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Research Article

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Production and analysis of protease from *Aspergillus niger* using fish scales as substrate

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ABSTRACT

Industrial enzymes are produced by various organisms using a wide variety of substrates. The present study was carried out to investigate the production of protease by Aspergillus niger using fish scale as the growth media. Fish scale, the chief waste material of fish processing industries, was enzymatically hydrolysed by protease produced by A. niger. Two mutated strains of A. niger, namely, AB30 and AB60 were also used apart from the wild type A. niger strain. Trichloroacetic acid buffer was used to maintain the optimum moisture content of the substrate. No separate nutrient sources apart from the fish scales were used. This proves the cost effectiveness and efficiency of this process. The enzyme activity was analyzed from the 5th day and it was further analyzed for three consecutive days and the results were plotted and analyzed.

Keywords: protease, fish scales, enzyme activity

INTRODUCTION

Proteolytic enzymes are ubiquitous in occurrence, being found in all living organisms and essential for cell growth and differentiation. The extracellular proteases are of commercial value and find multiple applications in various industrial sectors. Although there are many microbial sources available for producing proteases, only a few are recognized as commercial producers[1]. Annually over 100 million tons of fish are harvested worldwide, and about half of the total catch is discarded as processing waste[2]. Proteolytic enzymes from microorganisms may be located within the cell (intracellular), cell wall associated (periplasmic), or excreted into the media (extracellular)[3]. Extracellular enzymes are usually capable of digesting insoluble nutrient materials such as cellulose, protein and starch, and the digested products are transported into the cell where they are used as nutrients for growth[4,5].

Proteases, which account for about 60% of total enzyme market and among the most valuable commercial enzymes, are the single largest class of enzymes occupying a pivotal position due their wide application in the industrial processes[6, 7]. Plants, animals, algae and microbial sources are employed for protease production[8, 9]. Microbes serve as the preferred source of proteases because of their rapid growth, the limited space required for their cultivation, and the ease with which they can be genetically manipulated to generate new enzymes with altered properties. Microbial extracellular alkaline proteases are important enzymes and are mainly used in detergents to facilitate the release of proteinaceous stains such as blood, milk, egg and meat. They account for approximately 40 % of the total worldwide enzyme sale. Some extracellular enzymes are used in the food, dairy, pharmaceutical, and textile industries and are produced in large amounts by microbial synthesis[10, 11]. The major uses of free proteases occur in dry cleaning, detergents, meat processing, cheese making, silver recovery from photographic film, production of digestive and certain medical treatments of inflammation and virulent wounds[12].

Several attempts have been made to improve enzyme production through *A.niger* by strain selection using mutagenesis classical screening techniques[13,14,15]. Traditionally, strain development requires painstaking lengthy

and tedious procedures to identify superior isolates among a mutagen-treated population. Rational selection procedures are considerably more efficient than random screening for selecting improved producers, and usually have a biochemical basis. Special environmental condition, toxic to the majority of cell types but less toxic or non-toxic to a desired minority of cells have been often employed to enrich a cell population to obtain desired mutants[16,17]. The great advantage of this screening method is its simplicity; it does not require any profound understanding of molecular biology and physiology of the micro-organisms being manipulated. The main objective of this study was to determine the protease activity from *A. niger* grown on powdered fish scales and to compare the enzyme activity of the wild-type and the two mutated strains of *A. niger*.

EXPERIMENTAL SECTION

Test organism

A. niger wild type strain, obtained from infected onions, was used for the present study.It was cultured on potato dextrose agar (PDA) and stored at 4ºC in a refrigerator. From the pure culture mutant varieties of *A. niger* were obtained by exposing the colonies to 30 seconds (AB30) and 60 seconds (AB60) of UV radiation.

Processing of Fish Scale

In the present study, fish scales were collected from market and washed with deionised water, dried in mechanical drier for 4 h at 100°C. Then, after adding required quantity of distilled water, scales (1g scale/ml of water) were steamed for 30 min prior to fermentation. This was required to denature the collagen structure helps to moisture uptake and improves the diffusibility of enzyme in scale substrate to increase its susceptibility to hydrolysis[18]. After steaming, scales were dried in a mechanical drier and then crushed to almost powdered form. Crushed scale (5 g) was used in fermentation experiment.

Medium and culture conditions

Three sets of conical flasks, each set having three conical flasks of 150ml volume were taken. Surface solid state culture fermentation was carried out using 150 ml conical flasks. The powdered fish scales were taken and in each of the flasks 5g were added. TCA was added in all the flasks to wet the powder and also to act as a buffer. The above media was usedto grow all the three types of *A. niger* chosen. The enzyme activity was checked starting from the 5^{th} day for three consecutive days.

Enzyme Assay

After fermentation, mycelium was separated from the media by filtration through Whatman No. 1 filter paper and 0.5 ml of 1:10 diluted clear filtrate was taken to determine protease activity. The enzyme activity was determined spectrophotometrically using casein as a substrate[19]. One unit of enzyme activity was defined as the amount of enzyme liberating one µ mole of tyrosine/ml/min under the defined conditions.

$$\text{Units/ml Enzyme} = \frac{(\mu\text{mole tyrosine equivalents released})}{(1) \times (10) \times (2)} \times (11)$$

11= Total volume (in milliliters) of assay
10= Time of assay (in minutes) as per the Unit definition
1= Volume of Enzyme (in milliliters) of enzyme used
2= Volume (in milliliters) used in Colorimetric Determination

RESULTS AND DISCUSSION

In this present study protease activity of *A. niger* on fish scales was estimated. The solid state fermentation set up is shown in Fig 1.

Fig 1. Set up of Solid state fermentation

The optical density of the enzyme samples were measured spectrophotometrically at 660nm and the results are tabulated in Table 1. The results are also represented in the form of a bar diagram as shown in Fig 2. Based on the data from Table 1, it can be deduced that on Day 1, the AB60 strain of *A. niger* has the highest enzyme activity which can be attributed to its increased growth rate. AB30 has the least activity while the wild-type has the second fastest growth rate as well as protease enzymatic activity.

Table 1: Protease activity

Stage	*A. niger* wild type	*A. niger* AB30	*A. niger* AB60
Day 5	0.204	0.076	0.942
Day 6	1.282	0.51	1.482
Day 7	2.496	1.826	2.122

Note: AB30 denotes the mutated Aspergillus niger by exposure to 30 seconds of ultraviolet radiation. AB60 denotes the mutated Aspergillus niger by exposure to 60 seconds of ultraviolet radiation.

On day 2 however the growth and enzyme activity of the wild-type has comparatively increased and on the 3rd day the wild-type is found to have the highest enzyme activity. It can be observed that though the growth rate of AB60 was initially the highest and also its enzyme activity, the wild-type has caught up on the third day and even had seen a higher enzyme activity on day 3. AB30 has the lowest growth rate as well as enzymatic activity as well. Therefore it can be concluded that overall AB60 has the highest growth rate while the wild-type has the highest enzymatic activity.

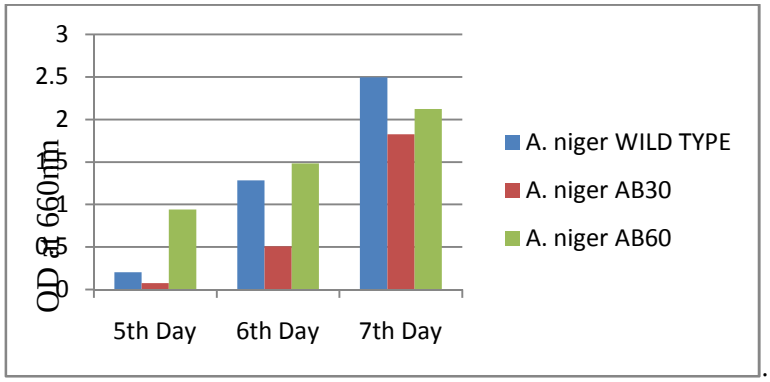

Fig.2 Protease activity

CONCLUSION

Thus three types of *A. niger* strains were cultures on fish scales and enzyme activity for three days were recorded and analysed. The growth rate of *A. niger* varied depending on the strain type that was used. This difference can enable one to select a strain of optimum growth rate for large scale production of protease.

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