Recent Research in Science and Technology 2011, 3(5): 80-83
ISSN: 2076-5061
www.recent-science.com

MICROBIOLOGY

ENZYMATIC ACTIVITY OF ACTINOMYCETES ISOLATED FROM MARINE SEDIMENTES

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Abstract

The marine environment represents a largely untapped source for isolation of new microorganisms. Gram positive actinomycete bacteria are of special interest, since they are known to produce chemically diverse compounds with a wide range of biological activities. Totally, 107 different actinomycetes were isolated from marine sediments, collected from five coastal sites of Konkan coast of Maharashtra.90 actinomycetal isolates were identified up to generic level. It was found that these actinomycetal isolates were belonging to *Streptomyces, Micromonospora, Intrasporangium, Saccharopolyspora, Streptosporangium, Rhodococcus, Saccharomonospora* and *Nocardia.* Enzymatic activities of 90 identified actinomycetal isolates were performed. It was found that out of 90 actinomycetes 76 (84.44 %), 70 (77.78%), 65 (72.22%), 39 (43.33%), 34 (37.78%) and 15 (16.67%) number of actinomycetes were possessing protease, gelatinase, amylase, lecithinase, cellulase and ureases activity respectively.

Keywords: Actinomycetes, Potease, Amylase, Marine sediments

Introduction

The world ocean with a coastline of 312,000 Km and volume of 137 X 106 Km ^{3}, is the largest ecosystem on the earth, and has been used for a variety of purpose by man for millennia. Microorganisms occur nearly everywhere in nature and occupy an important place in human view of life. Marine microbes represent a potential source for commercially important bioactive compounds and their bioremediation capabilities are also remarkable. Among the three major habitats of the biosphere that marine realm which covers 70% of the earth surface provides the largest inhabitable space for living microorganisms (Surajit Das et al., 2000).The marine environment represent a largely untapped source for isolation of new microorganisms with potential to produces biologically active secondary metabolites. Actinomycetes have been a source of a numerous useful products including pharmaceuticals, agrochemicals, enzymes for use in a number of industrial applications from food industry to paper making. Actinomycetes can be isolated form marine sediments, marine water, marine plants and animals. Studies on microbial diversity by 16 S r DNA gene analysis showed actinomycetes are dominant in marine sediments.

Many reports describe that in India, the East coast area is a major source of actinomycetes (Dhanasekaran et al., 2000). However, only few reports are available pertaining to actinomycetes diversity in west coast of India and mangrove soils of India.

Materials and Methods

Study area

The samples were collected from Konkan coast of Maharashtra. Konkan coast line lies in between Mumbai and Goa. It is 560 km long. Konkan region includes Mumbai, Thane, Raigad, Sindhudurg and Ratnagiri district. The Arabian Sea lies in the western side of Konkan region.

Collection of sediment samples

Twenty near-sea shore sediment samples were collected, 10 cm in depth in sterile Petri plates from sampling sites. Four sediment samples were collected from each site. Samples were collected from 20 feet away from the sea shore. All samples were labeled and were brought to the laboratory. They were stored at a temperature between 6^0C to 10^0C until further use.

Isolation of actinomycetes

Enrichment

The dilutions of collected sea sediment samples were made in sterile sea water (1:10 w/v). A temperature shock 70^0C for 5 minutes was given to each diluted sediment sample and 5ml of sample was inoculated in 250 ml conical flask containing 50 ml of

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enrichment medium. The medium was supplemented with antifungal antibiotic griseofulvin at 50µg/ml concentration (Nevine et al., 2000). The temperature shock and antibiotic added in medium depress associated gram negative bacteria which create problem during isolation (Ratnakala and Chandrika, 1993). The flask were incubated at 30^0 C for 10 days.

Isolation of actinomycetes

Actinomycetes from enrichment medium were isolated by streak plate method using starch nitrate agar, glycerol aspargine agar, actinomycetes isolation medium, starch casein agar prepared in sea water and supplemented with antifungal antibiotic griseofulvin at 50µg/ml concentration. Then these plates were incubated at 30 ^{0}C for 7 days.

Study of characteristics of isolates

Colonial characteristics

After incubation typical dry leathery colonies of actinomycetes were selected. Good growth was obtained on starch casein agar so it was used for further study. Colony characters were studied.

Morphological characters

The cover-slip cultures of actinomycetal isolates were prepared and morphological characteristics were studied. The isolates were grown on glycerol aspargine agar as simple cover –slip cultures (Kawato and Shinobu, 1959). A sterilized cover slip was carefully inserted at an angle of about 45^0 in glycerol aspargine agar plate until about an half of the cover slip was in the medium. An actinomycetes isolate was then inoculated along the line where the upper surface of the cover slip meets agar. The plates were incubated at 30^{0}C for 7 days. After incubation, the cover slip was carefully removed with respect to its orientation and placed in upward on a slide and used for microscopic observations.

Microscopic examination

The following microscopic observations were recorded using cover slip culture.

- Presence or absence of substrate mycelium
- Fragmentation of substrate mycelium
- Presence of sclerotia or sporangia
- Sporulation on substrate mycelium
- Spore chain morphology: rectiflexibiles, retinaculiaperti or spirals.

The generic level identification was carried out by using Bergey's manual of Determinative Bacteriology 9th edition (Holt, 1994).

Study of enzymatic activity

The actinomycetal isolates were inoculated on suitable medium by streak or spot inoculation method in order to check different enzymatic degradative activities. The plates were incubated at 30^{0}C for 4-7days. The details are given in table 1`.

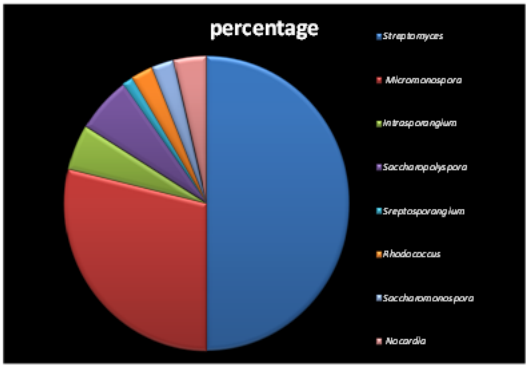
Table 1. Studies on the enzymatic activities of actinomycetes

Sr.No	Enzyme	Medium	Inoculation method	Incubation period (days)	Criteria of positive enzyme activity
1	Protease	Modified Bennett agar	Spot	7	Clearing around the growth
2	Gelatinase	Modified Bennett agar with 0.4% w/v gelatin	Spot	7	Clearing around the growth after flooding with 1.5% acidified $HgCl_2$ solution.
3	Amylase	Modified Bennett agar with 1% w/v starch	Spot	7	Clearing around the growth after flooding with 2% iodine
4	Lecithinase	Egg yolk agar	Spot	4-6	Opaque (cloudy) zone around the colony.
5	Cellulase	Mandel and Reese medium	Streak	7-21	Clearing around the growth.
6	Urease	Christenson's agar	Streak	7	Development of pink color.

Results and Discussion

Out of 107 actinomycetal isolates 90 actinomycetal isolates were identified up to generic level. It was found that out of 90 identified actinomycetal isolates were belonging to *Streptomyces, Micromonospora, Intrasporangium, Saccharopolyspora, Strepto sporangium, Rhodococcus, Saccharomonospora* and *Nocardia* respectively (Fig.1).

Fig. 1: Generic distribution of actinomycetes isolated from Konkan coast



Enzymatic activity of actinomycetes

Enzymatic activities of 90 identified actinomycetal isolates were performed and resultes are given in table 2 and Fig.2. It was found that out of 90 actinomycetes 76(84.44 %), 70 (77.78%), 65 (72.22%), 39(43.33%), 34(37.78%) and 15(16.67%) number of actinomycetes were possessing protease, gelatinase, amylase, lecithinase, cellulase and urease activity respectively. It was found that members of genera *Streptomyces, Micromonospora, Nocardia* were possessing protease, gelatinase, amylase, lacithinase, cellulase and urease activity. Members of *Streptosporangium* were lacking lecithinase activity. Members of *Intrasporangium*, *Sccharopolyspora, Streptosporangium*, *Rhodococcus* and *Saccharomonospora* were lacking urease activity. Thus it was found that most of the genera were enzymatically active.

It was thus seen from the table 2 that high numbers of actinomycetes were biochemically active in sea sediments. These actinomycetes plays significant role in decomposition of complex organic matter.

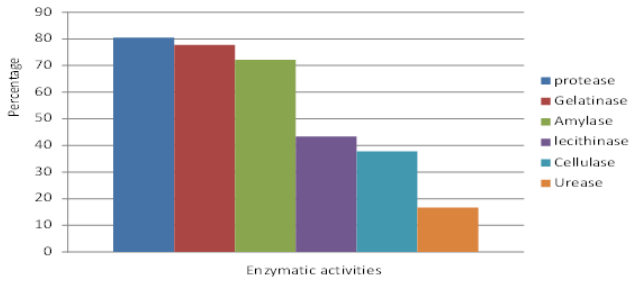
A number of research workers in earlier investigation have also reported that actinomycete from soil and water bodies possess high number of enzymatically active actinomycetes. Patke and Dey (1996), Chaphalkar and Dey (1993) have reported protease activity in isolated actinomycetes. Stapp (1953), Shejul (1999) have reported gelatinase activity in isolated actinomycetes. Amylase activity in actinomycetes has been reported by Abraham and Herr (1964), Fogarty (1983), Williams et al. (1983), Obi and Obido (1984), Stanford et al. (2001) and Nawani et al. (2002). Okawa and Yamaguchi (1975) have detected lecithinese activity in actinomycetal isolates. Cellulase activity in actinomycetes has been reported by Stutzenberger (1972), Konde (1978), Hagerdal et al. (1980), Moreira et al. (1981), Lee and Fan (1981), Deobald and Crowford (1987).

Table 2. Generic distribution of actinomycetes genera on the basis of enzymatic activities

Sr No.	Actinomycetes	Number tested	Protease	Gelatinase	Amylase	Lecithinase	Cellulase	Urease
1	*Streptomyces*	50	40	34	32	19	16	13
2	*Micromonospora*	23	19	20	17	11	9	2
3	*Intrasporangium*	4	4	4	4	3	2	-
5	*Saccharopolyspora*	5	5	5	5	2	3	-
6	*Streptosporangium*	1	1	1	1	-	-	-
8	*Rhodococcus*	2	2	1	2	1	1	-
9	*Sacchromonospora*	2	2	2	1	1	1	-
10	*Nocardia*	3	3	3	3	2	2	2
11	Total	90	76	70	65	39	34	15

--- No enzymatic activity

Fig. 2: Percentage of enzymatic activity in marine actinomycetal isolate



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