



Original Research Article

Isolation and Partial Identification of Sediment derived Actinomycetes from Turmeric Field of Erode District, India

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ABSTRACT

The actinomycetes strains were isolated from the soil sample of Erode, Kodumudi turmeric crop plantation. Then the soil samples were air dried under *In vitro* lab condition for 3 – 4 days. After 4 days contaminants and dust particles were removed from the soil samples. The samples was subjected to the basic microbiology procedures like, Serial dilution, pour plate, and spread plate technique, that is to identify the specific growth of actinomycetes and it is identified that it is present in the 10^{-3} and 10^{-4} . Followed by the microbiology procedure, finally the samples were undergone for identifying the biochemical characterization and amylase activity. Several parameters of the biochemical like Macconkey, Starch hydrolysis and Gelatin hydrolysis were clearly performed for the identification. Samples underwent for all the basic microbiological and biochemical studies and each studies shown the excellent result towards all the examinations.

Keywords

Actinomycetes,
Turmeric
soil,
Amylase

Introduction

Actinomycetes are the group of Gram positive filamentous bacteria which are primarily recognized as organism of academic curiosity, potential degraders and also as a potential source for antibiotics. Although actinomycetes are well exploited for antibiotics and other high value metabolites, they are less exploited in terms of nanoparticles (bala *et al.*, 2011). Aerobic actinomycetes are found in soils in different parts of the world (Subbaiya *et al.*, 2014). Actinomycetes have provided many

important bioactive compounds of high commercial value and continue to be routinely screened for new bioactive compounds (Duraipandiyan *et al.*, 2010). Actinomycetes are one of the most important source for new bioactive compounds such as antibiotics and enzymes (Ravikumar *et al.*, 2012). This is due to their capability to produce biologically active secondary metabolites, with many of them as potent antibiotics and/or lead compounds that would otherwise not be

discovered in terrestrial microorganisms (Xiaochun Gao., *et al* 2012).

The majority of these antibiotic substances are antibacterial in nature and are indispensable for the treatment of bacterial infections of plants and animals including the human beings (S. Rayadapadar, *et al.*, 2001). Living organisms have the endogenous ability to regulate the synthesis of inorganic materials (Sathya sadhasivam *et al.*, 2012). These microorganisms (bacteria and actinomycetes) are virtually unlimited sources of novel compounds with many therapeutic applications (Adinarayana Gorjana *et al.*, 2006). Investigation of actinomycete diversity in tropical rainforests using a combination of 16S rDNA analysis and morphological examination revealed that many of the isolates had only adjutant relationship with known species, indicating the presence of new taxa and an excellent source for novel natural product discovery (*J. sarah et al.*, 2010).

Actinomycetes capable of producing many types of secondary metabolites are a group of prokaryotic organisms, which are gram-positive bacteria that grow extensively in soils with rich organic matter (Kozue Azai *et al.*, 2008). In the field of investigational search for new biologically active substances, where more than 10,000 secondary metabolites of microbial origin have been discovered (Takefumi Hamaki *et al.*, 2005). Over 10,000 of these compounds are produced by actinomycetes, representing 45% of all bioactive microbial metabolites discovered, 80% if we only consider those compounds in practical use (Carlos Olano *et al.*, 2008). Despite this apparent success, most of the actinomycete-based screening programs at big pharmaceutical companies have been abandoned in the recent years due to several reasons (Sergey B. Zotchev., 2012). Actinomycetes are the most

economically and biotechnologically valuable prokaryotes (M. Rajeshmuthu *et al.*, 2013).

Materials and Methods

Collection of soil sample

The soil samples were collected from Erode, Kodumudi, where turmeric plantation crop is enormous. The soil sample was air dried for 3 to 4 days under *in vitro* lab condition. Serial dilution techniques were carried out followed by the pour plate method was done. Morphology shows that the colonies were present in 10^{-3} and 10^{-4} and finally the biochemical methods were done.

Gram Staining

A loopful of culture was taken in a clean glass slide and heated gently over a flame. The smear was covered with a thin film of crystal violet for 1 min and washed gently in slow running tap water. Gram's iodine solution was flooded over the smear for 1 min and washed with tap water. Alcohol was used to decolorize the smear until the violet color ceased to flow away. The slide was washed with water and counter stain safranin was flooded over the smear for 2 min, then the slide was washed, drained, air dried, and viewed under microscope. The culture retaining the violet color indicated that it was Gram-positive organism. (Dhuraipantiyan *et al.*, 2010)

Starch hydrolysis test

Actinomycetes have the ability to produce the amylase enzyme. This was done to confirm whether the isolated organism have the ability to produce the amylase and hence to determine whether this organisms are actinomycete. The isolated colony was subjected to simple streaking in the SCN media and it was incubated for 4 days at 30°C.

MacConkey agar test

This test is used to test whether the organism are gram positive or gram Negative. The media used here is MacConkey agar. This media support only the growth of gram negative bacteria. The media was prepared in sterilized condition and poured in the Petri plates. It was allowed for solidification for few minutes and then the culture was inoculated in the media under sterile condition and kept for incubation for 4 days at room temperature.(Selvakumar *et al.*, 2012)

Casein hydrolysis test

Casein hydrolysis test carried out in order to test whether the organism has the capacity to produce the protease enzyme. Casein is an exoenzyme. A sterilized media called casein agar was prepared for this test. (Selva kumar *et al.*, 2012).

Gelatin hydrolysis test

Gelatinase test was used to detect the whether the organism has the capacity to produce gelatinase enzyme. The nutrient gelatin media was used as a differential media.

Results and Discussion

Isolation of Actinomycetes

After the incubation period of spread plate, a single colony was chosen based upon its morphology. This identified colony was sub cultured using the simple streaking method. In order to obtain the pure culture, quadrant streaking was done and it was sub cultured and preserved for the future use. It was preserved in the freezer so that the rate of contamination will be lesser.

Gram Staining

The organism was confirmed as gram

positive bacteria. In order to confirm whether it is an actinomycetes, mycelium and spores were seen under the microscopical view.

Starch hydrolysis test

When the iodine solution poured on the sample and waited for few seconds. A clear zone around the organism is seen while the other area was clearly covered with iodine solution. This is because the organism was surrounded by the amylase enzyme produce by it. This enzyme replace those iodine solution. The amylase was produced in order to degrade the starch present in the media.

MacConkey agar test

After the incubation period of inoculum in MacConkey agar media, the growth of the organism was not seen. This concludes that the isolated organisms are gram positive bacteria.

Casein hydrolysis test

The casein presented in the media is degraded by the protease. This protease presence was detected when a clear zone was seen around the organism and it shows that it is having the ability to produce protease.

Gelatin hydrolysis test

The organism was tested for the gelatinase production, were the gelatin is used in media. The gelatin was used as clotting factor. After the incubation period the sample was incubated in the ice for half an hour where the media still remains unsolidified which shows that the organisms produces gelatinase which hydrolysis the gelatin.

Fig.1 Simple streak



Fig. 2. Continuous streak



Fig.3 Quadrant streak



Fig.3 Starch hydrolysis test



Fig.4 MacConkey agar test

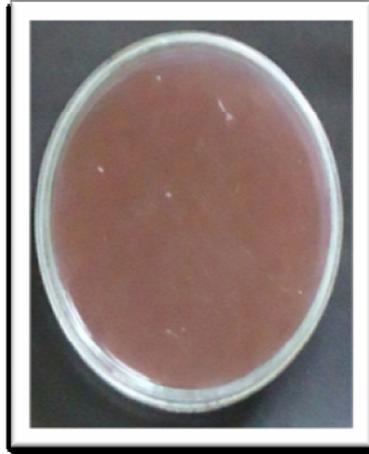


Fig.5 Casein hydrolysis test

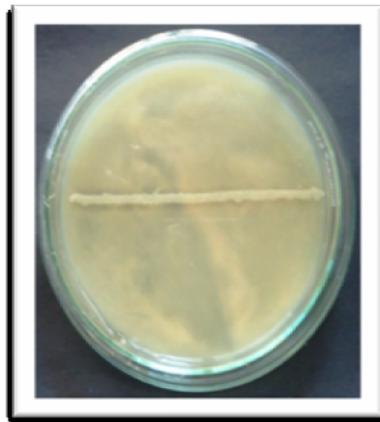
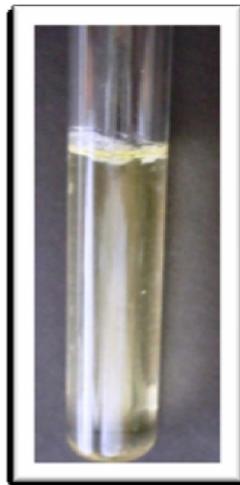


Fig.6 Gelatin hydrolysis test



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