Isolation and Screening of Actinomycetes for Bioactive Compounds from the Marine Coast of South- Gujarat Region

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***Abstract*-Actinomycetes are the most economically and biotechnologically valuable prokaryotes able to produce wide range of bioactive secondary metabolites. Around 23 000 bioactive secondary metabolites produced by microorganisms have been reported and over 10 000 of these compounds are produced by Actinomycetes, representing 45% of all bioactive microbial metabolites discovered. Studies have shown that Actinomycetes isolated from the marine environment are metabolically active and have adapted to life in the sea. As marine environmental conditions are extremely different from terrestrial ones and also most potential source for isolation of Actinomycetes ,marine soil is collected from less unscreened west- coast area of Arabian sea (Tithal) and Salt pan region of Charwada , Valsad district of South- Gujarat region. A Physical and Chemical pretreatment of collected marine soil samples resulted into good isolation of 17 species belonging to the Actinomycetes group. These isolates were screened for the production of bioactive compounds including enzymes such as amylase, gelatinase , caseinase, lipase and urease. & antibacterial compounds. However, of the 17 isolates obtained only four isolates namely ACT-2, ACT-8, ACT-10 and ACT-11 showed significant antibacterial activity against both tested gram-positive and gram-negative bacteria.**

Key Words: Marine soil, Actinomycetes, pretreatment, enzymatic activity, antimicrobial activity.

I. 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. Marine microbes represent a potential source for commercially important bioactive compounds and their bioremediation capabilities are also remarkable. Many reports describe that in India, the East coast area is a major source of actinomycetes. However, only few reports are available pertaining to actinomycetes diversity in west coast of India. Actinomycetes forms the dominant and significant group among soil microbes.

Actinomycetes are Gram-positive, mycelium-forming soil bacteria, represents a ubiquitous group of microbes that are the most economically and biotechnologically valuable prokaryotes. They include many species that provide a potential source for novel products including pharmaceuticals, agrochemicals, enzymes of industrial interest. As marine environmental conditions are extremely different from terrestrial ones, it is summarized that marine Actinomycetes have different characteristics from those of terrestrial counterparts and therefore might produce different types of bioactive compounds

In view to the significance of marine ecosystems , the present study is aimed for isolation of actinomycetes using various pretreatment including enrichment , physical and selective medias with special focus on the production of bioactive compounds (enzymes) using specific medias & antibacterial compound by the isolates obtained.

II. MATERIALS AND METHOD

A. Study area :

The samples were collected from 2 different marine environments, West- coast of Arabian sea (Tithal), and Salt pan of Charwada -Valsad district , India.

B. Collection of soil samples:

Twenty soil samples are collected quarterly from marine environments at a depth of 10-15 cm from soil surface and placed in sterile polythene bags, sealed tightly and brought to the laboratory and stored in refrigerator at 40C after labeling until further use.

C. Pretreatment of sample:

The soil samples are given a physical treatment. In physical treatment – 1gram soil sample is mixed with sterile 9.0 ml distil water , mix properly and exposed at high temperature of 700C for 15minutes to kill the contaminants .

D. Enrichment of soil sample:

In 250 ml conical flask 5ml of the Pretreated soil sample was added to the 50 ml of the Actinomycetes Isolation broth medium. The medium was supplemented with Nalidixic acid (50 µg/ml) and Amphotericin B (50µg/ml) concentration. The flask were incubated at 300 C for 07 days.

E. Isolation of Actinomycetes :

Enrichment medium prepared for isolation of actinomycetes were plated on actinomycetes isolation agar medium / starch casein agar medium using 10 fold serial dilution & Spread plate technique . During media preparation antibacterial an antifungal antibiotics was added to inhibit bacterial and fungal contamination, respectively. The plates were incubated at 300C for 07 days. (Deshmukh,2011). During incubation, Plates were observed for the growth of typical actinomycetes colonies for up to 07 days.

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Isolated actinomycetes colonies obtained were purified and further identified morphologically by Gram staining based on Bergey's Manual of Systematic Bacteriology. Isolates so obtained were further subjected to screening of enzymatic and antibacterial activity.

F. Screening of enzymatic activity:

All the actinomycetes isolates were inoculated on suitable medium by streak/spot inoculation method in order to check different enzymatic degradative activities.The plates were incubated at 300C for 4-7days. The details of tests & results of enzyme activity obtained are given in table 2 and Chart 1.

G. Screening of Antibacterial activity:

All the actinomycetes isolates obtained were tested for their antibacterial activity against several bacteria Gram-negative bacteria and Gram -positive bacteria. Screening for antimicrobial activity of pure isolates was determined by perpendicular streak method using Nutrient Agar Medium.

III. RESULTS AND DISCUSSION

A total of 17 isolates were isolated from marine soil samples. The numbers of samples and isolates in each sample were presented in Table 1.

Table 1 : Isolation of Actinomycetes from Marine soil samples

Sr. No.	Geographical location	Types of sample	Number of Isolates
1	Valsad -Tithal	Soil with water	08
2	Valsad-Salt pan Charwada	Dry Soil sample	09

Out of the 17 isolates, 09 isolates showed amylase,10 isolates showed gelatinase , 08 isolates of caseinase,05 isolates showed lipase and 07 isolates showed Urease production. The results of enzymatic activity are shown in Table 2.

Among Antimicrobial activity, only 05 isolates were identified to showed antibacterial activity against the selected test organisms. The results of which are shown in Table 3 and Colony characteristics of the 05 potential antibacterial actinomycetes are given in Table 4.

Table 2 : Results for study of enzymatic activities of Actinomycetes

1	Amylase	Starch agar medium	Clearing around the growth after flooding with 2% iodine.	ACT-1,2,,4,5, 6,7,10,11, 13
2	Gelatinase	Gelatin agar medium	Clearing around the growth after flooding with Frazier's reagent	ACT-1,2,4,5,7, 8,9,10,11, 15
3	Caseinase	Skimmed milk agar medium	Clearing around the growth	ACT-1,2,3,5, 7,8,9,11
4	Lipase	Tributyrene agar medium	Clearing around the growth	ACT-4,9,11, 12, 13
5	Urease	Urea broth	Pink coloration of urea broth	ACT-4,5,6,7, 10,12 ,16

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Table3. The results of study of antibacterial activity of Actinomycetes isolates against the selected test organisms.

Actinomycetes isolates	E.coli	E. aerogenes	Klebsiella pneumoniae	Proteus vulgaris	Pseudmonas aeruginosa	S. typhi	Shigella	Bacillus subtilis	Bacillus megaterium	S. aureus
ACT-1	-	-	-	-	-	-	-	-	-	-
ACT-2	+	-	+	+	-	+	+	-	+	+
ACT-3	-	-	-	-	-	-	-	-	-	-
ACT-4	-	-	-	-	-	-	-	-	-	-
ACT-5	-	-	-	-	-	-	-	-	-	-
ACT-6	-	-	-	-	-	-	-	-	-	-
ACT-7	-	-	-	-	-	-	-	-	-	-
ACT-8	+	+	+	+	+	+	+	+	+	+
ACT-9	-	-	-	-	-	-	-	-	-	-
ACT-10	-	-	-	-	-	-	+	+	+	-
ACT-11	+	-	+	-	-	+	+	+	+	+
ACT-12	-	-	-	-	-	-	-	-	-	-
ACT-13	-	-	-	-	-	-	-	-	-	-
ACT-14	-	-	-	-	-	-	-	-	-	-
ACT-15	-	-	-	-	-	-	-	-	-	-
ACT-16	-	-	-	-	-	-	-	-	-	-
ACT-17	-	-	-	-	-	-	-	-	-	-

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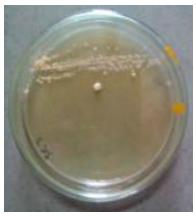
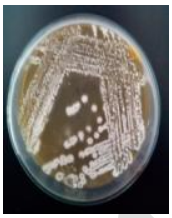
ACT-2 ACT-8 ACT-10 ACT-11

Table 4: Colony characteristics of these 05 potential antibacterial actinomycetes isolates.

Actinomycetes isolates	MS2	MS8	MS10	MS11
Colony Characteristics				
Size	Large	Large	Large	Large
Shape	Irregular	Round	Round	Round
Surface	Rough	Rough	Powdery	dry
Edge	Wavy	Irregular	Irregular	Irregular
Elevation	Convex	Convex	Raised	Convex
Consistency	Leathery	Tough	Dry	Tough
Opacity	Opaque	Opaque	Opaque	Opaque
Pigmentation	Chalk White	Chalk White	Chalk white	Chalk White
Gram Staining	Large rod shaped branched filamentous Gram positive bacteria	Large cocci and rod shaped branched filamentous Gram positive bacteria	Large cocci and rod shaped branched filamentous Gram positive bacteria	Large rod shaped branched filamentous Gram positive bacteria

IV. CONCLUSION

Considering the outcome of the present findings, it was concluded that marine environment of west coast of India is a rich source of deriving economically important actinomycetes that are able to produce different bioactive compounds such as enzymes and antibacterial compounds and so are capable of degrading numerous organic compounds as well as producing drugs used for treatment against pathogens. Identification of potential actinomycetes producing bioactive compounds to genus level and further studies along these findings are underway.

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REFERENCES

[1]. Gulve R. M. and Deshmukh A. M. (2011) Enzymatic activity of Actinomycetes isolated from marine sediments. Recent Research in Science and Technology, 3(5): 80-83.

[2]. Ahmed M. Reyad(2013) Diverse of enzymatically active actinomycetes associated with mangrove Rhizospher in Jazan coast. Annals of Biological Research, 2013, 4 (4):100-10.

[3]. Atta Houssam M ; El-Sayed, A. S; El-Desoukey, M. A; Mona Hassan, M.and Manal El- Gazar, M.(2011) Screening,

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Identification, Phylogenetic Characterization and Optimization of Antimicrobial Agents Biosynthesis Produced By Streptomyces rimosus World Rural Observations ;3(3)

[4]. Narendra Kumar, Singh Ravi Kant, Mishra S.K., Singh A.K., Pachouri U.C.(2010) Isolation and screening of soil Actinomycetes as source of antibiotics active against bacteria. International Journal of Microbiology Research, Volume 2, Issue 2,pp.12-16.

[5]. Remya Mohan, Ramasamy Vijayakumar (2008) Isolation and Characterization of Marine Antagonistic Actinomycetes from West Coast of India, Series: Medicine and Biology Vol.15, No1, pp. 13-19.

[6]. Pandey Amit, Imran Ali , Butola Kailash Singh, Chatterji Tanushri, Singh Vidyottma (2011) Isolation and characterization of Actinomycetes from soil and evaluation of antibacterial activities of Actinomycetes against Pathogens. International Journal of Applied Biology and Pharmaceutical Technology, Volume: 2: Issue-4.384-392.

[7]. Rahman Md. Ajijur, Islam Mohammad Zahidul and Islam1 Md. Anwar Ul (2011) Antibacterial Activities of Actinomycete Isolates Collected from Soils of Rajshahi, Bangladesh. Biotechnology Research International

[8]. Singh S.L., Baruah Indra and Bora T.C.(2006)Actinocycetes of Loktak Habitat: Isolation and Screening for Antimicrobial Activities.

[9]. Valli S, Suvathi Sugasini S, Aysha O S, Nirmala P, Vinoth Kumar P, Reena A (2012) Antimicrobial potential of Actinomycetes species isolated from marine environment. Asian Pacific Journal of Tropical Biomedicine, pp469-473.

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