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Screening of Endophytic Actinomycetes from Different Indigenous Medicinal Plants

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Abstract- ***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.***
Ayurveda, is the ancient science of life.
New chemical entities are requested in order to enable therapeutic innovation. Combinational chemistry and exploiting structural diversity derived from natural sources contribute to improved lead discovery. In particular low molecular mass natural products from bacterial fungi and plants either from terresteric, or marine environments perform unique structural diversity.
The greatest variety of antibiotics is produced by Actinomycetes among all microbes. More than 50% of the known natural antibiotics are produced by Actinomycetes. Actinomycetes are a diverse group of heterotrophic prokaryotes forming hyphae at some stage of their growth hence referred to as filamentous prokaryotes. They have different morphological, cultural, biochemical, and physiological characteristics. This group is a potential producer of many enzymes, enzyme inhibitors, growth-promoting substances, and antibiotics. In our study Collected plant parts like leaves, fruits, twigs, etc. were first rinsed with sterilized water then plant parts were kept in a sterile beaker consisting of 70% ethanol for 3-5 min. After that, the ethanol sterilized plant parts were kept in a beaker consisting of Sodium hypochlorite (5%) with tween 20(0.1%) for 5 minutes. At last plant, parts were rinsed with sterilized water. The surface sterilized plant parts like leaves, twigs, fruits, etc. were taken and crushed using a sterile pestle and mortar and spread on the different nutritional media like starch Casein agar, Albumin media, and YMA media. consisting of antifungal agent Nystatin 50 µg/ ml, by pour plate technique. The plates will be incubated at different temperatures for up to 7-10 days, for growth of actinomycetes were observed each day, and produced actinomycetes colony were purified on the Petri dishes using streak methods on the same media. A total of 8 actinomycetes were isolated from different plants on different media. The potent actinomycetes were characterized by morphological and biochemical methods.

Keywords— *Endophytica Actinomycetes, Plant parts, Surface Sterilization, Nutrient Media, Identification.*

Introduction

Natural products are naturally derived metabolites and/or by-products from microorganisms, plants, or animals. 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.[1]

Actinomycetes

These are the organisms with characteristics common to both bacteria fungi but yet possessing distinctive features to delimit them into a distinct category. In the strict taxonomic sense, actinomycetes are clubbed with bacteria the same class of Schizomycetes and confined belonging to the important order of Actinomycetales.

They are unicellular like bacteria, but produce a mycelium which is non-septate (coenocytic) and more slender, tike true bacteria they do not have distinct cell-wall and their cell wall is without chitin and cellulose (commonly found in the cell wall of fungi). On culture media unlike slimy distinct colonies of true bacteria which grow quickly, actinomycetes colonies grow slowly, show powdery consistency and stick firmly to agar surface. They produce hyphae and conidia / sporangia like fungi. Certain actinomycetes whose hyphae undergo segmentation resemble bacteria, both morphologically and physiologically.

Actinomycetes[1,8] are numerous and widely distributed in soil and are next to bacteria in abundance. They are widely distributed in the soil, compost etc. Plate count estimates give values ranging from 10^4 to 10^8 per gram of soil. They are sensitive to acidity / low PH (optimum PH range 6.5 to 8.0) and waterlogged soil conditions. The population of actinomycetes increases with depth of soil even up to horizon 'C' of a soil profiler They are heterotrophic, aerobic and mesophilic (25-30 ^{O}C) organisms and some species are commonly present in compost and manures are thermophilic growing at 55-65°C temperature (eg. Thermoatinomycetes, Streptomyces).

Actinomycetes belonging to the order of Actinomycetales are grouped four families viz Mycobacteriaceae, Actinomycetaceae, Streptomycetaceae and Actinoplanaceae.

Actinomycetous genera which are agriculturally and industrially important are present in only two families of Actinomycetaceae and Strepotmycetaceae.

Functions /Role of actinomycetes:

1. Degrade/decompose all sorts of o rganic substances like cellulose, polysaccharides, protein fats, organic-acids etc.
2. Organic residues / substances added soil are first attacked by bacteria and fungi and later by actinomycetes, because they are slow in activity and growth than bacteria and fungi.
3. They decompose / degrade the more resistant and indecomposable organic substance /matter and produce a number of dark black to brown pigments which contribute to the dark colour of soil humus.

4. They are also responsible for subsequent further decomposition of humus (resistant material) in soil.

5. They are responsible for earthy / musty odor / smell of freshly ploughed soils.

6. Many genera species and strains (eg. Streptomyces if actinomycetes produce /synthesize number of antibiotics like Streptomycin, Terramycin, Aureomycin etc.

7. One of the species of actinomycetes *Streptomyces scabies* causes disease "Potato scab" in potato.

Nowadays efforts are being made to explore rare actinomycetes like

Actinomadura

Actinoplanes

Actinosynnema

Dactylosporangium

Kibdilosporangium etc.

Actinomycetes are diverse group of heterotrophic prokaryotes forming hyphae at some stage of their growth hence refereed as filamentous prokaryotes. They have been different morphological, cultural, biochemical and physiological characters. This group is a potential producer of many enzymes, enzyme inhibitors, growth promoting substances 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

Nutrient Agar

Trypticase Agar

Blood Agar

Starch Casein Agar

Albumin Agar etc

MATERIALS AND METHODS:

Collection of sample:

The different parts of plants were collected from different regions of India and stored into sterile plastic bags and were kept in aseptic condition.

There were following plant samples collected -:

➢ *Catharanthus rosea -: I. Twig*

II. Roots

III. Leaves

➢ *Brassica sps.*

I. Fruits

II. Leaves

III. Twig

➢ *Calotropis procera*

I. Fruits

II. Leaves

III. Twig

➢ *Eugenia caryophyllus*

I. Fruits

➢ *Emblica officinalis*

I. Twig

II. Fruit

III. Leaves

2. SURFACE STERILIZATION Collected plant parts like leaves, fruits, twig etc. were first rinsed with sterilized water then plant parts were kept in sterile beaker consists of 70% ethanol for 3-5 min. After that the ethanol sterilized plant parts were kept in beaker consist of Sodium hypochlorite (5%) with tween 20(0.1%) for 5 minutes. At last plant parts were rinsed with sterilized water.

3. ISOLATION OF ACTINOMYCETES BY USING DIFFERENT NUTRIENT MEDIA

The ingredients of media were accurately weighed for the each 1000 ml of the following type's media i.e.

- Starch Casein Agar Media
- YMA Media
- Albumin Media

Accurately weighed ingredients of different media were dissolved in required quantity of distilled water and sterilized at 121^{0}C (15 lbs) for 15 min by using autoclave. After sterilization the antifungal Nystatin was added (50µg/ml) then different media were poured into Petri dishes under sterile condition (laminar air flow) and allowed for cooling for sufficient time for the solidification of media.

The surface sterilized plant parts like leaves, twig, fruits etc. were taken and crushed using sterile pestle and mortar and spread on the three of the media and kept at 28^{0}C for 2-3 weeks, growth of actinomycetes were observed each day and produced actinomycetes colony were purified on the Petri dishes using streak methods on the same media.

Biochemical Test

Certain biochemical test were performed for identification of different strains producing antibacterial compound.

- **Melanoid Formation Test**

- 0.5 gm L-tyrosine was Suspended in 10 ml distilled water in culture tube mixed thoroughly by vortexing and autoclave at 121^{0}C (15 lbs) for 15 min.
- 100ml base was combined (Beef extract-3 gm, Peptone-5 gm, Agar-15 gm, distilled water-1000 ml), mixed thoroughly by gentle rotation of bottle 2 or 3 times.
- Aseptically 3-5 ml of media was dispensed into tubes with frequent mixing.
- Tubes were cooled rapidly to prevent separation of tyrosine.
- The isolated actinomycetes were inoculated and kept for incubation at 37^{0}C for 4 days.

- **Test for Nitrate Reduction**

- The composition of Organic nitrate broth media weighed and dissolved in required quantity of distilled water and sterilized at 121^{0}C (15 lbs) for 15 min by using autoclave. After sterilization the media were poured into Test tubes under sterile condition (laminar air flow) and allow to cool.
- The isolated actinomycetes were inoculated and kept for incubation at 37^{0}C for 4 days.
- After incubation, 1 ml of broth was taken and added 2 drops of alpha- naphthalene, 2-3 drops of H_2SO_4 and observed for any change in colour.

- **Test for Acid Production**

- The composition of Glucose nutrient broth media were weighed and dissolved in required quantity of distilled water and sterilized at 121^{0}C (15 lbs) for 15 min by using autoclave. After

sterilization the media were poured into Test tubes under sterile condition (laminar air flow) and allow to cool.

- The isolated actinomycetes were inoculated and kept for incubation at 37^0C for 4 days.
- The broth was checked daily for any change in colour.

- **Hydrogen Sulphide Production Test**

- The composition of SIM media were weighed and dissolved in required quantity of distilled water and sterilized at 121^0C (15 lbs) for 15 min by using autoclave.
- After sterilization the media were poured into Test tubes under sterile condition (laminar air flow) and allow to cool.
- Inoculated the isolated actinomycetes into its appropriately labeled tube by means of stab inoculation.
- Incubated the inoculated tubes at 35^0C.for about 48 hrs.
- Examined the tubes for the presence or absence of black coloration along the line of stab inoculation.

RESULTS AND DISCUSSION

Nearly 8 types of actinomycetes strains were isolated from different plant samples on different isolation media.

The following plant parts were collected from different regions of Uttar Pradesh as follows.

- *Catharanthus rosea*
- *Calotropis procera*
- *Brassica sps.*
- *Eugenia caryophyllus*
- *Emblica officinalis*

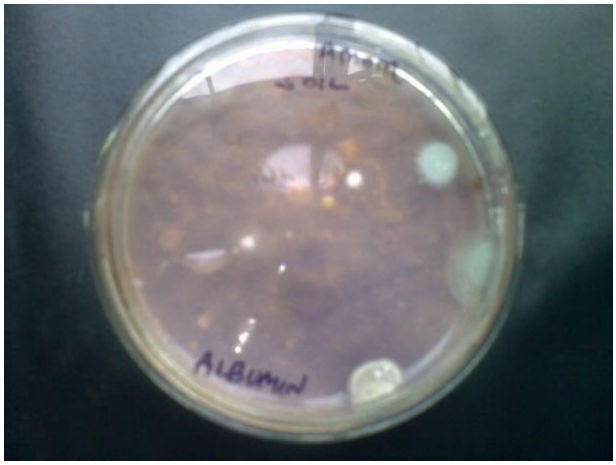

Fig 1: Showed actinomycetes growth on Albumin media

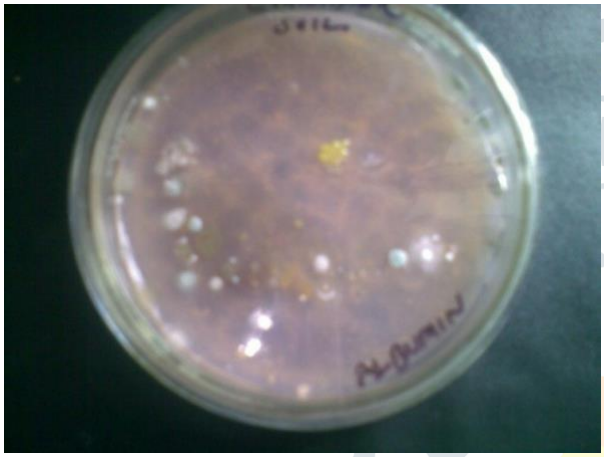

Fig 2: Showed actinomycetes growth on Albumin media

S.No.	Name of the plants	Nutrient Media	No. of Endophytic actinomycetes isolated
	➢***Catharanthus rosea -:***		
1.	*IV. Twig* *V. Roots* *VI. Leaves*	Albumin	3 actinomycetes
2.	➢*Brassica sps.* *I. Fruits* *II. Leaves III. Twig*	Albumin	5 actinomycetes
3.	➢*Calotropis procera* *I. Fruits* *II. Leaves III. Twig*	Starch Casein Agar Media	0
4.	➢*Eugenia caryophyllus* *I. Fruits*		0
5.	➢*Emblica officinalis* *I. Twig* *II. Fruit* *III. Leaves*		0

Table No.1. Shows different types of actinomycetes strains(CR1,CR2, CR3,BS1, BS2, BS3,BS4 and BS5) showing the growth on different nutrient media

Media	CR1	CR2	CR3	BS1	BS2	BS3	BS4	BS5
ISP2	+	-	-	-	-	+	+	+
ISP4	+	+	+	+	+	-	+	-
ISP5	+	+	+	+	-	-	-	+
ISP6	-	-	+	+	+	-	-	+
ISP7	-	-	-	+	-	+	-	+
ISP8	-	+	+	-	+	-	-	-

Table No.2. Shows different types of actinomycetes strains (CR1,CR2, CR3,BS1, BS2, BS3,BS4 and BS5) showing the growth on different ISP media.

The +ve sign indicate that these actinomycetes grow well on ISP media and –ve sign indicate that actinomycetes are unable to grow on the ISP media because of their culture characteristics of growth on different media.

BIOCHEMICAL TEST

There are certain biochemical test were performed for the identification of 5 strains of actinomycetes producing anti-bacterial compound given as-

- **MELANOID FORMATION**

The actinomycetes producing anti-microbial compound isolated from different soil samples showed different results, given in Table 4

- **TEST FOR NITRATE REDUCTION**

The actinomycetes producing anti-microbial compound isolated from different soil samples showed different results, given in Table No.5

- **TEST FOR ACID PRODUCTION**

The actinomycetes producing anti-microbial compound isolated from different soil samples showed different results, given in Table No.6.

Actinomycets strain	ISP-2		ISP-4	
	Colour of mycelium	Reverse Side Colour	Colour of mycelium	Reverse Side Colour
S1	-	-	-	-
S2	-	-	Yellow	Dark brown
S3	-	-		
S4	-	-	-	-
S5	-	-	-	-
S6	White	Brown	White	Brown
S7	-	-	-	-
S8	White	Brown	Yellow	Brown

Table No.3. Shows the colour of mycelium and culture media on ISP-2 and ISP-4 media.

The –ve sign indicate that actinomycetes are unable to grow on the ISP media because of their culture characteristics of growth on different media

Actinomycetes strain	ISP-5		ISP-6	
	Colour of mycelium	Reverse Side Colour	Colour of mycelium	Reverse Side Colour
S1	Orange	Yellow	-	-
S2	White	Brown	Orange white	Orange
S3	Yellow	Dark brown	Yellow	Dark brown
S4	-	-	-	-
S5	Yellow	White	orange	Yellow
S6	-	-	White	Brown
S7	Yellow	White	-	-
S8	-	-	-	-

Table No.4. Shows the colour of mycelium and culture media on ISP-5 and ISP-6 media.

The –ve sign indicate that actinomycetes are unable to grow on the ISP media because of their culture characteristics of growth on different media

Actinomycetes strain	ISP-7	
	Colour of Mycelium	Reverse Side Colour
S1	Yellow	White
S2	-	-
S3	White	Dark brown
S4	Orange	Brown
S5	White	White
S6	-	-
S7	-	-
S8	-	-

Table No.5.Shows the colour of mycelium and culture of media on ISP-7.

The –ve sign indicate that actinomycetes are unable to grow on the ISP media because of their culture characteristics of growth on different media

Table No.6. Shows the Melanoid formation test.

Actinomycetes strain	Reaction
S1	+ve
S2	+ve
S3	+ve
S4	-ve
S5	-ve
S6	+ve
S7	+ve
S8	-Ve

Table No. 7. Shows Nitrate reduction test.

Actinomycetes strain	Reaction
S1	-ve
S2	-ve
S3	-ve
S4	-ve
S5	-ve
S6	+ve
S7	-ve
S8	+ve

Table No.8. Shows Acid production test.

Actinomycetes strain	Reaction
S1	-ve
S2	-ve
S3	-ve
S4	-ve
S5	-ve
S6	+ve
S7	+ve
S8	-Ve

Table No. 9.Shows Hydrogen sulphide test.

Actinomycetes strain	Reaction
S1	-
S2	+
S3	+
S4	+
S5	-
S6	+
S7	-
S8	-

CONCLUSION:

Our study prove that actinomycetes may be isolated from different soil samples surrounding the medicinal plants and which may be further study for the production of different useful compounds like Antibiotics, Enzymes and Xenobiotics etc.

REFERENCES:

1. Ozgur C, Gulten O and Aysel U. Isolation of soil *Streptomyces* as source antibiotics active against antibiotic-resistant bacteria. Eur Asian Journal of BioSciences.2008; 2: 7382

2. Chi NS, Ji H C, and Keun S B. An Improved selective isolation of rare actinomycetes from forest soil. The Journal of Microbiology. 2001: 17-23

3. Mustafa O. Comparison of *Streptomyces* diversity between agricultural and nonagricultural soils by using various culture media. Scientific Research and Essay. 2009; 4:
997-1005

4. Sivakumar K. Centre of advanced study in marine biology. Annamalai University : 197-204

5. Lo CW, Lai NS, Cheah HY, Wong NKI. and Ho CC. Actinomycetes isolated from soil samples from the crocker range sabah. ASEAN Review of biodiversity and environmental conservation; 2002: 1-7.

6. Singh SL and Bora T C. Actinomycetes of Loktak habitat.Isolation and screening of antimicrobial activity. Biotechnology.2006; 5: 217-221.

7. Deepika L and Krishnan T. A report on antidermatophytic activity of actinomycetes. IIJB. 2009: 132-136.

8. Manjula C, Rajguru P and Muthuselvam M. Screening for antibiotic sensitivity of free and immobilized actinomycetes isolated from India. Advances in Biological Research.
2009; 3: 84-88.

9. Srivibool R, Kurakami K, Sukchotiratana M and Tokuyama S. Coastal soil actinomycetes:Thermotolerant strains producing *n*-acylamino acid racemase. *ScienceAsia.* 30 ;2004: 123-126

10. Sultan MZ, Khatune NA, Sathi ZS, Md. Shah and Bhuiyan A. Biotechnology. 2002; 1: 100-106.

11. Jongrungruangchok S, Tanasupawat S, Kitakoop P, Bavovada R, Kobayashi H, and Kudo T. Identification of *streptomyces* and *kitasatospora* strains from thai soils with geldanamycin production strain. Actinomycetologica. 2006: 20:10–14.

12. Igarashi Y, Miura S, Fujita T and Furumai T. Pterocidin :A Cytotoxic Compound from the *Endophytic Streptomyces hygroscopicus*. Journal of Antibiotics. 2006; 59: 193– 195.

13. Wenhan L, Liya Li, Hongzheng Fu, Isabel S , Xueshi H and Susanne G. New Cyclopentenone Derivatives from an Endophytic *Streptomyces* sp. Isolated from the Mangrove Plant *Aegiceras comiculatum.* J. Antibiot. 2005; 58: 594–598.

14. Gary S and Bryn D. Bioprospecting for Microbial Endophytes and Their Natural Products. Microbiology and molecular biology reviews. 2003;4: 491–502.

15. Oskay M, Tamer A. U and Azeri C. Antibacterial activity of some actinomycetes isolated from farming soils of Turkey. African Journal of Biotechnology. 2004; 3: 441446.