

Isolation and Identification of Protease Enzyme Producing *Bacillus subtilis* from Soil

Fakhar-un-Nisa Yunus^{1*}, Husna Malik¹, Saira Hadi¹, Mirza Imran Shahzad², Muhammad Irfan³, Shahzad Ali⁴, Ali Muhammad⁵

¹Department of Zoology, Lahore College for Women University, Jail Road Lahore 54000, Pakistan.

²University College of Veterinary and Animal Sciences, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan.

³Department of Biochemistry and Molecular Biology, University of Gujrat, HH Campus, Gujrat, Pakistan.

⁴Department of Wildlife and Ecology, University of Veterinary and Animal Sciences, Lahore, Pakistan.

⁵Department of Zoology, University of Poonch, Rawalakot 12350, Azad Kashmir, Pakistan.

Received: 30.Sep.2016; Accepted: 20.Jan.2017; Published Online: 30.Mar.2017

*Corresponding author: Fakhar-un-Nisa Yunus; Email: fakhar_yunus@yahoo.com



Abstract

Bacillus subtilis is commonly recovered from water, soil, air and decomposing plant residue. But the main habitat of *B. subtilis* is soil and a long term survival is expected to occur in soil. In this study, twenty samples of soil were collected from different industrial areas of Lahore. These soil samples were used to analyze the presence of microorganisms in it. Bacteria were grown on the specific medium for the colony identification. Then Biochemical test was performed to isolate and identify *B. subtilis* from the soil samples. In the present study, *Bacillus* species were found to be abundant in our soil samples. The bacteria isolated from the soil were rod shaped gram positive bacteria that form spores under stress conditions. The spores were appeared to be rounded and green in color inside the cell. Positive zone formation test confirmed the presence of *B. subtilis*. The protease enzyme was produced by *B. subtilis* in large quantities during vegetative growth. It was found that absorbance reached to its maximum value at 5µg/ml concentration of protease enzyme. The maximum values of absorbance were 0.45 and 0.5 at 550nm. It was concluded from the above results that soil is the main habitat of many microorganisms and among these microorganisms *Bacillus subtilis* was found to produce protease enzyme.

Keywords: *Bacillus subtilis*, industrial areas of Lahore, spores, zone formation test, protease enzyme, soil.

Cite this article: Yunus, F.N., Malik, H., Hadi, S., Shahzad, M.I., Irfan, M., Ali, S., Muhammad, A., 2017. Isolation and Identification of Protease Enzyme Producing *Bacillus subtilis* from Soil. PSM Microbiol., 2(1): 15-19.

INTRODUCTION

Bacillus subtilis is commonly recovered from water, soil, air and decomposing plant residue. But the main habitat of *B. subtilis* is soil and a long term survival is expected to occur in soil (Versar, 1992). Population level of *B. subtilis* in soil is 10^6 to 10^7 per gram of soil (Alexander, 1977). The cell of *B. subtilis* may occur singly or in chains which may be of considerable length. The rods may have rounded or square ends may be quite small ($0.5 \times 1.2 \mu\text{m}$) or rather large ($2.5 \times 10 \mu\text{m}$). The cytoplasm may be vacuolated or may be stain uniformly. The cell may contain parasporal bodies or protein crystals. The form of endospores is usually cylindrical or ellipsoidal or oval or round (Claus and Berkeley, 1986).

B. subtilis is potentially able to form resting cells after the end of exponential growth or if vegetative cells are transferred from rich to poor medium. The spores differ from vegetative cells in many respects like optical

refractility, ultrastructure and chemical composition and resistance to chemical and physical stresses which lead to vegetative cells being killed rapidly (Hobbs and Cross, 1983). *Bacillus* strains do not form endospores under all cultural conditions. Most *Bacillus* strains form endospores on nutrient agar supplemented with 10 – 50 mg manganous salts per liter of medium but may lose these property or sporulate only with a low frequency. Such cultures are referred as oligosporogenous (Osp.). Other factors affecting endospore formation include the temperature of growth, the pH of medium, aeration, presence of minerals and presence of certain carbon and nitrogen compounds and concentration of carbon and nitrogen sources. Formation of endospore is mutliaphasic process which is similar in all *Bacillus* strains so far studied: Stage 0 – vegetative cell growth; Stage I – presepation, the DNA forms an axial filament; Stage II – septation, separation of chromosomes resulting in asymmetric cell