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Isolation and Identification of Esterase Producing *Bacillus subtilis* from Soil

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Abstract
Bacillus subtilis is most widely used bacteria for the production of different enzymes and chemicals. The aim of this study was to isolate the esterase enzyme producing bacterial strains from soil samples in Lahore, Pakistan. A total of thirty soil samples collected from different industrial areas were examined microscopically. Starch hydrolysis test was performed to confirm the gram positive bacteria *Bacillus subtilis*. Primary screening of esterase activity on agar plates was done with tributyrin substrate and clear zones or opaques around the colonies indicated this activity. The bacterial colonies range from 1 to 2mm in diameter. Bacterial colonies isolated from all samples were purple in color and gram positive while sample number 10 and 26 were slightly positive and appeared cocci in shape under microscope. The spores appeared green and oval in shape. These spores were observed in all samples but absent in sample 10 and 26. The colonies were creamy white, shiny white or dull yellow in color. Colonies were irregular, wrinkled, sticky, serrate, umbonate, opaque, circular, rounded flat in appearance. Positive zone formation in tributyrin substrate confirmed the production of esterase enzyme by *Bacillus subtilis*. Sample number 10, 16, 18 and 26 showed negative zone formation test. It might be because the soil samples were collected from the site where the chemicals from the industry are falling on the soil. The chemicals might have killed some of the microorganisms in the soil.
Keywords: *Bacillus subtilis*, industrial areas of Lahore, tributyrin substrate, zone formation test, esterase enzyme, soil.

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INTRODUCTION

Bacillus subtilis are rod-shaped bacteria that are Gram-positive (Perez, 2000). It has the ability to form a tough, protective endospore, so the organisms tolerate environmental conditions. *B. subtilis* is capable of forming highly resistant dormant endospores in response to nutrient deprivation and other environmental stresses. These spores are easily made airborne and dispersed by wind (Merrill et al., 2006). *B. subtilis* has been classified as an obligate aerobe. *B. subtilis* inhabits the gut and should be considered as a normal gut commensal (Hong et al., 2009). The vegetative form of *B. subtilis* is prevalent in nutrient rich environments such as the rhizosphere. The cell wall forms the barrier between the environment and the bacterial cell. It is also responsible for maintaining the shape of the cell and also withstanding the high internal turgor pressure (Schaechter, 2006).

Bacillus subtilis strains can act as biofungicides for benefiting agricultural crops and antibacterial agents. *Bacillus subtilis* also reduces mild steel corrosion. *Bacillus subtilis* bacteria are non-pathogenic. They can contaminate food; however, they result in food poisoning. They are used on plants as a fungicide. *Bacillus subtilis* use as a fungicide fortunately does not affect humans. Some strains of *Bacillus subtilis* cause rots in potatoes. Some strains related to *Bacillus subtilis* are capable of producing toxins for insects. Those strains can also be used for protecting crops as well (Inatsu *et al.,* 2006).

Lipases act mainly on triacylglycerols composed by long chain fatty acids whereas esterases preferentially hydrolyze triacylglycerols composed by short chain fatty acids. Microbial lipases have a wide range of enzymatic properties and substrate specificities, making them very useful for industrial applications, such as the processing of fats and oils, additives, detergents, cosmetics, paper manufacturing, and pharmaceuticals. Digestive aids, treatment of malignant tumors,

