SCREENING OF ACTINOMYCETES FROM MARINE ENVIRONMENT

Sivasankari T , R. Sri Priya

PG and Research Department of Zoology, St. Mary's College (Autonomous), Thoothukudi, Affiliated to Manonmaniam Sundaranar University, Thirunelveli, Tamil Nadu, India

ABSTRACT

Actinobacteria are a group of gram-positive bacteria which share the characteristics of both bacteria and fungi, and distributed in both terrestrial and aquatic ecosystems, mainly in soil. They are considered as an important group of bacteria because of the spectrum of antibiotics they produce, that are exploited as antibiotics. Majority of the clinically important antibiotics are secreted by actinomycetes. Actinobacteria are also important in the standpoint of ecological benefits. The current study is focused on isolating actinobacteria from sea of Thoothukudi District and characterize them. Ofthe ten actinobacterial strains that were isolated from Marine soil all were found to be gram-positive bacteria. Twenty isolates were obtained in the experiment. All the strains produced protease enzyme and 10 strains produced the amylase enzyme. Ten strains out of the ten strains characterized, solubilized phosphate.

Key Words: Actinomycetes, Marine environment, Gram - positive, Antibiotics

INTRODUCTION

Actinomycetes are a group of prokaryotic organisms belonging to subdivision of the Gram-positive bacteria. Most of them belong to subclass *Actinobacteridae*, order *Actinomycetales*. All members of this order are characterized by high G+C content (>55 mol %) in their DNA[1]. They are filamentous bacteria which produce two kinds of branching mycelium, aerial mycelium and substrate mycelium. The aerial mycelium is important as the part of the organism that produces spores. For this reason, they have been considered as fungi, as is reflected in their name, akitino means ray and mykes means mushroom/fungus, so Actinomycete was called ray fungi. Actinomycetes are the most widely distributed group of microorganisms in nature and are also well known as saprophytic soil inhabitants[2]. They occur in a multiplicity of natural and made environments and a unique group having different morphological, cultural, biochemical and physiological characters. The soil Actinomycetes produces a volatile compound called geosmin[3]. This organic compound is responsible for the strong odor that occurs in the air when rain falls after a dry spell of weather. In natural habitats, Streptomyces are common and are usually a major component of the total Actinomycetes population. Several halophilic actinomycetes have special biological and chemical defense systems such as pH tolerance, and stress-tolerant metals present one of the

main sources for the discovery of novel metabolites, biosurfactants, several other chemicals, and commercially important enzymes with various applications. There are still a large number of actinobacterial species that have not been characterized. The current study focuses on isolation and characterization of novel actinobacteria.

MATERIALS AND METHOD

Collection of Sample:

Samples for the experiments were collected from the coast of Therespuram, Thoothukudi, and Marine soil samples were collected from the sea approximately at a depth of 1 meter. The samples were collected in bottles, and transported to the laboratory for the isolation of marine actinomycetes.

Characterization of culture:

Isolation of bacteria by serial dilution:

100 ml of sterilized double distilled water was taken separately in 250 ml conical flasks, ~~add~~ 10 g of the sediment sample was placed in it and incubated in a shaker at 37^0C, 150 rpm for 24 hrs to separate filamentous actinomycetes and also for detachment of spores. This sample was used for isolating Actinomycete species.

Plating of culture:

The starch casein agar was prepared and ~~was~~ autoclaved at 121°C for 15 minutes. The medium was poured into a petri dish and allowed to solidify. The samples were plated on the medium by spread plating. 100 µl of the samples from each dilution (10^{-1} and 10^{-6}) were plated using the spreading technique. Plates were incubated at 37°C till the growth of the colonies.

Selective Isolation of Actinomycete Strains:

From the marine soil samples, microorganisms were isolated by serial dilution followed by spreading and restreaking. Actinomycete isolates were obtained by adding 10 mg/l of nalidixic acid to the medium to inhibit the growth of other bacterial strains. The plates were incubated at 37°C till visible colonies appeared.

Isolation of pure bacterial colonies:

Single colonies were picked and streaked to obtain single pure colonies. Pure colonies were incubated at 37°C for 24 hours and stored at 4°C for further studies.

Characterization of isolates:

Morphological characteristics of colonies, such as shape, size, color, pigmentation, and gram staining, were recorded.

Staining of isolates:

Gram staining:

Gram staining was performed for isolated colonies according to standard procedure. A smear of bacterial cells was prepared on a clean glass slide by gentle heat fixation. The heat-fixed smear was filled with crystal violet solution for one minute. The smear was washed with distilled water, and then Gram iodine was washed with 95% ethanol and cleaned with water. Finally, safranin was used as a counterstain and allowed to stay for 60-80 seconds and washed with water. Then the slides were observed under a microscope.

Biochemical characterization:

Catalase test:

Bacterial culture was spread on a slide and a drop of hydrogen peroxide was added. Active effervescence indicates catalase production.

Phosphate solubilization:

Bacterial isolates were screened invitro for their phosphate solubilizing activity using potato dextrose rose Bengal agar. The cultures were streaked on the agar. Plates are incubated at 37°C for 24 hours. The growth of the bacterial colony indicates a positive result for phosphate solubilization[4].

Screening for Hydrolytic enzyme production:

Bacterial isolates were screened for their hydrolytic enzyme production like protease and amylase.

Protease production activity:

Bacterial isolates were screened for the ability to produce proteolytic enzymes in skim milk agar (SM medium). The medium was poured into a sterilized petridish and the isolated bacterial strain was streaked on the surface of the medium. Formation of a clear zone around the colonies is indicative of protease production.

Starch hydrolysis activity:

About 20 ml of starch medium was poured into the sterilized petridish. The isolate was streaked and incubated for 24 hours. At the end of the incubation period, two or three drops of Lugol's iodine solution were added on the surface of the medium. A clear zone around the area of growth indicates starch hydrolysis activity of the isolates.

Antibiotic production:

In order to check the antibiotic production by isolates, the antibiotic production assay was performed by streaking the actinomycetes isolates on a plate with *E. coli.* The colonies showed a zone of clearance indicative of production of antibiotic which could stop the growth of E. coli.

RESULTS

Isolation of bacteria:

Bacterial strains were isolated from the marine soil samples collected from he sea Coast of Therespuram, Thoothukudi. In order to obtain actinomycetes from the soil sample Starch Casein agar supplemented with Nalidixic acid was used to inhibit the growth of other bacterial strains. The colonies developed after incubation at 37°C for 7 days. Pure culture of the bacterial isolates that were isolated from the Marine sample using Starch casein agar supplemented with 10 mg/l of Nalidixic acid were maintained for further studies. Among the single colonies obtained in the serial dilution experiment, twenty colonies patched on Starch casein agar and maintained. The colonies were named as SS1 – SS20. Among the twenty colonies, first 10 colonies (SS1-SS10) were taken for further studies.

Morphological characterization of isolated bacteria:

Morphological characterization:

The bacterial isolates were characterized for the based on the colony morphology such as shape, colour, margin, elevation and opacity using compound microscope and the results are tabulated (Table 1).

Gram staining of the isolates:

Gram staining was done on the isolates. All the isolates were found to be Gram positive.

Biochemical analysis of isolated bacteria:

Biochemical tests were performed on the bacteria isolates

Catalase test:

Catalase test was done to identify strains that produce catalase enzyme. The isolates SS1-SS10 were used for catalase test. O/N liquid culture of the isolates was raised. The liquid culture was placed on a glass slide and a few drops of hydrogen peroxide was added. A brisk effervescence indicates a positive result. None of the colonies were positive for catalase test [Table 2].

Phosphate solubilization test:

Phosphate solubilization ability of bacteria can be detected by culturing the isolates on potato dextrose rose Bengal agar plate method. Growth on this medium confirms their phosphate solubilization activity. The isolates SS1-SS10 were checked for phosphate solubilization. All the colonies grew well on potato dextrose rose Bengal agar indicative of its phosphate solubilization ability [Table 2].

Protease production test:

To check the ability of the strains to produce protease enzyme, the colonies were patched on Skim milk (SM) agar medium. The colonies SS1- SS10 were used for the analysis. All the colonies grew well on the SM agar medium. This showed that the isolates confirming the production of protease[Table 2].

Starch hydrolysis test:

The starch hydrolysis test was performed to confirm the production of the enzyme amylase by the bacterial isolates. The isolates that produced amylase enzyme can hydrolyze the starch present in the Starch Agar and show a zone of clearance around their colony when iodine is added. The test was performed on 10 colonies (SS1-SS10) all of them showed a zone of clearance indicative of their ability to hydrolyze starch [Table 2].

Table 1 Morphology of the isolates

Isolates	Colour	Shape	Margin	Elevation	Opacity
SS1	White	Irregular	Undulate	Flat	Opaque
SS2	White	Irregular	Undulate	Flat	Opaque
SS3	White	Irregular	Undulate	Flat	Opaque
SS4	White	Irregular	Undulate	Flat	Opaque
SS5	White	Irregular	Undulate	Flat	Opaque
SS6	White	Irregular	Undulate	Flat	Opaque
SS7	White	Irregular	Undulate	Flat	Opaque
SS8	White	Irregular	Undulate	Flat	Transparent
SS9	White	Irregular	Undulate	Flat	Transparent
S10	White	Irregular	Undulate	Flat	Transparent

Table 2 Biochemical characterization of the bacterial isolates collected from the sea water

Isolates	Catalase test	Phosphate solubilization	Protease production	Starch hydrolysis
SS1	Negative	Positive	Positive	Positive
SS2	Negative	Positive	Positive	Positive
SS3	Negative	Positive	Positive	Positive
SS4	Negative	Positive	Positive	Positive
SS5	Negative	Positive	Positive	Positive
SS6	Negative	Positive	Positive	Positive

SS7	Negative	Positive	Positive	Positive
SS8	Negative	Positive	Positive	Positive
SS9	Negative	Positive	Positive	Positive
SS10	Negative	Positive	Positive	Positive

Antibiotic production:

In order to check the antibiotic production by isolates, the antibiotic production assay was performed by streaking the actinomycetes isolates on a plate with *E. coli*.The colonies SS2 and SS6 showed a zone of clearance indicative of production of antibiotic which could inhibit the growth of *E. coli*. (Fig.1).

DISCUSSION

The objective of the current study was to isolate actinobacterial drains. Hence soil samples from Sea were used to isolate the organisms. Initially the colonies were isolated on Starch Casein Agar. As this did not inhibit growth of other bacterial colonies Nalidixic acid was used. On the Actinomycete selection medium, many Actinomycetes bacterial strains were identified. For the analysis a few of the colonies which grew on Starch Casein Agar and a few colonies from the medium containing Starch casein agar supplemented with Nalidixic acid was used. Morphological and biochemical characterization of the colonies were done. The bacterial colonies showed diverse morphological characteristics as indicated from variation in shape, colour, margin, elevation and opacity. On the basis of their gram reaction all were gram positive. Some of the colonies also produced a wide range of bioactive compounds such as enzymes protease, amylase, catalase etc.,Sneath (1992)[5] revealed Gram staining technique used to classify bacteria based on their cell wall structure. Marine soil actinomycetes are typically Gram-positive bacteria.John and Sons (2015)[6] have reported that catalase test is an important test for detection of actinomycetes. Very few marine soil actinomycetes are known

to produce catalase enzyme.Holt *et.al.*,(1994)[7] have reported that protease production test is an important biochemical test for characterization of bacterial isolates. Proteases are enzymes that break down proteins into amino acids. Marine soil actinomycetes are known to produce proteases.The study of Staley (2001)[8] revealed that phosphate solubilization is another important characteristic of marine soil actinomycetes. These bacteria have the ability to solubilize insoluble forms of phosphate.Williams, (1994)[9] reveled that starch hydrolysis test can be used to characterize marine soil actinomycetes. Starch is a complex carbohydrate that can be broken down by certain enzymes produced by bacteria.

CONCULSION

Isolation and characterization of actinomycetes play a crucial role in various fields such as pharmaceuticals, agriculture, and bioremediation. Actinomycetes are a group of Gram-positive bacteria known for their ability to produce bioactive compounds with diverse biological activities. Present work is designed to isolate and characterize novel actinomycete species from marine samples.

Actinomycetes are widely distributed in nature, particularly in soil and aquatic environments. They have been a rich source of bioactive compounds such as antibiotics, antifungals, anticancer agents, and immunosuppressants. Isolation of actinomycetes involves selective media and culture techniques to isolate specific strains with desired properties. Characterization of actinomycetes includes morphological, biochemical, and molecular techniques to identify the genus and species of the isolated strains. Although there are several studies that focus on the isolation and characterization of actinomycetes from different environments, leading to the discovery of novel bioactive compounds there are still more actinomycetes with novel characteristic features.

The present work is designed to isolate such novel strains and utilize it for production of bioactive compounds. We have identified ten individual gram-positive bacterial colonies by selectively culturing the bacterial species on starch casein agar which favors the growth of actinomycete. These colonies were all gram positive and showed varied morphology. None of them were catalase positive, confirming that they are putative Actinomycetes. Biochemical tests were done for their initial screening, but further confirmation has to be done by performing 16s rRNA sequencing. Further research has to be done for presence of bioactive compounds in these strains.

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