Mohamed Seddik Benyahia University of Jijel
Faculty of Nature and Life Sciences
Department of Applied Microbiology and Food Sciences
Master I Applied Microbiology
Course: Industrial Microbiology (IM)

Lab. 3 Isolation and Primary Screening of Spore-Forming Bacteria Producing Protease

Introduction

In general, microorganisms are the most important source of proteases, as they can be cultivated on a large scale in a relatively short time using fermentation methods. This also provides a stable and abundant supply of the desired products. Moreover, microbial proteins have a long shelf life and can be stored under less-than-ideal conditions for weeks without significant loss of activity. Microorganisms produce various types of proteases, which can be either intracellular and/or extracellular. Intracellular proteases play a crucial role in various cellular and metabolic processes, such as sporulation and differentiation, protein turnover, enzyme and hormone maturation. Certain extracellular proteases are essential for the hydrolysis of environmental proteins, allowing the organism to absorb and utilize the hydrolysis products, while others act as toxins or factors involved in virulence.
Proteases of various types have applications in numerous industries, particularly in detergents, as well as in the food, pharmaceutical, medical, leather industries, and even in bioremediation processes.

Objective

The objective of this practical session is to isolate and select, through qualitative screening, a number of spore-forming bacteria from soil that are capable of producing a proteolytic enzyme.

Required Materials

- Soil samples
- Normal saline
- Nutrient agar containing 1% skimmed milk (for detecting protease)
- Storage agar
- Nutrient agar
- Sterile tubes
- Sterile toothpicks
- Sterile graduated pipettes
- Micropipettes with sterile tips
- Spreader
- Water bath at 80°C
- Incubator at 37°C

Experimental Protocol

- In a sterile glass tube, add 10 ml of sterile normal saline to 1 g of soil sample, and securely close the tube.
- Heat the tube in a water bath at 80°C for 10 minutes.
- Allow it to cool, then perform a serial dilution in sterile normal saline (up to 10^{-6}).
- From the last dilution tube, inoculate skimmed milk agar plates by spreading.
- Incubate at 37°C for 24 hours or longer.
- The presence of a clear hydrolysis zone around a colony indicates protease production.
- Subculture and further purify the protease-producing strains on nutrient agar, and store them on storage agar at 4°C.

Pr H. Ouled Haddar