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Research Article

Isolation and Screening of Actinomycetess from Different River Sediments

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Abstract

The research are going to design and production of a number of drugs. There is an increase or discovery of different diseases which causing great harm to society. The past researches indicated that huge number of antibiotics were produced by Gram +ve ike bacteria known as Actinomycetes. So we can say that among all microbes more than 50% of the known antimicrobial compounds were produced by Actinomycetes only. These are a specific type of class of prokaryotes forming thread like structure at some stage of their growth, so refereed as filamentous prokaryotes. This class or group is a actively produce of different types of enzymes, enzyme inhibitors, growth promoter and antibiotics etc. In modern era a number of microbes is getting or developing resistance against different infectious microorganisms (e.g., species of *Staphylococcus, Mycobacterium,* and *Streptococcus*) to existing compounds.

In our study screening of Actinomycetes was performed by using different river sediments. Soil samples was collected from river Godaveri and Krishna and stored in the U.V. and alcohol sterilized Poly bags.

Soil samples was serially diluted upto 10^{-6} and 1 ml from each dilution was plated on different isolation media like starch Casein agar, Albumin media and YMA media, consisting of antifungal agent Nystatin 50 µg/ ml, by pour plate technique. The plates were incubated at different temperature ranges 18 ^{0}C to 28 ^{0}C upto 7-14 days.

There were 3 actinomycetes were isolated and these were streak on solidified Bennet agar media at straight line and plates were kept for incubation in incubator at 37^{0}C for nearly about 3 days. Identification of actinomycetes was performed using Gram's staining.

Keywords: Actinomycetes, river sediments, Nutrient Media, Identification.

INTRODUCTION

Actinomycetes[1] are a widely distributed and successful group of bacteria which have a number of properties which favor them in competition with other saprophytic microorganisms and ensure their survival under unfavorable environmental conditions. Actinomycetes form an integral part of any balanced microbial community in soil, the majority of isolates being Streptomycetes which manly exist in the form of dormant spores. These spores germinate in presence of suitable plant and animal remains to form a limited branching mycelium bearing short chain of spores. The spores are continuously washed into aquatic habitats where they accumulate in sediments.

The past researches indicated that huge numbers of antibiotics were produced by Gram +ve ike bacteria known as Actinomycetes. So we can say that among all microbes more than 50% of the known antimicrobial compounds were produced by Actinomycetes only. These are a specific type of class of prokaryotes forming thread like structure at some stage of their growth, so refereed as filamentous prokaryotes. This class or group is an actively produce of different types of enzymes, enzyme inhibitors, growth promoter and antibiotics etc.

Actinomycetes production was almost exclusively confirmed to the group of Streptomyces. In moden days human efforts are being generated to broad and performing research about rare actinomycetes which belong to different group's like-

Actinomadura
Actinoplanes
Actinosynnema
Dactylosporangium
Kibdilosporangium etc.

Actinomycetes are a special group of heterotrophic prokaryotes forming hyphae at some stage of their growth hence refereed as filamentous prokaryotes. They have been specialized and different morphological, cultural, biochemical and physiological characters. This group is a potential producer of different enzymes, enzyme inhibitors, growth promoter and antibiotics etc. Actinomycetes are gram +ve bacteria belonging to the order of actinomycetales. Actinomycetes are characterized by the formation of normally branching threads or rods, frequently giving rise to a typical mycelium which is unicellular, especially during the early stages of growth. Actinomycetes are heterotrophic group in nature. Most of them are strict saprophytes, while some parasitic or mutualistic association with plants and animals. They are aerobic and most of them readily grow on the common bacteriological media like

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Number of Antibiotics Produced By Major Group Of Microorganisms[2]

Taxonomic groups	Number of antibiotics
Bacteria other than *actinomycetes*	950
Actinomycetes	4600
Fungi	1600

Above table showed that most of the drug mainly produced by Actinomycetes.

Important Microbes Producing Antibiotics [3]

S. No.	Name of microorganism	Name of antibiotics
1	*P. notatum*	Penicillin
2	*P. griseofulvum*	Griseofulvin
3	*P. chrysogenum*	Penicillin
4	*S. griseus*	Streptomycin
5	*S. venezuelae*	Chloramphenicol
6	*S. aureofacns*	Chlortetracycline
7	*S. virdofaciens*	Aureomycin
8	*S. rimosus*	Oxytetracycline
9	*S. texas*	Tetracycline
10	*S. aureofaciens*	Dimethyl-chlortetracycline
11	*S. erythricas*	Erythromycin
12	*S. halstedii*	Carbamycin
13	*S. ambofaciens*	Ravomycin
14	*S. noursei*	Nystatin
15	*S. griseus*	Cycloheximide

Nutrient Agar
Trypticase Agar
Blood Agar
Starch Casein Agar
Albumin Agar etc

Needs for New Medicines[2]

Nowadays human is facing great harming due to different diseases because a number of microbes got resistance against the available drugs.

These products have been exploited for human use for thousands of years, and plants have been the chief source of compounds used for medicine. Even today the largest users of traditional medicines are the Chinese, with more than 5,000 plants and plant products in their pharmacopoeia In fact, the world's best known and most universally used medicine is aspirin (salicylic acid), which has its natural origins from the glycoside salicin which is found in many species of the plant genera *Salix* and *Populus*. Examples abound of natural-product use, especially in small native populations in a myriad of remote locations on Earth. For instance, certain tribal groups in the Amazon basin, the highland peoples of Papua New Guinea, and the Aborigines of Australia each has identified More recently, the Benedictine monks (800 AD) began to apply *Papaver somniferum* as an anesthetic and pain reliever as the Greeks had done for years before Many people, in past times, realized that leaf, root, and stem concoctions had the potential to help them. These plant products, in general, enhanced the quality of life, reduced pain and suffering, and provided relief, even though an understanding of the chemical nature of bioactive compounds in these complex mixtures and how they functioned remained a mystery.

Scope of Herbal Drugs

India can play a major role in the coming years in the global market for herbal products based medicines, since there is a growing demand for plant based medicines and cosmetics, since pharmaceutical industry is plagued with increased cost of new drug development coupled with low serum rate. Scientific validation quality, quantity, consistency and good marketing network are quite essential for the growth of herbal plant industry in India. The absence of these in the country has affected growth of medicinal plant industry in the country. India has a big potential for the cultivation of herbal plants.

Both China and India share 38% in marketing of medicinal plants worldwide. While China's turnover in medicinal plants has been Rs.22,000crore, India's business is hardly about Rs.450 crore. In fact the country has a rich collection of medicinal plant in Uttaranchal, Himalayas, Kerala and North Eastern States, but hardly some medicinal plants have been marketed here.

As against a demand of 35,000 tonnes of medicinal plants, the supply is around 5000 tonnes. While 98% of herbal plants depending upon forest production.

In some Asian and African countries, 80% of the population depends on traditional medicine for primary health care.

Herbal medicines are the most lucrative form of traditional medicine, generating billions of dollars in revenue. Traditional medicine can treat various infectious and chronic conditions: new antimalarial drugs were developed from the discovery and isolation of artemisinin from *Artemisia annua* L., a plant used in China for almost 2000 years.

Counterfeit, poor quality or adulterated herbal products in international markets are serious patient safety threats.

More than 100 countries have regulations for herbal medicines.

Traditional medicine is the sum, total of knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures that are used to maintain health, as well as to prevent, diagnose, improve or treat physical and mental illnesses. Traditional medicine that has been adopted by other populations (outside its indigenous culture) is often termed alternative or complementary medicine. Herbal medicines include herbs, herbal materials, herbal preparations, and finished herbal products that contain parts of plants or other plant materials as active ingredients.

Who uses traditional medicine?

In some Asian and African countries, 80% of the population depends on traditional medicine for primary health care. In many developed countries, 70% to 80% of the population has used some form of alternative or complementary medicine (e.g. acupuncture). Herbal treatments are the most popular form of traditional medicine, and are highly lucrative in the international marketplace. Annual revenues in Western Europe reached US$ 5 billion in 2003-2004. In China sales of products

Identification of different actinomycetes
These are the following photographs showing the samples collected-

Figure 1: Showed sample collected

Figure 2: Showed sample collected

Figure 3: Showed sample collected

Figure 4: Showed actinomycetes growth on Starch Casein Agar media

There were 3 strains isolated from different soil nutrient media, after 14 days of inoculation of Nutrient media.

S.No.	Colour of Colony	Code
1	Yellow Colony	AS1
2	Dark Yellow Colony	As2
3	Milky White Colony	AS3

totaled US$ 14 billion in 2005. Herbal medicine revenue in Brazil was US$ 160 million in 2007.

Challenges

Traditional medicine has been used in some communities for thousands of years. As traditional medicine practices are adopted by new populations there are challenges.

The possibilities for developing new drugs from forest resources should figure heavily in any calculation of the forests true worth. All 119 plants derived drugs, used worldwide in 1991, came from fever than go of the 250, 000 plants species that have been identified, each such plant is a unique chemical factory as correctly mentioned by Norman R. Faransworth of the university of Illinois at Chicago, that are capable of synthesizing unlimited numbers of highly complex and unusual chemical substances whose structures could otherwise escape the imagination, scientist may be able to synthesize, these plants compounds in the laboratory, but dreaming them up, rather than plucking then from the forest and then replicating them is quite different.

The credit for having first recognized the ability of actinomycetes to destroy microbial cells is generally given to Gasperini (1890), who observed, in the course of his classical researches on Streptothrix foersteri Cohn, that the filaments of this organism may destroy the cell-membrane of several bacteria and fungi.

MATERIALS AND METHODS

Collection of sample: The different samples of soil sediments were collected from river Krishna and Godaveri after making 2 cm depth and stored in sterile polybags.

Sterilization of polybags : Airtight polybags were purchased from market and these were sterilized after application of ethyl alcohol and keeping into U.V. light for 5 minutes.

The actinomycetes strain AS1,AS2 and AS3 showing growths on different types of ISP media are shown in table as follows

Media	AS1	AS2	AS3
ISP2	+	+	+
ISP4	+	+	-
ISP5	+	-	+
ISP6	+	+	-
ISP7	-	+	+

All three actinomycetes were Violet in colour it means these showed Gram,s +ve in nature.

Our research showed that river soil are suitable fot the growth of Actinomycetes.

Storing of sediments: Collected soil sediments first about 20 gm were kept in polybags and stored in refrigerator.

Preparation of samples for isolation of actinomycetes: The different soil samples were taken and these were serially diluted upto 10^{-6}. Each of sample were prepared using different testube.

Isolation of actinomycetes by using different nutrient media

The ingredients of media were accurately weighed for the each 500 ml of the three type's media i.e.

Starch Casein Agar Media

YMA Media

Albumin Media

Weighed ingredient were dissolved in required quantity of distilled water and sterilized at 121 °C (15 lbs) for 15 min by using autoclave. After sterilization the antifungal Nystatin was added (50 µg/ml) then media were poured into Petri dishes under sterile condition (laminar air flow) and allow cooling for sufficient time for the solidification of media.

The surface sterilized plant parts were taken and crushed using sterile pestle and mortar and spread on the three of the media and kept at 28 °C for 2-3 weeks, growth of microbes were observed each day and produced actinomycetes colony were purified on the Petri dishes using streak methods on the same media.

Identification of different actinomycetes

Growth on different ISP media

Media composition were weighed and dissolved in water and sterilized at 121°C (15 lbs) for 15 min by using autoclave. After sterilization the media were poured into Petri-dishes under sterile condition (laminar air flow) and allow cooling for sufficient time for the solidification of media and after solidification isolated microbes were streaked on solidified media in zigzag fashion and kept for incubation in incubator at 37°C for about 24 hrs.

Gram's staining

The microbes' smears were taken on glass slide.

The smears were air dried.

Smears were covered with crystal violet for 30 seconds.

Covered each smear with Gram's Iodine solution for 60 seconds.

Washed off Iodine solution with 95% ethyl alcohol, ethyl alcohol was added drop by drop until no more colour flows from the smear.

The slides were washed with distilled water and drain.

Safranin was applied to smears for 30 seconds (counter staining).

The slides were washed with distilled water and blot dried with absorbent paper.

Let the stained slides air dry.

The slides were examined under microscope.

RESULTS AND DISCUSSION

Actinomycetes isolates: There were 3 types of the actinomycetes were isolated (given code) from different soil sediments on the three of nutrient media given in table 3 and these were identified a 3 actinomycetes. I

Growth of Actinomycetes strain on different ISP Media.

The actinomycetes strains isolated from soil sediments (AS1, AS2 and AS3) were allowed to grow on the different types of ISP (International *Streptomyces* Project) media as follows.

a. ISP-2
b. ISP-4
c. ISP-5
d. ISP-6
e. ISP-7

REFERENCES

1. Librado OO, Luis FB, Veronica Y. Biological, Biochemical, and Biomedical Aspects of Actinomycetes. Academic Press New York; 1984: 453.
2. Hung PQ, Kumar SM, Govindsamy V and Annapurna K. Isolation and characterization of Endophytic bacteria from wild and cultivated soybean varieties. Journal of Biol Fertil Soils. 2007; 44: 155–162.
3. Sivakumar K. Centre of advanced study in marine biology. Annamalai University: 197-204.
4. Debananda S, Ningthoujam, Suchitra S, and Salam N. Screening of Actinomycete Isolates from Niche Habitats in Manipur for Antibiotic Activity. American Journal of Biochem and Biotech. 2009; 5: 221-225.
5. Kavya D, Solomon S, Nagalakshmi D. Isolation and screening of *Streptomyces* sp. from coringa mangrove soils for enzyme production and antimicrobial activity. International journal of Pharmaceutical, chemical and biological sciences, 2012; 2:110-116.
6. Dharna S, Anjali S. Isolation and screening of *actinomycetes* from mangrove soil for enzyme production and antimicrobial activity. International Journal of Research and Scientific Innovation,2016; 2321–2705.
7. Silambarasan S, Praveen kumar E, Murugan T, Saravanan D, Balagurunathan R. Antibacterial and antifungal activities of actinobacteria isolated from Rathnagiri hills. Journal of Applied Pharmaceutical Science; 2012; 2: 099-103.
8. Kaur S, Kaur HP, Kaur G. Isolation and characterization of antibiotic producing *actinomycetes* from agriculture soil. World Journal Of Pharmacy And Pharmaceutical Sciences; 5: 1109-1117.
9. Gebreselema G, Feleke M, Samuel S, Nagappan R. Isolation and characterization of potential antibiotic producing actinomycetes from water and sediments of

Lake Tana, Ethiopia. Asian Pac J Trop Biomed. 2013; 3): 426-435.

10. Pradhan S, Mishra BB, Rout S. Screening of novel actinomycetes from near lake shore sediment of the chilika lake, Odisha, India.International Journal of Current Micobiology and Applied Science, 2015;4:66-82.
11. Lisboa MP, Bonatto D, Bizani D, Henriques J and Brandelli AP. Characterization of a Bacteriocin- like Substance produced by *Bacillus amyloliquefaciens* isolated from the Brazilian Atlantic Forest. Journal of International Microbiology, 2006; 9:111-118.
12. Hung PQ, Kumar SM, Govindsamy V and Annapurna K. Isolation and characterization of Endophytic bacteria from wild and cultivated soybean varieties. Journal of Biol Fertil Soils. 2007; 44: 155–162.
13. Tautorus T E and Townsley P M. Analysis of an Effective Antibiotic (Chaetomacin) Isolated from a Thermophilic Bacillus sp.Against Olive Green Mold. Applied and environmental microbiology; 1984:775-779.
14. Augustine SK, Bbhavsar SP and Kapadnis BP. A non-polyene antifungal antibiotic from *Streptomyces albidoflavus PU 23*. Journal of Biosciences. *2005;* 30: 201–211.