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Research Article

ANTIBACTERIAL ACTIVITY OF MARINE ACTINOMYCETES AGAINST DENTAL CARIES BACTERIA STREPTOCOCCUS MUTANS

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A B S T R A C T

'Antibacterial activity of Marine Actinomycetes against dental caries bacteria *Streptococcus mutans.'* is a work to recognize the Marine Actinomycetes is capable for control dental caries bacteria *Streptococcus mutans*. In this study there are 82 Marine Actinomycetes strains isolated from four marine sediment samples from Vizhinjam coastal region, Thiruvananthapuram district in Kerala. The agar overlay method and well and disc diffusion methods were used to check the antibacterial activity of isolated marine actinomycetes against dental caries bacteria *Streptococcus mutans*. 52 marine actinomycetes isolates showed antibacterial activity in primary screening agar overlay method and 6 marine actinomycetes showed significant antibacterial property in secondary screening well and disc diffusion methods against *Streptococcus mutans.* The result evidence that the isolated marine actinomycetes capable to producing some secondary metabolites with antibacterial properties against dental caries bacteria *Streptococcus mutans.*

INTRODUCTION

In microbial field Actinomycetes are one of the most exclusive prokaryotic organisms with widespread range of bioactive secondary metabolites. (1*)*. More than 23000 bioactive secondary metabolites produced from microorganisms and in excess of 10000 of these compounds are produced by Actinomycetes, 45% of every bioactive microbial metabolite revealed from Actinomycetes (2). Greater than 70% of earth is covered by oceans. In the deep sea floor estimation of the biological diversity is highly superior. Marine environmental circumstances are extremely miscellaneous and marine actinomycetes have dissimilar character from those of earthly complement so they have ability to produce diverse types of bioactive compounds (3). Marine actinomycetes are normally Gram-positive and filamentous microorganism known as significant secondary metabolite producers (4). The human oral cavity contains abundant varieties of microbes in altering proportions including bacteria, viruses, and fungi, organized into dental plaque as biofilms, this plaque formation helps microbes to resistant mechanical stress and antibiotic treatment (5). The tooth decay or cavity also known as Dental caries, it is a bacterial infection that causes demineralization and destruction of the hard tissues of teeth, this usually happens from the production of acid by bacterial fermentation of the food fragments accumulated on the tooth surface. Dental caries is one of the most extensive diseases in the world (6). Dentists recommend seven to eleven percentage of all general antibiotics for the treatment of dental caries but studies revealed that in the order of one-third of all out-patient antibiotic prescriptions are needless (7). Prolonged exposure to antibiotics and its improper dosages results in critical side effects and development of drug resistance pathogens. (8). To overcome such unpleasant situation there must be an imperative move towards the discovery of novel antibiotics and new therapeutic agents by continuous screening of secondary metabolites of microbial origin from usual as well as unusual environments. So the present study aimed to screen the secondary metabolites of marine actinomycetes to detect the possibility of presence of any antagonistic bio molecules to control the dental pathogens.

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MATERIALS AND METHODS

Sample collection

Four Marine sediment samples were collected in sterile, disposable plastic containers from seashore of Vizhinjam coastal region, Thiruvananthapuram district in Kerala. The collected samples were transported to the Laboratory and used for further studies.

Isolation of Actinomycetes from Marine Sediment Samples

One gram of each samples were aseptically diluted in 100 ml of sterile sea water and spread plated on to the starch casein agar (HIMEDIA, INDIA) plates. The plates were incubated at 28^0 C for one to three weeks in an inverted position. After proper incubation plates were observed and actinomycete colonies were morphologically analyzed for selection and enumeration (9).

Collection of Dental samples

Dental caries tooth sample from dental caries patients was collected in sterilized bottles and transported to the Laboratory, stored in the refrigerator at 4^0C for analysis.

Isolation of dental pathogen Streptococcus mutans

The sample of Dental caries tooth from dental caries patients was soaked in sterile saline solution and spread plated on to mitis salivarius agar (HIMEDIA, INDIA) plates. The plates were incubated 37^0 C for 24 – 48 hours. After proper incubation, plates were observed and the colonies formed were morphologically analyzed for further studies.

Selection, Purification and Preservation of Bacterial isolates

Dental pathogenic bacteria *Streptococcus mutans* colonies developed on mitis salivarious agar plates were selected on their macroscopic, microscopic and morphological properties and predominance. Selected colonies of dental pathogenic bacteria were purified by repeated sub-culture. The purified *Streptococcus mutans* isolates were streaked on to Nutrient agar (HIMEDIA, INDIA) slants and stored at 4^0 C for further studies.

Antibacterial activity of marine actinomycetes against Streptococcus mutans

Primary Screening - Agar Overlay Method

72 hours of actinomycete broth culture was streaked as a single line on the Muller Hinton Agar (HIMEDIA, INDIA) plates. The plate was incubated at 28^0 C for three days.10 ml of soft agar medium was prepared and 1ml of 12 hours isolated *Streptococcus mutans* culture was added and mixed thoroughly. It was poured over the actinomycete spotted growth on Muller Hinton Agar plates. The plates were incubated at 37^0C 24 hours (10).

Secondary Screening

Preparation of Cell Free Culture Filtrate and Cell Lysate

After primary screening actinomycete isolates with significant antagonistic effect were inoculated in 10 ml of nutrient broth (HIMEDIA, INDIA) and incubated at 28^0 C for 72 hours in shaker cum incubator (REMI, INDIA) at 120 rpm. After incubation the broth culture was centrifuged at 7000 rpm for 10 minutes using centrifuge (REMI, INDIA). The cell free supernatant filtered through Whatmann No: 1 filter paper. Dried and pelleted actinomycete cell mass was extracted with ethyl acetate by incubating its mixture at 37^0 C for one hour and then centrifuged at 7000 rpm for 15 minutes. The supernatant was taken as crude intra- cellular extract for further antagonistic activity study (11).

Well Diffusion and Disc Diffusion Method

12 hours young over-night *Streptococcus mutans* culture was effectively swabbed on the Muller Hinton Agar (HIMEDIA, INDIA) plates. Wells of 6mm diameter were made using well cutter. 50 µl of cell free culture filtrate as well as cell lysate extract were loaded in separate wells. The plates were incubated at 37^0 C for 24 hours. *Streptococcus mutans* culture was effectively swabbed on another Muller Hinton Agar plates. The cell free culture filtrate and intra cellular extract were loaded discs were placed aseptically on pre-inoculated agar plate surface using sterile forceps. The plates were incubated at 37.0^0C for 24 hours. After incubation the plates were observed and measured the zone of growth inhibition around the wells and discs.

RESULT

Isolation of Marine Actinomycetes

82 marine actinomycete isolates were obtained from marine sediment samples collected from Vizhinjam coastal region, Thiruvananthapuram. The obtained marine actinomycetes were purified through repeated subculture technique. The isolates were streaked on to starch casein agar slants and preserved at 4^0 C for further studies.

Isolation of Streptococcus mutans

A huge number of colonies of dental caries causing bacteria *Streptococcus mutans* developed on mitis salivarious agar plates. *Streptococcus mutans* were selected and isolated on their macroscopic, microscopic and morphological properties and predominance. Selected colonies of dental pathogenic bacteria were purified by repeated sub-culture. The purified *Streptococcus mutans* isolates were streaked on to Nutrient agar slants and stored at 4^0 C for further studies.

Primary Screening - Agar Overlay Method

In primary screening by agar overlay method 82 marine actinomycete isolates were screened to detect their antibacterial activity against dental caries causing pathogenic bacteria. From the 82 marine actinomycete isolates 52 isolates (63.4%) showed antibacterial activity but 30 (36.5%) isolates were not showed antibacterial activity to the dental caries causing pathogenic bacteria *Streptococcus mutans*. The trial product of antibacterial activity was expressed qualitatively; for the detection of antibacterial activity single or double plus in case of any minor to moderate response (+/++); effective antagonism by triple plus (+++) and absence of antagonism with negative sign (–). Out of the 52 actinomycete isolates antagonistic to the isolated dental pathogenic bacteria, six isolates showed significant activity, represented with '+++'. Other 46 isolates showed minor to modest response.

Secondary Screening

In secondary screening, well and disc diffusion methods were carried out using the cell free culture filtrate of selected six actinomycete isolates as crude extract against isolated dental pathogenic bacteria. In the six actinomycete isolates screened, two isolates showed significant activity in terms of zone formation against dental caries causing pathogenic bacteria *Streptococcus mutans*. The response of extract of actinomycetes isolates against the *Streptococcus mutans* was shown in Table 1. The remaining four isolates were also antibacterial to the bacterial pathogen but the level of inhibition was comparatively low to that of other two.

Table 1 Antibacterial activity of marine actinomycete isolates by secondary screening.

Actinomycetes	*Streptococcus mutans*	
	Well diffusion method (zone formation in mm)	Disk diffusion method (zone formation in mm)
1	26	26
2	23	20
3	18	15
4	17	14
5	19	17
6	20	17

DISCUSSION

The present study aims to find antibacterial activity of marine actinomycetes against dental caries causing bacteria *Streptococcus mutans*. 82 actinomycete isolates were obtained from four marine sediment samples collected from Vizhinjam coastal region, Thiruvananthapuram district in Kerala. Agar plating of samples followed by incubation at 28^0 C resulted in the development of isolated actinomycete colonies. The colony development was a gradual process and for the first two weeks no colony was noticed but in the third week colonies developed. In Another study sediment sample collected from the coastal region of Kerala with the aim of isolating actinomycetes and screen then for antagonistic activity against bacterial pathogen (12). The isolation of dental pathogen by swabbing of dental caries tooth samples in mitis salivarious agar. In another study revealed that three oral pathogenic bacteria isolated from decayed tooth sample collected from dental hospital (13). In primary screening by agar overlay method a sum of 82 actinomycete isolates were screened to detect their antibacterial activity against *Streptococcus mutans*. Among 82 marine actinomycete isolates 52 isolates (63.4%) were antagonistic and 30 (36.5%) isolates were non antagonistic to dental pathogen. There were 117 actinomycetes screened for its antagonistic property by agar spot method, among them 15 isolates showed significant antibacterial activity (14).In secondary screening, well and disc diffusion methods were carried out using the cell free culture filtrate of selected six actinomycete isolates as crude extract *Streptococcus mutans*. Among the six actinomycete isolates screened, two isolates showed significant activity in terms of production of zone of growth inhibition against *Streptococcus mutans*.

The result revealed that marine actinomycetes contain some secondary metabolites which have antibacterial activity against dental caries causing bacteria *Streptococcus mutans.*

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