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ANTIMICROBIAL ACTIVITY OF ACTINOMYCETES ISOLATED FROM RHIZOSPHERE SOIL OF INDIAN MEDICINAL PLANTS AGAINST HUMAN SKIN PATHOGENS

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ABSTRACT

Actinomycetes also called as "actinobacteria" are primarily distributed in natural ecosystems all over the world. The aim of this study is to search antimicrobial activity of actinomycetes isolated from some Indian medicinal plants rhizosphere soil samples. Total five rhizosphere soil samples of medicinal plants viz; *Aloe barbadense*, *Emblica officinalis*, *Zingiber officinale*, *Eucalyptus camaldulensis* and *Santalum album*. These medicinal plants were collected from local area at Barshi.Dist-Solapur, M.S.India. Total thirty-two isolates were obtained and studied by morphologically, culturally and biochemically. All isolates were identified as actinomycetes with the help of MICRO-IS Software and also few by 16rRNA analysis. All these isolates were belonged to *Streptomyces, Streptoverticillium, Nocardia, Micromonospora* and *Micropolyspora*. The antimicrobial activity were tested against standard culture of human skin pathogens such as *Candida albicans, Acinetobacter baumannii and Aspergillus niger*. All actinomycetes were tested for their antimicrobial activity against all pathogens by agar overlay technique. *Streptomyces enissocaesilis* (AL3) isolated from *Aloevera barbadense* was showed antimicrobial activity against *Candida albicans*, *Acinetobacter baumannii* and *Aspergillus niger*. *Streptomyces chattanoogensis* (SD3) isolated from *Santalum album* was showed maximum zone of inhibition against *Candida albicans*.

KEYWORDS: Indian medicinal plants, antimicrobial activity, human skin pathogens.

INTRODUCTION

Actinomycetes are a diverse group of Gram positive bacteria with high amount of G + C in their DNA. They are unicellular organisms found in terrestrial ecosystems. Actinomycetes are known as producers of antibiotics and other metabolites (Challis and Hopwood, 2003). They can produce secondary metabolites (with antifungal and antibacterial properties). Antibiotics are the substance that inhibit the growth of microorganisms (anti-metabolites) or their replication (a bacteriostatic effect). They were obtained by extracting them from cultures of microbes.

Human skin covers the entire external surface of the human body which contains hundreds of bacteria per square inch of skin. The majority of skin microbes are found in the first few layers of the epidermis and in upper region of the hair follicle. The common skin microflora are found in and on skin such as *Escherichia coli*, *Pseudomonas aeruginosa, Staphylococcus, Corynebacterium, Micrococcus*, *Mycobacterium* and *Pityrosporum*. These microflora normally known as resident of skin and it can also be a protective mechanism. They live on skin but at certain condition, they cause primary infection and in major case it converts into disease. The skin infection and disease causing bacteria and fungi can be passed on by touch to someone else directly or to a surface such as *Candida albicans*, *Acinetobacter baumannii* and *Aspergillus niger*.

Acinetobacter baumannii is an opportunistic pathogen in humans. It is commonly found in burn wounds. This bacterium spreads through in short distance air droplets, through skin outbreaks and transmit by direct contact with skin (touching). Now a days, it become a serious problem due to its multidrug resistance mechanism. The most common cause of fungal infection is a member of the genus Candida especially, the species *Candida albicans*. This fungus found on or in the human bodies, especially in the mouth and gastrointestinal system (Candidiasis).A number of other fungi cause other type of infections such as member of the genus Aspergillus and causes cutaneous aspergillosis infection (areas such as nail bed, eyes skin and ear canals). The infection is more if spores inhaled and in rare cases via the skin (Primary cutaneous aspergillosis). The infection primarily involving skin injury, burns or surgery. Infectious diseases are still the second leading cause of death worldwide (Luzhetskyy *et. al*, 2007).

In India, Ayurvedic system of medicine has its long history of therapeutic potential in which plants have been a valuable source of natural products. According to the World Health Organization (Santos *et., al*, 1995) medicinal plants would be the best source to obtain a variety of drugs. Many microbes are commonly live with plant root. The rhizosphere soil microbial community provides chemical compounds which are important by qualitatively and quantitatively. Many plants have been studied for their antimicrobial properties and some of them resulted in development of drugs (Patil and Ramajah, 2004).Now a days, drug discovery (for novel compounds) is essential due to the resistant pattern of pathogens to antimicrobial compounds. The goal of the present study is to screen the antimicrobial substance producing actinomycetes from rhizosphere soil of selected Indian medicinal plants and to test the antimicrobial activity against disease causing, multidrug resistant bacterial and fungal pathogens.

MATERIALS AND METHODS

MATERIALS

Rhizosphere soil samples from selected medicinal plants viz; *Aloevera barbadense, Emblica officinalis, Eucalyptus camaldulensis, Santalum album* and *Zingiber officinale.*

Glycerol asparagine agar.

Nutrient or Sabouraud agar (0.7%).

Chloroform (1.5 ml for each plate).

METHODS

Collection of soil samples and Isolation of Actinomycetes

Rhizosphere soil of medicinal plants viz; *Aloevera barbadense, Emblica officinalis, Eucalyptus camaldulensis, Santalum album* and *Zingiber officinale* were selected from local area around Barshi, Dist.-Solapur, M.S.India. The soil samples were collected at 10-20 cm depth from selected medicinal plants. For the isolation of actinomycetes, five soil samples were collected and stored in sterile ziploc bag. These soil samples were suspended in sterile distilled water (1g soil sample +100ml D/W). From this, 0.1 ml was transferred to glycerol asparagine agar (L-asparagine 0.1g, K_2HPO_4 -0.1g, glycerol-1gm, trace salt solution-0.1ml,agar-2.5,distilled water-100ml,pH-7.4).After incubation of 4-7 days, the powdery colonies were selected and transferred to glycerol asparagine agar slants. These pure culture slants were preserve at 4^0 C and used for further study.

Identification of actinomycetes isolates

The selected isolates were studied their morphological features, cultural characteristics as well as biochemical chararacteristics and identified as actinomycetes by MICRO-IS Software and few also by 16rRNA phylogenetic analysis.

Skin pathogens

Culture of test organisms such as *Acinetobacter baumannii, Candida albicans* and *Aspergillus niger* were obtained from local area hospital source, confirmed and used for further study.

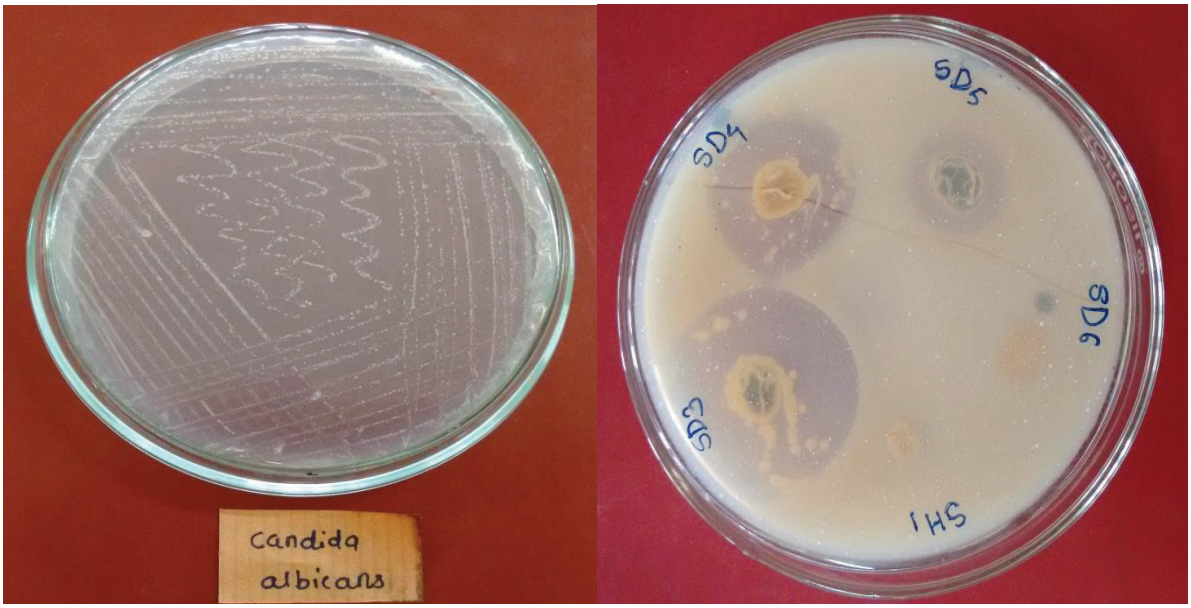
Antimicrobial test

Antimicrobial test was carried out using agar overlay technique in which isolates were spot inoculated on glycerol asparagine agar and petri plates were kept for incubation for 4-7 days. After incubation period, the growth of isolates were killed by inverting the plates over petri dish containing 1.5 ml chloroform for 40 min. After that, the excess chloroform vapours were removed and the surface was overlaid with 5 ml of 0.7 % nutrient agar (Peptone-1g, NaCl-0.5g, Yeast extract-0.3, agar -0.7 g, distilled water-100ml, pH- 7) for bacteria and sabouraud agar (Dextrose-4.0g, Peptone-1.0g, agar-0.7g,distilled water-100ml,pH-5.6) for fungi, incubated with 0.2 ml of suspension containing bacteria or fungi test organisms. After the incubation period, minimum 24 hrs. for bacteria and maximum 48 hrs.for fungi, the zone diameter of inhibition around the growth of the isolates were observed, measured in mm and recorded as sensitive.

RESULTS AND DISCUSSION

In present study, total thirty-two isolates were obtained from five rhizosphere soil of medicinal plants viz; *Aloevera barbadense, Emblica officinalis, Eucalyptus camaldulensis, Sanctalum album* and *Zingiber officinale.* All isolates were labelled as per their vernacular name *viz*; *Aloe barbadens* (Aloe Vera- AL), *Emblica officinalis* (Avala-AV), *Eucalyptus camaldulensis* (Nilgiri-NI), *Santalum album* (Sandal–SD) and *Zingiber officinale* (Ginger-GI). Total thirty-two isolates were obtained, studied for their characteristics (morphological cultural and biochemical) and identified as actionomycetes with the help of MICRO-IS Software. Among these, the isolates belonged to *Streptomyces, Streptoverticillium, Nocardia, Micromonospora* and *Micropolyspora*.

The test pathogens were obtained, confirmed (Gram staining), labelled and used for the study of antimicrobial activity test.The selective media were used for the isolation and maintenance of the pure culture of test pathogens. The isolation of test organism (*Candida albicans*) is shown in plate (i). The antimicrobial activity of *Streptomyces chattanoogensis* (SD3) and *Streptomyces aureofaciens* (SD4) isolated from *Santalum album* (Sandal) against *Candida albicans* are shown in Plate (ii).



(i) **(ii)**

Plate (i) – Isolation of *Candida albicans*.

Plate (ii) -Antimicrobial activity of actinomycetes isolated from *Santalum album* (Sandal) against *Candida albicans*.

Out of thirty- two isolates, twenty-two actinomycetes were showed zone of inhibition around the isolates. Out of twenty-two isolates thirteen isolates were showed the fungal activity to *Candida albicans*, among these three isolates from *Aloevera barbadense*, two isolates from *Emblica officinalis*, two isolates from *Zingiber officinale*, three isolates from *Eucalyptus camaldulensis* and three isolates from *Santalum album*. *Streptomyces chattanoogensis* (SD3) isolated from *Santalum album* was showed the maximum zone of inhibition as compared to all thirteen isolates.

Total seven isolates were showed zone of inhibition against *Acinetobacter baumannii*. Out of seven isolates, one isolate from *Aloevera barbadense*, two isolates from *Zingiber officinale*, one from *Eucalyptus camaldulensis* and three isolates from *Santalum album*. *Streptomyces*

fulvissimus isolated from *Zingiber officinale* was showed maximum zone of inhibition as compared to all seven isolates. The remaining two isolates, one isolate from *Aloevera barbadense* and one from *Eucalyptus camaldulensis* were showed antimicrobial activity against *Aspergillus* niger.

Table 1: Antimicrobial activity of actinomycetes.

Sr. No.	Actinomycetes	Zone diameter of inhibition against pathogens in mm		
		Candida albicans	***Acinetobacter baumannii***	***Aspergillus niger***
1.	*Streptomyces enissocaesilis* (AL3)	15 (10)*	20 (6)*	10 (10)*
2.	*Streptomyces antibioticus* (GI3)	30 (7)*	15 (10)*	R
3.	*Streptomyces thermovulgaris* (NI3)	35(10)*	R	15(10)*
4.	*Streptomyces chattanoogensis* (NI5)	10 (10)*	20 (10)*	R
5.	*Streptomyces aureofaciens* (SD4)	30 (11)*	15 (8)*	R

*=Zone of growth of isolates on media.

R= Resistance (No zone around the isolates).

Out of twenty-two sensitive actinomycetes isolates few were showed the antimicrobial activity against all test pathogens viz; *Streptomyces enissocaesilis* (AL3) isolated from *Aloevera barbadense* was showed antimicrobial activity against *Candida albicans*, *Acinetobacter baumannii* and *Aspergillus niger* and recorded as sensitive to all three pathogens.

Streptomyces antibioticus isolated from *Zingiber officinale* was showed antimicrobial activity against *Candida albicans* and *Acinetobacter baumannii* while resistant to *Aspergillus niger*. *Streptomyces thermovulgaris* isolated from *Eucalyptus camaldulensis* was showed zone of inhibition against *Candida albicans* and *Aspergillus niger* but resistant to *Acinetobacter baumannii*. *Streptomyces chattanoogensis* isolated from *Eucalyptus camaldulensis* was sensitive to *Candida albicans* and *Acinetobacter baumannii* while resistant to *Aspergillus niger*. *Streptomyces aureofaciens* isolated from *Santalum album* was sensitive to *Candida albicans* and *Acinetobacter baumannii* but resistant to *Aspergillus niger*.

Actinomycetes are primarily distributed in natural ecosystems around the world (Srinivasan *et., al,* 1991). There are 23,000 microbial synthesized bioactive secondary metabolites over 10,000 are being produced by actinomycetes (Subathra *et. al*, 2012). More than 70% of the naturally derived antibiotics were derived from soil actinomycetes which are in clinical use (Atta *et. al*, 2011). The polyenes (amphotericin B,nystatin, and natamycin) derived from

Streptomyces species have a broad spectrum (*in vitro*) of activity against a wide range of fungi including the *Aspergillus* species and *Candida* species (Hay,2003). They are widely used for the treatment of Candidiasis, histoplasmosis, coccidiodal meningitis and cutaneous dermatophytes. There is continues and urgent need to search new antimicrobial compounds for new re-emerging infectious diseases (Rojas *et. al*, 2003). Hence, this report gives a clear idea about the actinomycetes from medicinal plants rhizosphere soil would be the best source of novel antibiotics for infectious pathogens.

REFRENCES

1. Atta H.M, EI-Sehrawi M.H, Bahobail A.S (2011). Antifungal macrodiode production by *Streptomyces albidoflavus*-143: Fermentation, purification and biological activities. J. Am. Sci, 7(3): 13-22.
2. Challis, G.L. and D.A. Hopwood, (2003).Synergy and contingency as driving forces for the evolution of multiple secondary metabolite production by *Streptomyces* species. Proc. Natl. Acad. Sci. US, 100: 14516-14555. DOI: 10.1073/pnas.1934677100.
3. Hay RJ (2003). Antifungal drugs used for systemic mycoses. Dermatol. Clin, 21: 577-587.
4. Luzhetskyy, A., S. Pelzer and A. Bechthold (2007). The future of natural products as a source of new antibiotics. Current Opinion in Investigational Drugs, 8(8): 608-613.
5. Patil MB and Ramajah PV (2004).Ethnomedicines for human skin diseases from tribal areas of Nandurbar District of Maharashtra, India, In: Proceedings of the National Seminar on Ethnobotany and Sacred Groves, Agharkar Research Institute, Pune, India, 218-222.
6. Rojas R, Bustamante B, Bauer J (2003). Antimicrobial activity of selected Peruvian medicinal plants.J. Ethanopharm, 88: 199-204.
7. Santos PRV, Oliveira ACX, Tomassini TCB (1995). Control microbiogicode productors. Fitoterapicos.Rev.Farm.Bioquim, 31: 35-38.
8. Srinivasan, M. C, Laxman, R. S., Deshpande, M. V. (1991): Physiology and nutritional aspects of actinomycetes: an overview. World J. Microbiol. Biotechnol, 7: 171-184.
9. Subathra Devi, C: Amrita and Nitin J. (2012). Novel bioactive compounds from mangrove derived actinomycetes. International Research Journal of Pharmacy, 3(9): 25-29.