Isolation and Characterization of Actinomycetes from Surrounding Soil of Medicinal Plants

by

Aagat Awasthi
Pranita Kaphle
Pratima Pandey
Sabita Kadel
Shristi Neupane

A project report submitted in partial fulfillment of the requirements for the degree of Bachelor of Technology in Biotechnology

Department of Biotechnology
School of Science
Kathmandu University

September 2011

Isolation and Characterization of Actinomycetes from Surrounding Soil of Medicinal Plants

by

Aagat Awasthi
Pranita Kaphle
Pratima Pandey
Sabita Kadel
Shristi Neupane

Supervisor:
Janardan Lamichhane, Ph.D.
Associate Professor
Department of Biotechnology
Kathmandu University

A project report submitted in partial fulfillment
of the requirements for the degree of
Bachelor of Technology in Biotechnology

Department of Biotechnology
School of Science
Kathmandu University

September 2011

Letter of Recommendation

I, Janardan Lamichhane, hereby declare that the work assembled herein, submitted in the partial fulfillment of the requirements for the degree of Bachelor of Technology in Biotechnology of the School of Science at the Kathmandu University during the Academic Year 2011, is a genuine work done by Aagat Awasthi, Pranita Kaphle, Pratima Pandey, Sabita Kadel and Shristi Neupane, under my supervision. The work presented here has not been published elsewhere for the requirements of any degree programme. Any literature, data or work done by others are cited within this report and listed in reference.

Janardan Lamichhane, Ph.D.
Associate Professor
Department of Biotechnology
School of Science
Kathmandu University

September 2011

Board of Examiners

Recommended by:

Janardan Lamichhane, Ph.D.
Associate Professor
Department of Biotechnology
Kathmandu University
(Project Supervisor)

Approved by:

Tika Bahadur Karki, Ph.D.
Professor and Head
Department of Biotechnology
Kathmandu University

Examined by:

Rajani Malla, Ph.D.
Associate Professor
Central Department of Biotechnology
Tribhuvan University
(External Examiner)

September 2011

Declaration by the Students

We, Aagat Awasthi, Pranita Kaphle, Pratima Pandey, Sabita Kadel and Shristi Neupane, hereby declare that this project report entitled **"Isolation and Characterization of Actinomycetes from Surrounding Soil of Medicinal Plants"**, submitted in the partial fulfillment of the requirements for the degree of Bachelor of Technology in Biotechnology of the School of Science at the Kathmandu University during the Academic Year 2011, is a genuine work done by us under the supervision of Dr. Janardan Lamichhane. The work presented here has not been published or submitted elsewhere for the requirements of any degree programme. Any literature, data or work done by others are cited within this report and listed in reference.

Aagat Awasthi	Pranita Kaphle	Pratima Pandey

Sabita Kadel	Shristi Neupane

Department of Biotechnology
School of Science
Kathmandu University

September 2011

Acknowledgements

We wish to express our deepest and sincere gratitude to our supervisor, Dr. Janardan Lamichhane, Associate Professor, Department of Biotechnology, Kathmandu University whose encouragement, guidance and support from the initial to the final level enabled us to develop an understanding of the subject. Sincere appreciation goes to Dr. Tika Bahadur Karki, Professor and Head of Department for his valuable instructions and suggestions. We would also like to thank Dr. Bhupal Govinda Shrestha, Mr. Subodh Kumar Upadhyaya, Ms. Sangita Shakya, Dr. Dhurva Prasad Gauchan, Mr. Parash Mani Timilsina, Mr. Pritish Shrestha, Ms. Aashna Dhakal, and Mr. Hemanta Raj Mainali of Department of Biotechnology, Kathmandu University for their guidance and help during the project. We also appreciate the help provided by Ms. Tirtha Maiya Shrestha, Department of Pharmacy, Kathmandu University and Ms. Pooja Manandhar, Department of Natural Sciences, Kathmandu University.

Our sincere thanks go to all the staffs of Department of Biotechnology for providing a prosperous atmosphere for learning and research. We are grateful to Mr. Mitra Oli, Mr. Sunil Maharjan, Mr. Babu Kaji Shrestha, Mr. Pancha Narayan Maharjan and Ms. Binita Basnet for their technical help and support. More importantly, we also would like to extend our gratitude to the residents of the sample collecting areas for helping us identify the medicinal plants.

Finally, this work would not have been possible without the help and support from our family and friends. We thank all of them for their valuable advice and kindness.

(Abstract)

Isolation and Characterization of Actinomycetes from Surrounding Soil of Medicinal Plants

Aagat Awasthi, Pranita Kaphle, Pratima Pandey, Sabita Kadel, Shristi Neupane
Department of Biotechnology
School of Science
Kathmandu University

Supervisor: Janardan Lamichhane, Ph.D.

In this study, the rhizospheric and non rhizospheric soil samples of locally available medicinal plants were collected from three regions of Nepal: Suryakunda and Gosainkunda (high altitude areas), and forest area in Rautahat (low altitude area). Study of the morphological characteristics of all the isolates suggested that 22 out of 27 isolated species might be actinomycetes. Various biochemical tests of these 22 isolates were performed. These 22 isolates were also cultured in broth, for 7 days at 28°C. 15 of these isolates showed various pigmentations. The secondary metabolites were extracted with ethyl acetate from culture broth, by solvent extraction method. These extracts were assessed for their antibacterial activity against seven pathogenic bacteria (*S. aureus, P. aeroginosa, E. coli, B. subtilis, K. pneumoniae, B. thuringenesis, S. paratyphi*) and also screened for anti-fungal activity against five fungus (*Candida albicans, Penicillium, Rhizopus, Aspergilus*, *Alternaria*), using disc diffusion method. Among the seven test organisms of bacteria, *E.coli* was significantly inhibited by 13% of isolates, *S. aureus* by 33%, *B. subtilis* by 53%, *K. pneumoniae* by 67%, *S. paratyphi* by 27%, *P. aeroginosa* by 33% and *B. thurengenesis* by 60%. When screened for antifungal activities against five different fungus, significant zone of inhibition was shown against *Rhizopus, Aspergilus* and *Alternaria* only. The secondary metabolite produced by the isolates of soil from the surrounding of locally used medicinal plant, Chare Dabai showed good antibacterial and anti-fungal property. Extract from isolates of the rhizospheric soil of local medicinal plant used as antipyretics, showed best result as an anti-bacterial agent, inhibiting five among seven pathogenic bacteria. High anti-fungal activity was shown by extracts of isolates from

low altitude sample whereas high anti-bacterial activity was shown by extracts of isolates from high altitude sample. Secondary metabolite produced by the isolates might be some novel compound and the isolate might emerge as a potent antibiotic component producer. Also, isolates with significant anti-microbial activity are derived from the rhizospheric soil of the local plants having high medicinal value. This study, thus, also contributes to the identification of these plants.

Key words: *Streptomyces*, actinomycetes, anti-bacterial, anti-fungal, secondary metabolites, selective isolation

Contents

List of Tables

List of Illustrations

List of Figures

List of Graphs

Abbreviations

°C	degree Celsius
cm	centimeter
ELISA	Enzyme-linked Immunosorbent Assay
IAA	Indole-3-Acetic Acid
IMViC	Indole Test, Methyl Red Test, Voges-Proskauer Test, Citrate Utilization Test
MH	Mueller Hinton Agar
ml	milliliters
MR	Methyl Red Test
MRVP	Methyl Red and Voges-Proskauer Media
NA	Negligible Amount
NIZ	No Inhibition Zone
OD	Optical Density
PDA	Potato Dextrose Agar
SC	Starch Casein
SIM	Sulfide-Indole-Motility Media
TSI	Triple Sugar Iron Test
VP	Voges-Proskauer Test

Chapter I

Introduction

1.1 Soil Bacteria

Bacteria are some of the smallest and most abundant microbes in the soil. In a single gram of soil, there can be billions of bacteria. There are an estimated 60,000 different bacteria species, most which are yet to be even named, and each has its own particular roles and capabilities.

Types of bacteria

Decomposers

Bacteria play an important role in decomposition of organic materials, especially in the early stages of decomposition when moisture levels are high. In the later stages of decomposition, fungi tend to dominate.

Nitrogen fixers

Rhizobium bacteria can be inoculated onto legume seeds to fix nitrogen in the soil. These nitrogen-fixing bacteria live in special root nodules on legumes such as clover, beans, medic, wattles etc. They extract nitrogen gas from the air and convert it into forms that plants can use. This form of nitrogen fixation can add the equivalent of more than 100kg of nitrogen per hectare per year.

Disease suppressors

Bacillus megaterium is an example of a bacterium that has been used on some crops to suppress the disease-causing fungus *Rhizoctonia solani*. *Pseudomonas fluorescens* may also be useful against this disease. *Bacillus subtilis* has been used to suppress seedling blight of sunflowers, caused by *Alternaria helianthi*.

Aerobes and anaerobes

Aerobic bacteria are those that need oxygen, so where soil is well drained aerobes tend to dominate. Anaerobes are bacteria that do not need oxygen and may find it toxic. This group includes very ancient types of bacteria that live inside soil aggregates. Anaerobic bacteria favour wet, poorly drained soils and can produce toxic compounds that can limit root growth and predispose plants to root diseases.