

Inhibition of Fish Bacterial Pathogens by Antagonistic Marine Actinomycetes

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Abstract: Marine Actinomycetes from Chennai (Tamil Nadu) coastal area was isolated. A total of 10 potential marine actinomycete strains from 34 isolates was screened, against five fish pathogenic bacteria by cross streak method, the ECR3 strain showed good inhibition. Crude anti-bacterial compound from the potential strain ECR3 was extracted with different solvents by sub-merged fermentation method. The extract was tested against fish bacterial pathogens by disc diffusion method. Ethyl acetate extract showed a good inhibition range of 6-15 mm in diameter. The potential actinomycete strain was characterized and tentatively identified as *Streptomyces* spp. Production optimization, purification and structure elucidation of active bioactive compounds is in progress to prove its potential further for fish industry.

Key words: Marine actinomycetes · *Streptomyces* · Fish pathogens · Antagonistic activity

INTRODUCTION

Disease control and management in fish culture system has become one of the major problems as the fish bacterial pathogens are becoming more and more resistant to the conventional therapeutic drugs used in the industry and thus the fish farmers suffer from heavy financial losses. So, there is a need for the search of novel bio-active compounds with therapeutic potential which can be used to control the bacterial disease in an eco-friendly manner. Among bacteria, Actinomycetes the most widely distributed group of microorganisms in nature, are a particularly interesting group, responsible for the production of majority of clinically useful antibiotics. Nearly 9,500 antibiotics from actinomycetes have been reported by 2008, of which 85% are from *Streptomyces* species and remaining 15% are from other actinomycetes genera. Indeed, the actinomycetes represent the clade of microbes with the greatest potential to provide useful bioactive molecules. Several workers have isolated actinomycetes from marine environment [1-3]. Only few works have reported the occurrence of the antagonistic actinomycetes in marine sediment. Therefore the present study was undertaken to isolate and characterization of actinomycetes from the marine environment, which are antagonistic to bacterial pathogens associated with fish diseases.

MATERIALS AND METHODS

Collection and Isolation of Marine Actinomycetes: Marine sediment samples were collected from Chennai coast area, Tamil Nadu. The central portions of sediments were aseptically transferred into sterile bottles. The samples were air dried for 5-7 days and used for the isolation of actinomycetes. Actinomycetes were isolated by plating on starch casein agar medium. Triplicate plates of arch casein agar were used for actinomycetes count. Plates were incubated at 28°C and the numbers of colonies were determined after 2 to 3 weeks. The starch casein agar medium containing 50% sea water was supplemented with rifampicin 2.5 µg/ml and cyclohexamide 50 µg/ml to inhibit the bacterial and fungal contamination, respectively. The selected colonies were picked up and sub cultured on starch casein agar slants and used for antagonistic potential.

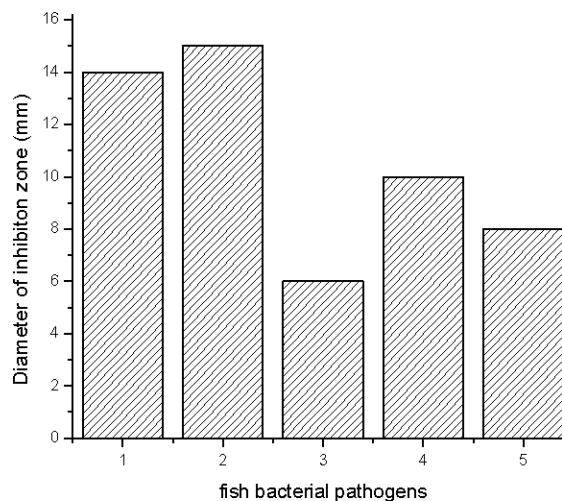
Screening of Marine Actinomycetes Against Bacterial Fish Pathogens: The antimicrobial activity was preliminarily studied by cross streak method against five fish pathogenic bacteria, the 10 isolated Actinomycetes strains were streaked as parallel line on Modified Nutrient Glucose Agar (MNGA) plates and incubated at 28°C for 5 days. Based on the results of antagonistic activity, one potential actinomycete strain was selected for the bioactive compound production.

Antibacterial Assay: After preliminary testing of the isolates for their antimicrobial activities, further studies for the production of antibacterial compound were carried out by shake flask method. The pure culture were inoculated in to 100 ml of soybean meal inoculation medium and incubated in rotary shaker with 120 rpm at 28°C for 48 hours. About 10 % of inoculum was transferred in to soybean meal production medium (1 litre) and incubated in rotary shaker with 120 rpm at 28°C for 120 hours. After fermentation, production medium was centrifuged at 4°C with 8,000 rpm for 30 minutes. The supernatant was collected and crude antimicrobial compound was extracted by sequential liquid-liquid extraction method using different solvents. Antibacterial activity of crude compound was tested against fish pathogens viz, *Vibrio harvei*, *Vibrio parahaemolyticus*, *Pseudomonas sp*, *Aeromonas hydrophilia* and *Vibrio alginolyticus* were tested by disc diffusion method.

Characterization of Potential Actinomycetes Strain: Based on the studied phenotypic characteristics the potential actinomycete was identified at species level with the help of Nonomura's key [4].

RESULTS AND DISCUSSION

Earlier a similar result was observed, in which it has been found that about 75% of their actinomycetes isolates were inhibitory [5]. However, in another study, it has been reported that only 27% of actinomycetes cultures isolated from marine sediments of Sagami Bay were antagonistic to various microorganisms [6]. Elliah *et al.* [7] reported that 25% of the isolates collected from marine sediments of Bay of Bengal near Machilipatnam exhibited antimicrobial activity. Totally 10 actinomycetes strain were isolated from starch casein agar plates. The isolated strains were tested by cross streak method. Especially, strain ECR3 showed prominent activity against all the 5 fish bacterial pathogens. Based on this result, strain ECR3 was selected for the production of bioactive compounds. In another study, it has been observed that the isolates of *Streptomyces* from marine sediments were inhibitory to *Bacillus cereus*, *B. subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* [8]. In the present study, high amount of crude compound from fermentation broth was obtained in ethyl acetate (520 mg/l). Among the various solvent extracts tested, ethyl acetate extract showed good activity (6-15 mm inhibition) against the fish pathogens (Graph 1).



Fish pathogens: 1. *Vibrio harvei*, 2. *Vibrio parahaemolyticus*, 3. *Pseudomonas sp*, 4. *Aeromonas hydrophilia* and 5. *Vibrio alginolyticus*.

Graph 1: Antagonistic activity of ECR3 strain

Table 1: Characteristics of potential Actinomycete strain ECR3

Characteristics	Result
Micromorphology	
Aerial Mass color	White
Melanoid pigment	--
Reverse side pigment	--
Soluble pigment	--
Spore chain	Rectiflexible
Spore surface	Smooth
Utilization of carbon source	
Arabinose	--
Xylose	--
Inositol	--
Mannitol	--
Fructose	--
Rhamnose	--
Sucrose	--
Raffinose	--

After preliminary screening the antibacterial activity were checked for their crude compound ability. The fish pathogens viz, *Vibrio harvei*, *Vibrio parahaemolyticus*, *Pseudomonas sp*, *Aeromonas hydrophilia* and *Vibrio alginolyticus* were tested against the isolated potent strain ECR3 by paper disc method. The maximum zone was obtained in *Vibrio harvei* and *Vibrio parahaemolyticus* 14mm and 15 mm zone, respectively. Lower 6 mm in pseudomonas sp. (Graph1). However, Jensen and Fenical [2] isolated antagonistic actinomycetes from the seawater Trischman *et al.* [9]

also reported the isolation of inhibitory actinomycetes from marine environment and isolated rare class of antibiotic substances. The potent isolated strain possessed LL-diaminopimelic acid (DAP) and glycine in the cell wall and tested negative for meso-DAP, indication that the cell wall was of chemotype-1 that has no characteristic sugar pattern [10]. Based on the observations of the ECR3 strains revealed that belongs to the genus *Streptomyces sp.* Morphological, micromorphological, physiological and biochemical characteristics (Table 1) of the potent strain were compared with those of *Streptomyces key* (4) and species described in the Bergey's Manual of Determinative Bacteriology [11]. Therefore, ECR3 tentatively identified *Streptomyces sp.*, but it resemble with *Streptomyces galtieri*. These strains, especially ECR3 which was very active against all five fish bacterial pathogens. In future investigation to characterization of the potent compound and it will be used to aquaculture industry.

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