

**EXTRACTION OF ANTIBIOTICS BY
ACTINOMYCETES ISOLATED FROM EASTERN
MOUNTAIN REGION OF NEPAL**



A

Dissertation

Submitted to the **Department of Microbiology,**
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in Partial Fulfillment of the Requirements for the Award of Degree of Masters of Science in
Microbiology (**Public Health**).

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ABSTRACT

Bioactive compounds like antibiotic can be considered as a substance, produced by one microorganism, which inhibits the growth of another microorganism. Actinomycetes, slow growing gram-positive bacteria, are the major sources of bioactive compounds. The aim of this study was to screen antibiotic producing Actinomycetes for antibiosis from the soils of Eastern Mountain region of Nepal. Primary screening and secondary screening were performed by perpendicular streak method and agar well assay method respectively. Microbiological characterization was performed for identification of presumptive genera. Characterization of the antibacterial substances extracted from this isolate was done by Thin-layer chromatography (TLC) using Chloroform: Methanol (10:90) as the solvent system and iodine vapor as the visualizing agent. Altogether forty-one actinomycetes were isolated from soil samples but only one (2.4%) of the isolate showed antibacterial activity against both Gram positive (*Staphylococcus aureus*) and Gram negative (*Escherichia coli*, *Salmonella* Typhi *Shigella* spp., and *Pseudomonas aeruginosa*) test-bacteria in primary screening. Isolate M₃ was selected for secondary screening because of its potent antibacterial activity. Minimum inhibitory concentration (MIC) of crude antibacterial substances was carried out by serial dilution method and found to be 2.5 mg/mL against test organisms. The chromatogram in TLC showed only one spot with R_f value 0.81 by the isolate suggesting that the isolate produced only one compound which was completely different from the spot with R_f value 0.89 produced by gentamycin. According to identification by Microscopy (1000X) and overall biochemical, and physiological characteristics, the isolate was considered as *Streptomyces* spp, a distinct taxonomic group.

Key words: Actinomycetes, Antibacterial activity, Minimum inhibitory concentration, thin layer chromatography.

LIST OF CONTENTS

TITLE PAGE	i
RECOMMENDATION	ii
CERTIFICATE OF APPROVAL	iii
ACKNOWLEDGEMENT	iv
BOARD OF EXAMINER	v
ABSTRACT	VI
TABLE OF CONTENTS	vii-ix
LIST OF TABLES	x
LIST OF FIGURES	xi
LIST OF APPENDICES	xii
LIST OF PHOTOGRAPHS	xiii
LIST OF ABBREVIATIONS	xiv
CHAPTER I	1-4
INTRODUCTION	1-3
1.2 Objectives	4
1.2.1 General Objectives	4
1.2.1 Specific Objectives	4
CHAPTER II	
LITERATURE REVIEW	5-18
2.1 Actinomycetes	5
2.2 Taxonomy	5-6
2.3 Taxonomic classification of the order actinomycetes	6
2.3.1 Micromonosporinia	6
2.3.2 Frankineae	7
2.3.3 Pseudonocardineae	7
2.3.4 Streptomyceiae	7
2.3.5 Corynebacterium	7

2.3.6 Actinomyineae	7
2.3.7 Prepionibacterianeae	8
2.3.8 Streptosporangineae	8
2.3.9 Glycomycineae	8
2.4.1 Isolation of Actinomycetes from soil	9-11
2.4.2 Screening of Actinomycetes for antimicrobial activity	11-12
2.4.3 Fermentation and Antibiotic production	12-13
2.4.4 Media Composition	13-14
2.4.6 Extraction and characterization of antimicrobial Compounds	14-15
2.5.1 History of Antibiotic Development	16-17
2.5.2 Mechanism of antibiotic resistance	17-18
2.5.3 Confirmation of antibiotic presence	18
CHAPTER III	
MATERIALS AND METHODS	19-25
3.1 Materials required	19
3.2 Research methodology	19
3.2.1 Research design	19
3.2.2 Population and Sample	19
3.2.3 Sample size	19
3.2.4 Laboratory Set up	19
3.2.5 Data collection procedure and data analysis plan	20
3.3 Method	20
3.3.1 Collection of soil samples	20
3.3.2 Isolation of actinomycetes	20
3.3.3 Screening of Actinomycetes for Antimicrobial activity	21
3.3.4 Characterization of Actinomycetes	22
3.3.5 Biochemical characterization	22
3.3.6 Fermentation	22

3.3.7 Extraction of antibacterial metabolites by using Ethyl acetate	23
3.3.8 Determination of Antimicrobial Activity	23-24
3.3.9 Determination of Minimum Inhibitory Concentration	24
Flow chart for the screening of actinomycetes for antibiosis	25
CHAPTER IV	25-33
RESULTS	26-34
4.1 Screening of Actinomycetes for Antimicrobial activity	25-26
4.1.1. Primary Screening of Actinomycetes	26-27
4.2.2 Secondary Screening of Actinomycetes	28
4.2 Characteristics of the active isolates	29
4.2.1. Macroscopic Characteristics	29
4.2.2. Microscopic Characteristics	30
4.2.3. Biochemical and physiological characteristics	30-33
4.3 Fermentation	33
4.4 Minimum Inhibitory Concentration of active compounds	33-34
4.5 Characteristics of the Antimicrobial Substances	34
4.6 Thin layer chromatography	34
PHOTOGRAPH	
CHAPTER V	
DISCUSSION	35-39
CHAPTER VI	
CONCLUSION RECOMMENDATIONS	40-41
6.1 Conclusion	40
6.2 Recommendations	41
REFERENCES	42-51
APPENDICES	I-XIV

LIST OF TABLES

Table 1: Number of actinomycetes isolate from soil samples.

Table 2: Zone of inhibition of active isolates in Primary screening against test bacteria

Table 3: Zone of inhibition of active isolates in Secondary screening against test bacteria

Table 4: Macroscopic characteristics of the active isolate on SCA

Table 5: Microscopic characterization of active isolate

Table 6: Carbohydrate utilization by active isolate

Table 7: Substrate utilization tests of the active isolate

Table 8: Other biochemical tests of the active isolate

Table 9: Physiological tests of the active isolate

Table 10: Concentration of antibacterial substances

Table 11: Thin layer chromatography of the antibacterial substance

LIST OF FIGURES

Figure 1: Flow chart for the screening of actinomycetes for antibiosis

Figure 2: Pie chart showing actinomycetes isolates with or without antibacterial activity.

Figure 3: Antibacterial activity shown by Actinomycetes isolates in primary Screening.

LIST OF PHOTOGRAPHS

Photograph 1: Primary screening of isolate M₃ by perpendicular streaking method.

Photograph 2: Extraction of antimicrobial metabolites by using organic (ethyl acetate) in a separating funnel.

Photograph 3: Secondary screening of active isolate against *Escherichia coli* by agar well assay method.

Photograph 4: Thin Layer Chromatography of extracted metabolites.

LIST OF ABBREVIATIONS

16 S rRNA: 16 Svedberg unit ribosomal Ribonucleic acid

ATCC: American Type Culture Collection

ATP: Adenosine Tri phosphate

BLAST: Basic local Alignment Search Tool

DABA: Diamino butyric Acid

DAP: Diaminopimelic Acid

DNA: Deoxyribonucleic Acid

G + C: Guanine + Cytosine

HPLC: High Pressure liquid Chromatography

MHA: Mueller Hinton Agar

MIC: Minimum Inhibitory Concentration

MRSA: Methicillin Resistant *Staphylococcus aureus*

NA: Nutrient Agar

Rf: Retention Factor

SCA: Starch Casein Agar

SIM: Sulphide Indole Motility

TLC: Thin Layer Chromatography

UV: Ultra violet

Viz: Name

LIST OF APPENDICES

Appendix - A: Materials used

Appendix - B: Composition of Media

Appendix - C: Methodology of biochemical test for the identification of
Actinomycetes.

Appendix - D: Procedure of 0.5 Mc Farland Nephelometer