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ORIGINAL RESEARCH ARTICLE

Antagonistic Study on *Streptomyces* spp. Isolated from Marine Fish and its Antibiogram Spectrum against Human and Fish Pathogens

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ABSTRACT

Antibiotics are the bioactive compounds which are isolated from many microorganisms. Many of the derived from *Streptomyces* sp. In the present investigation, the antibiogram of *Streptomyces* from marine fish red snapper. Here to studied the interaction of microbes particularly *Streptomyces* spp in the gut of fish of marine biotopes and their ability of producing antibacterial compounds against fish and human pathogens. (*Pseudomonas* and *Vibrio* spp). The result of active extract clearly proved from antibiogram, TLC and Spectral analysis that these strains are very much effective producers of antibacterial components against pathogens. The inhibition zone measurement ranging from 2 to 5.8 mm was observed. The TLC Rf values exhibited the same. It ranges from 0.40 to 0.78.The bioactive components revealed the maximal UV absorption peaks ranging from 215 to 300nm.These strains are produced a broad spectrum of antimicrobial activity. It is a new venture for the development of Biotechnological exploration and exploitation in the years to come.

Key words: Antibiotics, *Streptomyces*, Human pathogens and TLC.

1. INTRODUCTION

In recent years, the search for the novel antimicrobial compounds has been found in *Streptomyces* spp and the association with fish, shell fish, sediments and seaweeds is seeking the importance of the genus. Microorganisms have important role in industrial applications where they are involved in production of antibiotics, herbicides, pesticides, insecticides and even solvents as precursors for the manufacture of plastics. Because of their ability to synthesize varieties of valuable products of commercial importance, *Streptomyces* spp. are major groups of industrial importance. Collection of *Streptomyces* from various untapped resources is for new and novel bioactive molecules [1, 2].

An important part of the natural products from the group of small molecular secondary metabolites of microorganisms usually exhibits some kinds of biological activities and their compounds preferably the bioactive secondary microbial metabolites exclusively most characteristics feature of secondary metabolites are their incredible array of unique chemical structures and their very frequent occurrence and versatile bioactivities [3]. *Streptomyces* spp. are metabolically diverse and eats almost anything including sugars, alcohols, amino acids and aromatic compounds. This is achieved by producing extra cellular enzymes and they play a major role in the degradation of complex organic molecules [4]. It is also used as a biochemical agent against soil borne fungal plant pathogen.

Majority of the antibiotics are synthesized by *Streptomyces* spp. [5]. Historically streptomycin is a broad spectrum of antibiotic and is significant as the first effective antibiotic against tuberculosis disease of human beings. Genus Streptomyces in the family streptomycetaceae was classified by [6]. That also included a number of taxa that were in initially morphological in concept and important families of antibacterial activity available today. Issatchenko [7] studied on the occurrence of actinomycetes in salt lakes.

Subsequently sensitivity to antibiotics as a taxonomic feature was attempted by Burkholder *et al*. [8]. Krasilinikov [9] worked on the antibiotic producing actinomycetes which could be identified as the basis of inter and intra specific antagonisms. Waksman [10] worked on the occurrence of asparaginase in actinomycetes.

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Halstead [11] found many marine organisms and are known to contain antibiotic substances and less than 1% of aquatic strains have been examined for production of pharmacologically active substances. AnnieMathew *et al*. [12] observed that *Streptomyces* spp. isolated from shellfish, *Villorita cyprinoides*, have antagonistic property against *Vibrio anguillarum*, *Staphylococcus aureus* and *Aspergillus niger*.

Sadar and Gales [13] isolated a new antibiotic, Ertapenem from *Streptomyces cattleya* with a broad spectrum of antibacterial activity and improved stability to hydrolysis by renal dehydropeptidase enzyme, located in the brush border of the kidneys. It exhibits excellent antibacterial activity against clinically relevant Enterobacteriaceae including *E.coli*, *Klebsiella* sp., *Enterobacter* sp. and *Proteus* sp.

The antibacterial agent; Daptomycin derived from *S.roseosporos* binds to bacterial cell membranes and then disrupts the membrane potential leading to blocking of the synthesis of proteins, DNA and RNA. In the present investigation, the antagonistic property of *Streptomyces* sp. isolated from marine fish red snapper and its antibiogram spectrum analysis [14].

2. MATERIALS AND METHODS

2.1. Isolation of *Streptomyces* from fish gut

The marine fish red snapper was collected from east region of Akkaraipettai at Nagappatinam harbor of Tamil Nadu, India. The sample (fish) is aseptically transferred in to laboratory without contamination. The gut of the fish were removed aseptically and transferred in to the sterile flask and the serial dilution was carried out independently for each sample.1 ml of sample from appropriate dilution was pipette out in to the sterile petridish.15 ml of the Glycerol Aspargine Agar medium were poured in to the same petridish and mixed thoroughly by rotating the petridish both clock and anticlockwise directions. After the isolation of the *Streptomyces* cultures were tested for the colour production by employing media such as nutrient agar, actinomyces agar, glucose yeast extract medium, Tryptophan medium and Streptomyces medium.

2.2. Enumeration and Maintenance of Cultures

In each selective medium, numbers of coloured colonies of *Streptomyces* spp. were found. These selective colonies of Streptomyces species were sub cultured in slants by using Kuster's agar medium enriched with nutrients. Later they were kept in refrigerator (4^{o}C) till further analysis.

2.3. Characterization of selected *Streptomyces* spp.

The characterization of the selected *Streptomyces* sp. were carried out according to the methods employed by collaborator in International Streptomyces project (ISP) [15].

2.4. Colour Determination

For colour determination cultures were streaked on different media such as Glycerol Asparagine and Actinomyces Agar media and they were observed after seven days. Aerial and subrate mycelial colours were recorded in a simple (Grey, White, Red, orange, Green, Cream etc.) observation.

2.5. Mass culturing

The four selected *Streptomyces* sp. were mass cultured in Actinomyces broth (5.72 in 100 ml) and incubated for one week. After one week the culture was inoculated in fermentation broth. The inoculated broth was placed in shaker for 120 hrs i.e. 5 days.

2.6. Antibacterial Activity of Selected *Streptomyces* spp.

A loopfull of Streptomyces strains was inoculated into Actinomyces broth and incubated for seven days at 28^{o}C. After 7 days the culture was well developed . 2 ml of the cells was transferred into fermentation broth (100ml) and incubated at 28^{o}C for 120 hrs done in shaker. After growth it was centrifuged at 5000 rpm for 20 minutes to separate the mycelial biomass. Then the supernatant was mixed with equal volume of methanol. Then it was shaken for 2 hrs in shaker and transferred to separating funnel and solvent was separated. Then, the solvent was dried in water bath of 80-90^{o}C and residue was weighed. The residue was mixed and concentrated with little ethanol. Then, it was impregnated with filter paper disk (6mm diameter) and dried.

2.7. TLC Analysis of Antibacterial compounds

Quantitative analysis of antibacterial compounds in the experimental sample was carried out by using Thin Layer Chromatography (TLC). Then, applying slurry made by silica gel G for TLC grade and applied over the glass slides, TLC slides were made. This was dried at 60°C for an hour of period. The dried slides were pre-activation base was drawn on the TLC slides 1.0 cm away from the base line on the portion of the TLC slides.

After that sample were spotted on the baseline of the TLC slides at 1.0 cm and then allowed to dry at room temperature. Then sample applied TLC slides were placed in pre-saturated TLC chamber contains mobile phase of two combination

solvents like ethyl acetate (3.5 ml): chloroform (1.5 ml) for marine fish. Then the chromatogram was developed up to a mark. Then the slides were taken out dried for few minutes. Using iodine vapour the slides were stained and spots were marked. The distances traveled by each spot in base line and relative R_f values were calculated.

$$R_f = \frac{\text{Distance traveled by the solute}}{\text{Distance traveled by the solvent}}$$

2.8. Spectral analysis

The residue mixed and diluted with ethanol was taken for spectral analysis at 200-400nm using UV spectrophotometer.

3. RESULTS AND DISCUSSION

The present investigation was an attempt to understand the distribution pattern of *Streptomyces* sp. in the of gut regions of fish of marine environment. The primary isolation of *Streptomyces* was carried out under selective media like Glycerol asparagine agar [2]. In that bacterial and fungal colonies were minimum in numbers because of these selective medium (Glycerol asparagine agar) has glycerol in the medium, which inhibits the growth of bacterial and fungal population [16]. This occurrence of *Streptomyces* colonies inhibited the growth of bacteria because it has already been proved that marine *Streptomyces* synthesized antibiotics, anticancer agents, L-asparaginase enzyme as reported earlier [2,17]. This results has been observed for 14 days, the colouration pattern were entirely different in 7th day and 14th day this may be because of maximal secondary metabolite production. This may be due to that the enriched medium provides certain nutrients for triggering of genes for the conversion as well as expression of other metabolic products, which in turn, leads to different aerial and substrate mycelial colourations. This colouration difference may be due to other primary as well as secondary metabolites production provided by the enriched media [2]. These primary and secondary metabolites of different colouration are the rich source of certain compounds like amino acids, sugars, fatty acids, terpenes, antibiotics etc.

(**Table 1**) exhibited the antibacterial activity. The antibacterial activities of some Streptomyces strains were found to be active against test organism like, *Pseudomonas* and *Vibrio* spp. The maximum inhibition zone (2-5.8mm) occurred in strains obtained from gut of fishes. Antagonistic *Streptomyces* spp are famous for their antibiotic production against bacteria, fungi and virus [1,2,18]. Suja Devan [19] screened *Streptomyces* from Veli Lake for antagonistic property but in the present study the components were isolated antibacterial from fermentation media and were tested for antagonism against *Pseudomonas* and *Vibrio* spp. The inhibition zone proves that these strains showed effective antagonism against particular pathogen. Further purification of components can be done and be studied for individual compound's activity against any other pathogens [20, 21]. Our findings are corroborated the above observations.

Then TLC was carried out Table- 2 for the ethyl acetate, hexane, and petroleum ether extract samples of the selected strains. The R_f values ranged from 0.40 to 0.78. The similar results were observed by Llic *et al*. [22] their bioactive regions were detected on TLC plates and the R_f values were ranges from 0.50 to0.88.

The U.V spectral data for the ethanol extract of selected active strains from fermented broth are shown in Figure 3. Maximum absorbance peaks ranged from 215 to 300nm. The range of peaks was observed from 200 to 400nm and the characteristics of absorption peaks indicate a highly polyene nature. These strains produced either a broad spectrum antibacterial compound or several compounds with different activities. The spectral data are consistent with those obtained by Swaadoun *et al*. [23]. It is quite obvious that the *Streptomyces* spp are inherent in marine ornamental fish, synthesizing commercially valuable bio active compound

Table 1: Antibiogram of *Streptomyces* sp. associated in gut of marine fish (Red snapper) against human and fish pathogens

Strains of Fishes	Zone of inhibition (mm)
RSS-1	4.7
RSS-2	5.3
RSS-3	2.0
RSS-4	4.0

Table 2: Thin layer chromatographic separations of Antibacterial components

Strains of Fishes	Rf Values
RSS-1	0.63,0.69
RSS-2	0.67
RSS-3	0.78
RSS-4	0.51,0.46

4. CONCLUSION

Here we studied the interaction or association of microbes particularly *Streptomyces* spp in the gut and sediment of fishes of marine environmental biotopes and their ability of producing antibacterial components against fish and human pathogen (*Pseudomonas* and *Vibrio* spp). The result of active extracts clearly proved from antibiogram, TLC, and spectral analysis that these strains are very much effective producers of antibacterial components against pathogens. It has already been shown that our *Streptomyces* spp

fermented active extracts are effective against other bacteria also *viz*., *Bacillus subtilis*, *Staphylococcus aureus*, *Mycobacterium smegmates*, and *E. coli*. The similar result was obtained against *Pseudomonas* and *Vibrio* spp. Further investigation is needed in order to determine the structure of active components. In domestic markets, no toxic bio-fungicides and teratogenes do exist. The preparation gained from the investigated isolates would have been a great advantage over the existing commercial preparations. With increasing advancement in Science and Technology, there is greater demand for novel bioactive components from various sources. No wonder there is a search for the bio-active components from untapped resources like fishes of commercial importance. It is a new venture for the development of Biotechnological exploration and exploitation in the years to come.

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REFERENCES

1. Annie Mathew A.K.1995. Studies on antagonistic *Streptomyces* associated with fish and self fish of veli lake, Kerala. Ph.D Thesis. University of Kerala.
2. Dhevendran, K and Annie Mathew, K. 1999. Systematics of *Streptomyces* associated with the gut of alimentary canal of esturine fish and shell fish of India. *Fish Technol*., 36: 90 - 95.
3. Demain A.L and Fang A. 2000. The natural functions of secondary metabolites. In Advances in Biochemical engineering/Biotechnology. Vol.69.Ed schaper T Springer verlag. Berlin: 1-39
4. Krasilnikov, N.A. 1958. Biology of special group of actinomycetes (Russian) inst. microbial. akad. Moscow, 377 pp.
5. Williams ST and Vickers J.C. 1998. Detection of actinomycetes in the natural environment problems and prospectives in: Biology of actinomycetes. Japan. Sci. Soc. Tokyo, Japan: 265-270.
6. Waksman, S.A and Henrici, A.T. 1943. *Journal of Bacteriology*, 46: 337 – 341.
7. Issatchenko,V.L. 1938. Actinomycetes volume 1 nature occurrence and activation S.A.Walks man, The William and wilkini co Baltimore, 38.
8. Burkholder,P., R. Sun, and S.H. Anderson.1954 Criteria of speciation in the genus *Streptomyces*, *Ann.,New Tork Acad, Sci*., 60-102
9. Krasilnikov, N.A. 1958. Biology of special group of actinomycetes (Russian) inst. microbial. akad. Moscow, 377 pp.
10. Waksman SA 1959 actinomycetes vol 1, nature, occurance and activities Williams and wilkini co., Baltimore, 38.
11. Halatead GW. 1965. Poisonous and venomous marine animals of the world U.S. govt. printing office, Washington DC, 994.
12. Annie Mathew, A, Dhevendran, K, Georgekutty, M.I and Natrajan, P. 1994. L-asparaginase activity in antagonistic *Streptomyces* associated with clam villorita cyprinoides (Hanley). *Indian. J Mar. Sci*., 23: 204 – 208.
13. Sardar H.S and Gales, A.C. 2001. Emerging stratergies in infectious diseases: new carbapenem and trinem antibacterial agents. *Drugs*, 61: 553 – 564.
14. Dhevendran, K and Annie Mathew, K. 1999. Systematics of *Streptomyces* associated with the gut of alimentary canal of esturine fish and shell fish of India. *Fish Technol*., 36: 90 - 95.
15. Klevanskaya, I.H. 1960. Growth of soil actinomycetes in media of varying osmotic pressure. *Microbial*. 215-219.
16. Sudha K, Thampuran N and Surendran P.K. 2002. Prevalence of *Vibrio* species on fish from pelagic and demersal habitats. *Fish Tech*, 39: 150-154.
17. Anithakumari,Y.K and Dhevendran, K. 2004. Studies on *Streptomyces* associated with 3 selected weeds of Kovalam coast, Kerala. *Seaweeds Res. Utilin*., 26: 253-259.
18. Hood and Mayers, 1973. Microbial aspects of penaoid shrimp digestion. proc. Gulf caribb.fish.Inst., 26: 81 – 92.
19. Waksman, S.A (ed). 1959. The actinomycetes Vol.1 Nature, occurrence and activities. Williams and Wilkins co Baltimore, USA.
20. Manju, K.G and Dhevendran, K. 1997. Effect of bacteria and actinomycetes as single cell protein on growth of juveniles

of *Macrobacterium idella*. *Indian journal of Exp. Biology*, 35: 53-55.

21. Annie Mathew, A, Dhevendran, K, Georgekutty, M.I and Natrajan, P. 1994. L-asparaginase activity in antagonistic *Streptomyces* associated with clam villorita cyprinoides (Hanley). *Indian. J Mar. Sci*., 23: 204 – 208.
22. Demain A.L and Fang A. 2000. The natural functions of secondary metabolites. In Advances in Biochemical engineering/Biotechnology. Vol.69.Ed schaper T Springer verlag. Berlin: 1-39
23. Kuster, E. 1972. Simple working key for the classification and identification of named taxa included in the *Streptomyces* project. *Int. Jou. Sys. Bacteriology*, 22: 139-148