Student Minor Research Project

Isolation and Screening of Antimicrobial spectra of Actinomycetes from Soil

Under RUSA 2.0 Scheme

(Through Ch.S.D.St. Theresa's College for Women (Autonomous), Eluru, A.P)

Submitted by

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SRI Y N COLLEGE
(AUTONOMOUS)
Thrice Accredited by NAAC at 'A' Grade
Recognized by UGC as "College with Potential for Excellence"
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CERTIFICATE

This is to certify that the project work entitled **"Isolation and Screening of Antimicrobial spectra of Actinomycetes from Soil"** is bonafied work carried out by Ms G.Sushma Devi (Reg.No. 11710003), Ms Ch.Anusha (Reg.No. 11710004), Ms P.Divya Sree(Reg.No. 11710018).submitted in Third year of the degree B,Sc in Biotechnology during the year 2019 – 2020 is an authentic work under my supervision and guidance.

To the best of my knowledge the matter embodied in the project work has not been submitted to any other College/Institution.

Date: 29-12-2019

Mr.G.SAM BABU
PROJECT ADVISOR

DECLARATION

We, the undersigned, declare that the project entitled "**Isolation and Screening of Antimicrobial spectra of Actinomycetes from Soil**", being submitted in Third Year of Bachelor of Science in Biotechnology, Sri Y N College (Autonomous), is the work carried out by us

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CONTENTS

Abstract

Actinomycetes are considered as one of the most diverse groups of filamentous bacteria capable of thriving into different types of ecological niches due to their bioactive potential. The main objective of the present study was Isolation and Screening of Actinomycetes from soil of valander river of Narasapur and characterized for morphological identification and evaluated for antibacterial activity against human pathogens. Total 50 Actinomycetes were isolated and characterized for morphological identification and evaluated for antibacterial activity. Out of these isolated, five actinomycetes showed antimicrobial activity against selected bacterial pathogens. Screening using agar diffusion assay was carried out and total five actinomycetes isolated were found active against MTCC cultures Including Bacillus subtilis (MTCC 441), Pseudomonas aeruginosa (MTCC 4673), Staphylococcus aureus (MTCC 3160), Pseudomonas fluorescens (MTCC 103),Mycobacterium smegmatis (MTCC 994), E. coli (MTCC 443) and Streptococcus mutans (MTCC 890). The study indicated that Valander river of Narasapur had diverse group of actinomycetes with broad spectrum antimicrobial activity.

OBJECTIVES

To isolate and characterize novel actinomycetes and to evaluate their antibacterial activity against drug-resistant pathogenic bacteria

Isolation and morphological characterization of actinomicrobial producing Actinomycetes from soil sample

Observation of pinpoint colonies with the zone of inhibition

Introduction

Actinomycetes are gram positive bacteria frequently filamentous and sporulating with DNA rich in G+C from 55-75 %. Actinomycetes have provided many important bioactive compounds of high commercial value and are being routinely screened for new bioactive substances. Actinomycetes are the most widely distributed group of microorganisms in nature which primarily inhabit the soil. They are one of the major groups of soil population. The number and types of actinomycetes present in a particular soil would be greatly influenced by geographical location such as soil temperature, soil type, soil pH, organic matter content, cultivation, aeration and moisture content. The diversity of terrestrial Actinomycetes are of extraordinary significance in several areas of science and medicine, particularly in antibiotic production. Actinomycetes are prolific producers of novel antimicrobial agents. Vast numbers of these antimicrobial agents are discovered from Actinomycetes by screening natural habitat such as soils and water bodies. Actinomycetes have the ability to produce secondary metabolites with biological activities such as antibiotic, antifungal, antiviral, anticancer, enzyme, immunosuppressant and other industrially useful compounds. The 80% of the world's antibiotics are known to come from Actinomycetes, mostly from the genera Streptomyces and Micromonospora. The genus, Streptomycetes, is responsible for the formation of more than 60 % of

known antibiotics while a further 15 % are made by a number of related Actinomycetes, Micromonospora, Actinomadura, Streptoverticillium and Thermoactinomycetes.On the whole, the last 55 years have seen the discovery of more than 12,000 antibiotics. The actinomycetes yielded about 70 % of these, and the remaining 30 % are products of filamentous fungi and non-actinomycete bacteria and rare Actinomycetes. The non-Streptomycetes are called rare actinomycetes, comprising approximately 100 genera.

Review of Literature

The term antibiotic appeared as early as 1928 in the French microbiological literature as antibiosis. The phenomenon of antagonism between living organisms was frequently observed even since 1877, when Pasteur and Joubert noticed that aerobic bacteria antagonized the growth of Bacillus anthracis. However, the word in its present restrictive meaning, “a chemical substance derived from microorganisms, which has the capacity of inhibiting growth and even destroying other organisms in dilute solutions” was introduced by Selman and Waksman in 1942 (Waksman 1954). The majority of antibacterial and antifungal agents in clinical use nowadays were discovered during the ‘Golden Age’ of antibiotics in the 1940s–1960s through massive isolation and screening of soil actinomycetes and fungi. It has been assessed that almost 12,000 bioactive secondary metabolites were discovered during that time, and about 160 of them then reached clinical use as natural products or as semisynthetic derivatives. In fact, 55% of them were produced by the genus Streptomyces, 11% from other actinomycetes, 12% from non-filamentous bacteria and 22% from filamentous fungi. Penicillins, cephalosporins, tetracyclines, aminoglycosides, glycopeptides, macrolides and polyenes were all discovered in that period and have been useful in the battle against bacterial and fungal infections over the past 50 years (Marinelli 2009)

Antibiotics	**Producer organism**	**Isolated**	**Discovered**	**Active profile**
Penicillin G	P. notatum	Sir Alexander Fleming 1928	1928	Antibacterial
Actinomycin	S.antibiotics	Waksman & Woodruff	1940	Anticancer
Streptomycin	Streptomyces griseus	Waksman et al.	1944	Antimicrobial

CHEMICALS AND EQUIPMENTS

A. Autoclave

B. Hot air oven

C. Laminar air flow

D. Incubator

E. 250 ml Conical Flask

F. Petri plates

G. 1ml, 10ml Pipettes

H. Test tubes

I. Measuring Cylinder

J. Soyabean – Casein digest medium

K. Actinomycetes - Isolation agar medium

L. Starch Casein agar medium

M. Distilled water

N. Gloves

O. Plastic bag

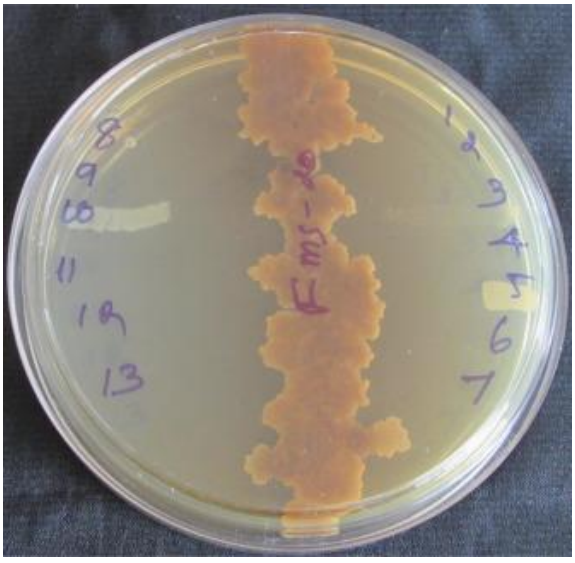
PROCEDURE

Collection of soil samples

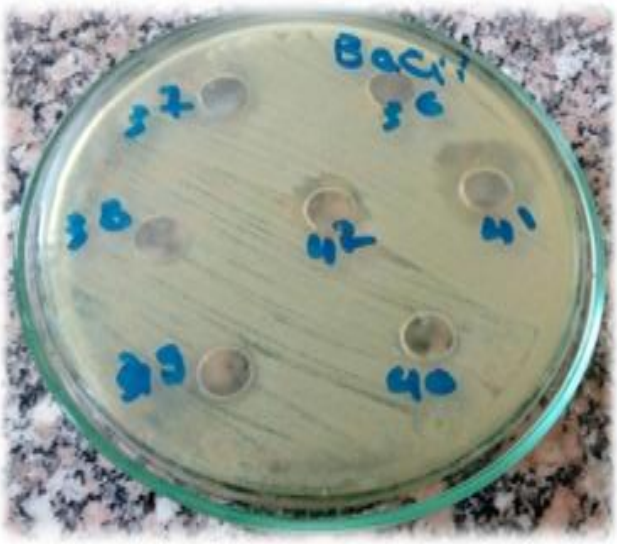
- Soil samples was collected from various locations of Narasapur Town
- I gm of soil sample was added to conical flask containing 100 ml of sterile water and few drops of Tween 80.
- A series of culture tubes containing 9 ml of sterile water was taken.
- From the stock culture 1ml was transferred to the first test tube 10^{-1} and mix well.
- Further serial dilutions were made to produce10^{-5} suspension were made.
- Suspension from each culture tube was spread on sterile SBCD Plates, AIA medium plates and starch casein agar medium plates aseptically in laminar air flow cabinet.
- The plates were incubated at 27^{o}C for 48 Hrs. observe intermittently during incubation
- After 48 hrs whitish pin point colonies clear zone of inhibition were observed.
- The Actinomycetes colonies were analyzed by agar streak method on SBCD medium and incubated at 27 ^{o}C for 4 Days.
- The zone of inhibition against each test organisms was noted.
- The isolated Actinomycetes were screened against some microorganisms like staphylococcus aureus, Escherichia coli.

OBSERVATION

- Based on their antimicrobial properties, isolates were chosen for the further biochemical observation.
- Morphological properties such as colony characteristics, types of areal hyphae, growth of vegetative hyhae, fragmentation pattern and spore formation were observed.



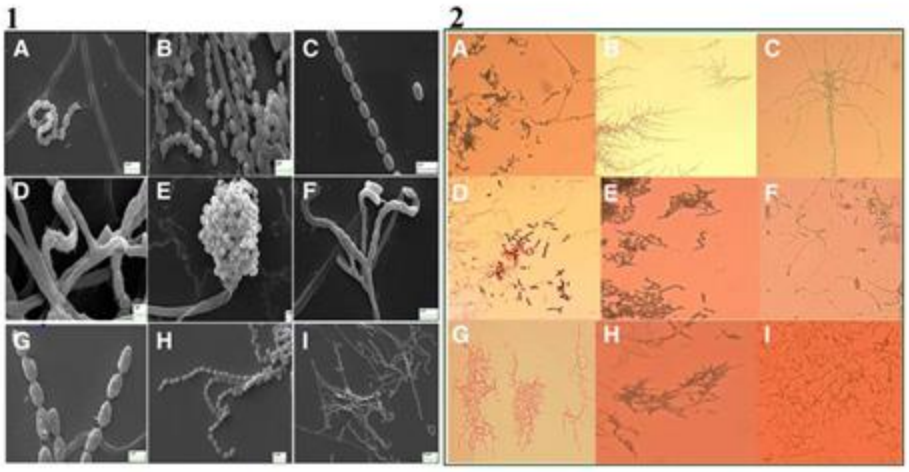
Antibacterial activity shown by B1& B4 against E.coli



Antibacterial activity shown by 33 & 34 against Pseudomonas fluoresces.

RESULTS

Actinomycetes Isolation agar medium is best as compared to the Starch casein agar medium, Wateryeast extract-agar (WYE), in terms of yield. Colonies having characteristic features such as powdery appearance with convex, concave or flat surface and color ranging from white, gray to pinkish and yellowish were selected. Furthermore, bacterial configuration same as Actinomycetes were accepted from gram staining. Fifty selected isolates were examined microscopically and identified by their morphological and culture characteristics. The Fifty isolates found come under three genera such as Actinomycetes, Micromonospora and Streptomyces, out of which 34 belong to Actinomycetes, 15 from Streptomyces and 1 from Micromonospora. Morphologically distinct Actinomycetes isolates were selected for anti-bacterial activity screening against the pathogenic test organisms. Out of Fifty isolates screened for antibacterial activity, only 4 showed positive results. B1, B4 showed the antibacterial activity against E.coli. 33 and 34 showed antibacterial activity against Pseudomonas fluorescens. Their zone of inhibition was measured and the results were noted. Further study will be needed to find out the active molecules from the actinomycetes.



Panel 1- Morphology of sporophores of nine Actinomycetes studied by scanning electron microscopy [(a) SMS_B, (b) SMS_5, (c) SMS_7, (d) SMS_9, (e) SMS_10, (f) SMS_13, (g) SMS_SU13, (h) SMS_SU21, (i) SMS_SU23]. Scale bar shown on each photomicrograph. Panel 2- Arrangement of mycelium studied by phase contrast microscopy. [Phase contrast micrographs of nine actinomycets (a) SMS_B (b) SMS_5 (c) SMS_7 (d) SMS_9 (e) SMS_10 (f) SMS_13 (g) SMS_SU13 (h) SMS_SU21 (i) SMS_SU23 . Magnification 40 × .]

Secondary Screening of antifungal activity

Sr. No	Test strains	Soil isolates (zone of Inhibition in mm)														PC
		ZE 18	ZE 19	YE 21	YE 22	YE 23	ZA 25	ZA 26	YD 28	RS 25	XD1	H1	V1	N1	S1	K1	E^{15}
1	*Bacillus subtilis* MTCC 441	16	16	13	27	22	23	21	–	–	20	17	25	12	13	11	30
2	*Bacillus subtilis* MTCC 121	16	16	–	17	16	19	19	–	–	17	16	25	–	12	–	30
3	*Bacillus pumilus* MTCC 1607	21	21	18	25	21	26	15	13	–	–	17	30	–	–	–	–
4	*Staphylococcus aureus* MTCC 96	23	17	–	20	17	25	24	–	24	24	20	21	–	13	11	25
5	*Staphylococcus aureus*, MTCC 902	–	–	–	14	17	–	–	12	25	20	15	30	–	14	11	17
6	*Escherichia coli* MTCC 1304	–	–	–	–	12	16	–	–	–	19	17	–	–	–	–	20
7	*Pseudomonas aeruginosa* MTCC 741	–	–	13	27	15	18	27	–	20	20	15	28		–	–	37
8	*Proteus vulgaris* MTCC 426	16	16	–	15	18	–	–	14	–	14	18	30	–	12	–	12
9	*Salmonella typhi* MTCC 581	–	–	–	15	15	18	17	12	–	16	16	29	–	–	–	13
10	*Klebsiella pneumonia* MTCC 13883	15	14	–	16	17	18	–	12	23	17	15	25	–	–	–	–

(–), not active; PC, Positive Control; E^{15}, Erythromycin and well diameter = 10 mm.

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