterodonorbis*

III. RESULTS

3.1. Total heterotrophic Bacteria (THB)

Totally, 320 individual marine bacterial strains were isolated from all fish gills, of these, 55, 61, 44, 50, 41 and 69 strains were isolated from Trachurus, indicus, Lepidsmismultitaeniata, Thryssawhiteheadi, Scarusfalcipinnis, Pempheriskuriamuria and Tripterodonorbis are respectively. Name of the strain was assigned by based on the fish name, for example, TI named strains were isolated Trachurus indicus (First letter of Genus and first letter of species) likewise, assigned names for all strains.

3.2. Antagonistic activity strains

Out of 320, there are 27 marine strains were showed antagonistic activity against maximum of the pathogenic bacteria. Strains, PK 11 and TO 12 were isolated from Pempheriskuriamuria and Tripterodonorbisandshowedprominent inhibition than

other strains. The moderate inhibition was observed from TI 22, TI 30, TW 30, SF 19 and SF 29. The remaining potential strains were notice trace amount of inhibition and even for very few pathogens (Tab.1). Fig. 8 showing the antagonistic activity of the marine strains against the many pathogens, here observed different level of inhibitory activity amount the marine bacteria. Few marine strains having prominent inhibitory action, some stains shown moderate activity and trace amount of inhibitory also observed by the few marine strains.

Table 1. Antagonistic activity of gut bacteria

S. No.	Strain No.	Pathogens (Inhibitory level)				
		Aeromonas salmonicida	Staphylococcus auroea	Aeromonas vronii	Vibrio vulnificus	Vibrio parahemolyticus
1	TI 11	TI	TI	TI	NI	TI
2	LM 06	NI	TI	TI	NI	TI
3	TW 09	NI	TI	TI	NI	TI
4	TI 22	MI	MI	MI	MI	TI
5	SF 10	TI	TI	TI	NI	TI
6	TO 12	PI	PI	MI	PI	MI
7	TI 30	MI	MI	MI	MI	TI
8	TW 16	TI	TI	TI	NI	TI
9	SF 19	NI	TI	TI	NI	TI
10	SF 29	TI	TI	TI	NI	NI
11	PK 11	PI	PI	MI	PI	TI
12	TO 20	TI	TI	TI	TI	TI
13	TI 46	TI	TI	TI	TI	TI
14	TO 33	TI	TI	TI	TI	TI
15	LM 43	NI	TI	TI	TI	TI
16	TI 51	NI	TI	TI	TI	NI
17	LM 32	NI	TI	TI	TI	NI
18	SF 29	MI	MI	MI	MI	TI
19	PK 16	TI	TI	TI	NI	TI
20	TW 29	TI	TI	TI	NI	TI
21	TO 41	TI	TI	TI	NI	NI
22	TI 52	TI	TI	TI	TI	NI
23	LM 48	TI	TI	TI	TI	NI
24	TO 49	TI	TI	TI	TI	TI
25	TW 30	MI	MI	MI	MI	TI
26	PK 34	TI	TI	MI	TI	TI
27	TW 49	TI	MI	TI	TI	TI

Note: PI – Prominent Inhibitory, MI -Moderate Inhibitory, TI – Trace Inhibitory, NI: No Inhibitory.

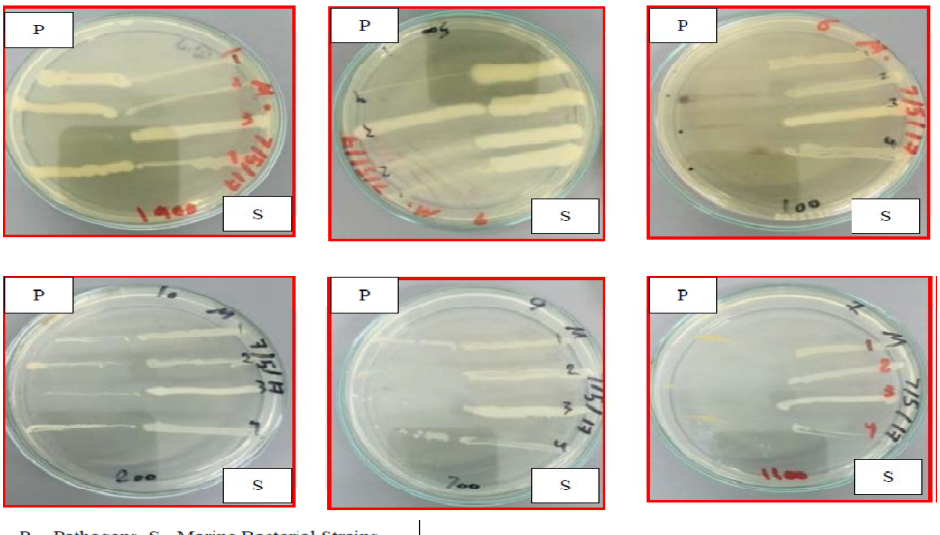


Fig. 8. Inhibitory activity levels of between gut bacteria and animal pathogens

IV. DISCUSSION

There are 320 individual marine bacterial strains were isolated from different types of fish gills, Out of these, 55, 61, 44, 50, 41 and 69 strains were isolated from Trachurus, indicus, Lepidsmismultitaeniata, Thryssawhiteheadi, Scarusfalcipinnis, Pempheriskuriamuria and Tripterodonorbis are respectively. Lemoset al, (1991)isolated 224 epiphytic bacterial strains from intertidal seaweeds, out of which 38 strains (16.9%) displayed antibacterial activity (9). Spragget al, (1997)isolated 51 strains from marine algae Fucusresiculosus, and in that, 13 (25%) exhibited activity against Methicillin resistant Staphylococcus aureus, (MRSA). Out of that 13 strains, onlyone exhibited good activity against MRSA (7). Jayanth et al, (2002) isolated 166 pigmented marine bacteria from different sources like seawater, sediment, algae (2 species) and bivalves (2 species) and found that 62 bacterial strains exhibited antibacterial activity (10).

Epiphytic bacteria from sponges were intensively screened for the production of antibiotics (11). In the present investigation, bacteria present in the fish guts were screened for antibiotic production. A total of 320 bacterial strains were isolated and 27 were found to be antibiotic producers. Two potential strains were found to exhibit higher activity against Aeromonas salmonicida, Staphylococcus auroea andVibrio vulnificus, five strains were found moderate activity against Aeromonas salmonicida, Staphylococcus auroea, Aeromonas vronii and Vibrio vulnificus and other active strains shown trace activity against few pathogens. It is necessary to discover new classes of antibiotics to increase due to the incidence of multiple resistance among pathogenic microorganisms to drugs that are currently in clinical use [12].

CONCLUSION

Further studies along these lines should be undertaken in the near future in order to find an effective way of these strains. The potential strains could be identified by molecular method and pure and active compounds could be separated by advanced spectroscopy methods. Finally, further more research could be taken on this field and commercialize in the Oman, once achieving this, it could be helpful to make the entrepreneurship for people of the Oman.

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