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Streptomycetes Sp From Mangrove Forest: Their Antimicrobial And Antibiotic Compounds

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ABSTRACT

Members of the genus Streptomyces are of great importance for biotechnology due to their ability to produce a large array of natural products, including antibiotics, anticancer agents and immunosuppressants. Recent research has increased our knowledge and understanding of the antimicrobial effect of mangrove Streptomyces. In a systematic screening effect, Streptomyces isolates from different mangrove forest were investigated for antimicrobial activity against bacterial pathogens and potentially active secondary metabolites. The different solvent extracts (Butanol, Ethyl acetate,ethanol, hexane) of selected isolates were screened for their antimicrobial activity by well cut method. The Zone of inhibition was measured in all extracts revealed a wide range of antimicrobial activity against pathogenic bacteria. The overall results of the antimicrobial activity indicates the presence of the antibiotics in Streptomycetes sp from mangrove forest evidenced by the presence of the compounds that can active against bacterial pathogens.

Key words: Mangrove, Streptomyces, Compounds, Antibiotics, Bacteria.

INTRODUCTION

Mangroves are coastal wetland forests mainly comprising of an assemblage of tropical trees and shrubs that inhabit the intertidal zone of tropical and subtropical latitudes. Mangrove ecosystems are well known potent areas for distribution and occurrence of microbes (Gupta et al. 2007 and Xu et al. 2009). Mangroves inhibit intertidal zones and can tolerate a wide range of salinities (Liang et al. 2008). They are regarded as highly productive ecosystems and abode to unexplored microbial diversity including actinobacteria (Hong et al. 2009).

Mangrove environment contains a wide range of distinct microorganisms that are lack in terrestrial environment. Though some reports were available on antibiotic and enzyme production by marine actinobacteria, marine environment is still a prospective source for new actinobacteria, which can yield novel bioactive compounds and industrially important enzymes (Sharma and Pant 2001).

Antibiotics are widely distributed in the nature, where they play an important role in regulating the microbial population of soil, water, sewage, and compost. The mangrove environment is a potent

source for the isolation of antibiotic-producing actinomycetes. Approximately 80% of naturally occurring antibiotics, including many of medical importance, have been isolated from actinomycetes, a major group of aerobic, gram-positive soil bacteria that are very widely distributed.

The antimicrobial potential of extracellular metabolites of actinomycetes strains against bacterial pathogens is abundant. However, there are less scientific literatures on the antagonistic effects of fungi on bacteria and the antifungal activities of actinomycetes in a mangrove community. Thus the present study aims at exploring the antimicrobial activities of the rhizosphere microbial communities from the Pichavaram mangrove ecosystems of Tamilnadu, which can be rich sources of useful metabolites.

Materials and methods

Sample collection:

The soil samples were collected from Pichavaram mangrove ecosystem situated along the southeast coast of India. Samples were taken in a zipped polythene bags and were carried to the laboratory under aseptic conditions for further studies. They were serially diluted in sterile distilled water and plated on starch casein agar plates. The plates were then incubated at 28ºC for 7 days.

Identification and characterization of the actinomycetes isolates:

The strain was carried out for gram staining, shape, size by under light microscope. Various biochemical tests were performed for the identification of various actinomycetes isolates. The mycelium structure, colour and arrangement of spore on the mycelium were examined under oil immersion.

Preparation of sample for Antimicrobial activity:

The selected strains were grown in 250ml flask containing 50ml of fermentation medium. The flasks were inoculated with active culture and incubated at 28±2ºC for 120hr. After sufficient growth, the contents of the each flask were extracted twice with n-Butanol and ethyl acetate (1-2.5v/v). The Butanol and ethyl acetate extracts containing bioactive components used for testing antimicrobial activity. Then the Butanol extracts were concentrated in water bath at 80ºC. The concentrated residue was dissolved in ethyl acetate and also used as a sample for antimicrobial activity.

Antibacterial activity by well cut method:

The sterile Muller Hintor agar medium was poured into each sterile petriplates and allowed to solidify. Using a sterile cotton swabs, fresh bacterial cultures with known population count was speared over the plates by following speared plate technique. The well cut prepared are placed over the media inoculated with appropriate bacteria, then the crude extract of these actinomycetes cultures is added in to the well. All the plates were incubated at 37° C for 24-48 hours. After the incubation period the results were observed and measured the diameter of clear zone was recorded after 3 days.

Preparation of sample for GCMS analysis:

The partially purified active compound was analysed by gas liquid chromatography-mass spectrometry (GC-MS). GC-MS system was equipped with a fused silica capillary column (DB 5 - MS Capillary Standard Non - Polar Column) was used to analyse the compound. The data was processed with GC/MS DSQ II (Thermo GC-MS DSQ II). Column condition was programmed as column oven temperature 70°C rose to 260° C at 6°C /Min. (Roy et al., 2006). The peaks of compounds in gas chromatography were subjected

to mass-spectral analysis. The spectra were analyzed from the available library data, Trace Ultra search (version 5.0).

Results and discussion

The soil samples collected from different areas of pichavaram, actinomycetes isolates were obtained in pure form and analysed for their antibacterial activities. All the isolates were found to be gram positive and showed branched mycelium in their cell morphology .The isolates which exhibited activity against the pathogens were selected for the further studies. According to Bergey's manual of determinative bacteriology and the laboratory manual for identification of actinomycetes, the isolates were identified as Streptomyces sp.

Table 1: Growth of isolated Streptomyces sp in Actinomycetes agar, Starch casein nitrate agar and Peptone agar

Strain code	Growth on actinomycetes agar	Growth on starch casein nitrate agar	Peptone agar
1	Pale pink, brown pigmentation, non-diffusable	Pink coloured, brown pigment, non-diffusable	Pale pink colonies
2	Sandal white, pale brown	Grey to white, dark brown pigment, diffusable	Sandal white colonies
3	Dull white, rough	Grey, powdery, brown pigment, diffusable	Dull white colonies
4	White colour	Whitish colonies with pale yellow pigment	White rough colonies
5	White colour, powdery, smooth colonies	Sandal colour, powdery, smooth colonies with dark brown pigment	White colonies with brown pigment

Antibacterial activity by well cut method:

With the increasing use of antibiotics, the serious problem of antibiotic resistance is gradually increasing. All the strains were able to produce antibiotic against gram-positive and gram negative organisms. The formation of inhibition zone around the pathogenic strains is due to the production of secondary metabolites by actinomycetes isolates. Thus the result of present investigation revealed that soil actinomycetes are the potent source of novel antibiotics and were found to be of potential activity against test organisms which can control variety of pathogenic organisms.

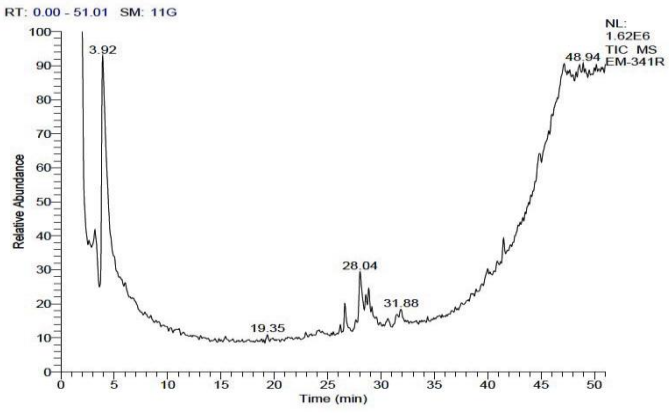
Table 2: **Antibacterial activity of selected active strains**

Human pathogen	Zone of Inhibition (mm)														
	1			2			3			4			5		
	50 µl	100 µl	200 µl	50 µl	100 µl	200 µl	50 µl	100 µl	200 µl	50 µl	100 µl	200 µl	50 µl	100 µl	200 µl
Acinetobacter sp,	10	14	22	9	15	24	-	-	20	-	10	23	13	21	35
E.coli	12	19	30	11	20	29	-	-	18	12	16	28	9	18	27
K.pneumoniae	13	21	35	15	23	35	12	21	35	-	11	23	11	20	29
S.aureus	8	17	3	10	21	35	-	-	20	-	8	17	12	22	35
S.marsecens	10	17	27	7	16	22	-	-	9	-	9	20	-	-	-
B.cereus	-	12	22	10	18	26	-	12	25	5	12	21	-	11	24
S.typhi	12	24	35	9	17	29	-	12	23	12	19	27	11	24	36

Gas Liquid Chromatography-Mass Spectrometry:

An attempt was made to identify the chemical nature of the compounds produced by the effective strain of Streptomyces sp and it was successfully determined by GC-MS. The GC-MS result of ethyl acetate extract showed 18 considerable compounds with different retention time intervals. Of these identified compounds, some compounds were previously determined and studied with their antibacterial and cytotoxic properties and thus indicates the presence of antimicrobial compound. Because of these features, further studies were undertaken on this Streptomyces sp.

Figure 1: Represents the different peaks obtained from GCMS analysis of the extract of Streptomyces sp, peaks showing the representative compounds



The important group of Quinone rich compounds was identified through GC-MS analysis from the effective strain. And the compound identified from the sample was named as 3-Chloro-2, 2, 5, 8-tetrahydroxy-3, 6, 7-trimethyl-2,3-dihydro-1,4-naphthoquinone along with the quinone as their one of the functional group. This quinone groups are widely distributed in nature and they are also synthesized by some microorganisms. Herein, the identified compound contains Naphtoquinones as their derivative and this naphthoquinone are bicyclic aromatic quinones and 1,4 naphthoquinones are the most common type of naphthoquinone present in nature with potent activity in a variety of biological targets such as cytotoxic, antimicrobial, antifungal, antiviral, and antiparasitic activities. This 1, 4 Naphthoquinones derivatives are the main class of natural products known to be produced by members of the MAR 4 clade. The MAR 4 is a new clade related to the genus Streptomyces (Verma, R.P, 2006 and Jensen, P.R et al., 2005) these microorganisms isolated from mangrove sediment has an unusual ability among Actinomycetes to produce meroterpenoids as their biologically active products.

These are the important compounds were identified from the ethyl acetate extract of Streptomyces sp isolate which showed sound antibacterial activity against selected bacterial pathogens. The identified compounds were used with their diverse derivatives in the pharmaceutical and agro industries and it showed significant biological activities such as antagonistic agents**,** including antibacterials, antifungals, antiprotozoans as well as antivirals, pharmacological agents, including antitumorals, immunomodulators, neurological agents and enzyme inhibitors, agrobiologicals, including insecticides, pesticides and herbicides, and compounds with regulatory activities, such as growth factors, siderophores or morphogenic agents. Thus it is concluded that the soil inhabiting Streptomyces sp have a great potential to produce secondary metabolite against bacterial pathogens.

Conclusion

The present study concluded that continuous isolation, screening of rare Streptomyces sp from mangrove source is the simple way to discover the novel drugs or bioactive metabolites. The ethyl acetate extract of Streptomyces sp showed better antimicrobial activity against pathogenic bacteria and also the producer of antibiotic producing compounds. So the present study also concludes that Streptomyces sp isolated from mangrove will be a potent source of antibiotic compounds. It is expected that the current attempt of isolation, characterization and the study on mangrove actinomycetes of the local area of Pichavaram will be useful for identification of new antibiotics effective against challenging pathogens.

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References

1. Asolkar RN, Maskey RP, Helmke E, Laatsch H, Chalcomycin B (2002) A new macrolide antibiotic from the marine isolate Streptomyces sp. B7064. J. Antibiot. 55: 893-898.

2. Aszalos AS, Frost DS (1968) Classification of crude antibiotics by instant thin layer chromatography (ITLC) J. Chromat., 37: 487-488.
3. Balagurunathan R, Xu L, Jiang C (1996) Diversity of soil actinomycetes from South India and South China. Actino. 4:89-94

4. Baldacci, E., Spalla, C., and Grein, A. **(**1955) The classification of the Actinomyces species. Arch. Mikrobiol., 20, 347-357
5. Baltz RH (2008) Renaissance in antibacterial discovery from actinomycetes. Curr. Opin. Pharmacol. 557-563.
6. Cross T (1982). Actinomycetes: a continuing source of new metabolites. Dev. Ind. Microbiol. 23: 1-18.
7. Davies FL, Williams ST (1970). Studies on the ecology of actinomycetes in soil. The occurrence and distribution of actinomycetes in a pine forest soil. Soil Biochem. 2: 227-238.
8. Donadio S, Carrano L, Brandi L, Serina S and Soffientini A et al., (2002a). Target and assays for discovering novel antibacterial agents. J. Biotechol., 99: 861-872
9. Gerner EW and Meyskens FL (2004) Jr. Polyamines and cancer: old molecules, new understanding. Nat. Rev. Cancer 4: 781-792.
10. Goodfellow M and Lechevalier MP (1989). Genus Nocardia. Trevisan. 9AL. In: Bergey's Manual of Systematic Bacteriology. Edited by ST Williams ME, Sharpe JG. Holt. Baltimore: Williams & Wilkins. 2: 1458-1471.
11. Hucker GJ and Conn HJ (1923) Methods of Gram staining. Technical Bulletin of the New York State, Agriculture Experimental Station 93.
12. Imada C and Simidu U (1988) Isolation and characterization of an alpha amylase inhibitor producing actinomycetes from marine environment. Nippon Suisan Gakkaishi 54: 1839–1845.
13. Jahir AK and Anand SP (2011) Extraction and Purification of Antimicrobial Metabolites from Actinomycetes spp. Isolated from soil sample. International journal of pharmaceutical research and development 63-7.
14. Kim TK and Fuerst JA (2006) Diversity of polyketide synthase genes from bacteria associated with the marine sponge Pseudoceratina clavata: culture-dependent and culture-independent approaches. Environ. Microbiol. 8: 1460-1470.
15. Kock I, Maskey RP, Biabani MAF, Helmke E and Laatsch H (2005) 1-hydroxy-1-norresistomycin and resistofl avine methyl ether new antibiotics from marine derived Streptomycetes. J Antibiot (Tokyo) 58:530–534.
16. Narendra K, Sing RK, Mishra SK, Singh AK and Pachouri UC (2010) Isolation and screening of soil Actinomycetes as source of antibiotics active against bacteria. International Journal of Microbiology Research Volume 2(2):12-16
17. Newman DJ and Cragg GM (2007) Natural products as sources of new drugs over the last 25 years. J. Nat. Prod. 70: 461-477.
18. Raja A and Prabakaran P (2011) Preliminary screening of actimycobacterial effect of psycrophillic actinomycetes isolated from manali ice point: Himachal predesh. Journal of Microbiology and Antimicrobials 3(2):41-46.
19. Sutthinan K and Akira Y (2009) L-Asparaginase production by actinomycetes isolated from some Thai medical plant rhizosphere soils International Journal of Integratyive Biology 6: 1, 22
20. Williams, P.G. Panning for chemical gold: marine bacteria as a source of new therapeutics. Trends Biotechnol. 2009, 27, 45-52.
21. Wu SJ, Fotso S, Li F, Qin S and Laatsch H (2007) Amorphane sesquiterpenes from a marine Streptomyces sp. J Nat Prod 70:304–306

22. Zengler K, Paradkar A and Keller M (2005) New methods to access microbial diversity for small molecule discovery. In: Drug Discovery and Therapeutic Medicine from Natural Products. Zhang L, Demain AL (Eds.), Humana Press, New Jersey, USA, pp 275–293.