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Course: Fermentation Microbiology

Lab. 4 Effect of medium composition and culture method on protease production by *Bacillus sp.*

Introduction

Proteases represent one of the most significant groups of industrial enzymes, widely applied in food processing, detergents, pharmaceuticals, and leather industries. Among microbial producers, *Bacillus* species are recognized as efficient sources of extracellular proteases due to their high secretion capacity and stability under various conditions. The synthesis of these enzymes is a growth-associated and inducible process that depends on the physicochemical properties and nutrient composition of the culture medium. Parameters such as carbon and nitrogen sources, mineral content, pH, temperature, water activity and aeration play a crucial role in regulating protease production.

Objective

This lab session aims to study how different culture media and methods (submerged and solid state fermentation) impact the production of protease by *Bacillus* sp., in order to determine the optimal conditions for enzyme yield.

Required materials

- *Bacillus* sp. strain (previously isolated and selected, fresh active culture)
- Nutrient broth and sterile normal saline for inoculum preparation
- Four different media :
  - Nutrient Broth (rich and complex)
  - Basal Medium II (chemically defined)
  - Wheat bran (autoclaved) (20% moisture)
  - Wheat bran–based poultry feed (autoclaved)(20% moisture)
- Folin–Ciocalteu protease assay reagents (see lab protocol 3)
- Sterile Erlenmeyer flasks
- Shaker incubator
- Static incubator for solid-state fermentation
- Centrifuge
- Spectrophotometer (for enzyme assay)
- Gauze and funnels for solid extraction
- Sterile filter paper Whatman
- Gloves

Experimental protocol

Use aseptic technique throughout. Run all conditions in triplicate.

1. Inoculum preparation

From a freshly streaked plate, pick a single colony of *Bacillus* sp. and inoculate 20 mL Nutrient Broth; incubate for 18 h at 37 °C, 180 rpm to obtain an active overnight culture.
Measure optical density (OD_{600}). Adjust cell density to obtain a standard inoculum (1×10^8 CFU·mL^{-1} or $OD_{600} \approx 0.8$) using sterile normal saline.

2. Fermentation protocol

* Submerged fermentation (SmF) (Nutrient Broth and Basal Medium II)

Inoculate each flask (250 ml) with 1 mL inoculum into 50 mL medium (2% v/v).
Incubate flasks at 37 °C, 180 rpm for 24 hours.
At the end of the incubation period : withdraw an aliquot (5mL), centrifuge at 6,000 × g for 10 min at 4 °C to remove

cells. Collect supernatant (crude extracellular enzyme) and keep at 4 °C until assay.

* Solid-state fermentation (SSF) (Wheat bran and Wheat bran–based poultry feed)

Substrate preparation: weigh 50 g of substrate into 250 mL Erlenmeyer flasks. Adjust moisture content to 20% (w/w) using sterile distilled water. Mix thoroughly to distribute moisture.

Sterilize substrates by autoclaving (121 °C, 20 min).

Add 1 mL of inoculum per 50 g substrate (2% v/w) and mix aseptically.

Incubate statically at 37 °C for 24 or 48h.

At the end of the incubation period, add 10 ml distilled water to the fermented substrate, shake vigorously at room temperature, then filter through sterile gauze and centrifuge filtrate at 6,000 × g for 10 min. Collect supernatant (crude enzyme extract).

4. Protease activity assay

Measure the protease activity of the four obtained crude enzyme extracts according to the method described in lab session 3.

Calculate the enzyme activity based on the previous standard curve.

Plot protease activity (IU) versus type of medium and culture mode, compare and assess the effect of culture medium on protease production.