

# **Isolation and Characterization of Actinomycetes from Surrounding Soil of Medicinal Plants**

by

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Pranita Kaphle  
Pratima Pandey  
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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  
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**September 2011**

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## **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.

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## 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.

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(Abstract)

## **Isolation and Characterization of Actinomycetes from Surrounding Soil of Medicinal Plants**

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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. aeruginosa*, *E. coli*, *B. subtilis*, *K. pneumoniae*, *B. thuringensis*, *S. paratyphi*) and also screened for anti-fungal activity against five fungus (*Candida albicans*, *Penicillium*, *Rhizopus*, *Aspergillus*, *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. aeruginosa* by 33% and *B. thurengensis* by 60%. When screened for antifungal activities against five different fungus, significant zone of inhibition was shown against *Rhizopus*, *Aspergillus* 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



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## 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.