JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue

JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)

An International Scholarly Open Access, Peer-reviewed, Refereed Journal

PRODUCTION AND ANALYSIS OF PROTEASE FROM ASPERGILLUS NIGER USING FISH SCALES AS SUBSTRATE.

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Abstract : The ultimate aim of this study is to evaluate the production of protease from Aspergillus Niger under solid state fermentation by utilizing black spotted croaker fish (Ghol fish) scales powder as a solid substrate Fish scale, the chief waste material of fish processing industries, was enzymatically hydrolysed by protease produced by A. Niger. 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 these processes. The O.D was taken at 275 nm spectrophotometrically. Solid-state fermentation (SSF) is recognized as an effective method to produce protease. The ultimate aim of this study is to optimize the production of protease from Aspergillus Niger under solid-state fermentation (SSF) by utilizing shrimp shell powder as a solid substrate. It was found that the produced protease from SSF was slightly alkaline. The correlation between factors operating parameters (incubation temperature, inoculums size, moisture content) for enzyme production is analyzed using statistical software.

Keywords: proteases,A.niger,Proteases application,chymosin,therapeutic agents.

I. INTRODUCTION

Proteolytic enzymes catalyzed the cleavage of peptide bonds in their protease; proteolytic enzymes can be classified as acidic natural and alkaline protease with the regard to their PH working range. Natural and alkaline proteases hold great potential for application in the detergent and leather tanning industrial due to increasing trend in developing environment friendly technologist. Alkaline proteases have numerous applications in food industry(M.Ravi.et al , Int.J.Curr.Microbiol.App.sci,2015).

Proteases occupy a central position in commerce, accounting for nearly 65% of the global enzyme market. They are used extensively in the detergent, leather, pharmaceutical, and food industries. Food applications of proteases include their use in cheese-making, beer clarification, protein hydrolysate production, pharmaceutical, and cosmetic industries. Acid proteases find application in the production of seasoning materials, protein hydrolysate, fermentation of soy sauce, and as digestive aids (Ahmed.M.,Medcrave,2018)

Filamentous fungi are exploited for the production of industrial enzymes due to their ability to grow on solid substrate and produce a wide range of extracellular enzymes. (K.s vishwanatha. et al, Int .J.Microbiol ,2009)Among the many advantages offered by the production of enzymes by fungi are low material costs coupled with high productivity, faster production, and the ease with which the enzymes can be modified. Further, the enzymes, being normally extracellular, are easily recoverable from the media. Although several reports have appeared recently about isolation of acid proteases from different fungi, Aspergillus oryzae is an organism of choice, due to it's generally regarded as safe (GRAS) status. (Paula Monterio.D et al, Brazilian journal ofMicrobiology,2015).

II. MATERIALS AND METHODS

1. SAMPLE COLLECTION:-

• Sample of Ghol fish (Protonibea Diacanthus) scale Collected from local fish market.

2. PREPARATION OF MATERIALS:-

- Media preparation: - potato dextrose agar media was prepared and an A Niger organism was cultured for Spore suspension.
- TCA (Trichloroacetic acid) preparation: - 5g TCA + 100ml distilled water.
- Potato Dextrose Agar: - To prepare potato infusion, boil 200 g sliced, unpeeled potatoes in 1 litre distilled water for 30 min. Filter through cheesecloth, saving effluent, which is potato infusion (or use commercial dehydrated form). Mix with Dextrose, Agar and Water and boil to dissolve. Autoclave 15 min at 121°C. Dispense 20-25 ml portions into sterile 15 × 100 mm Petri dishes. Final pH, 5.6 ± 0.
- Saline Preparation:- 0.48 g NaCl + 100 ml distilled water + autoclave
- Spore Suspension:- Saline + A .Niger culture

III. PROCEDURE:-

Test organism:-

A. Niger was obtained from infected onion. It was cultured on potato dextrose agar and stored at 4°C in a refrigerator.

Processing of fish scales:-

For this study, ghol fish scales were collected from market and washed scale with distilled water. Then Keep for drying in sunlight.

After drying the fish scales crush the fish scales into fine powder

Medium and culture condition:-

Take 2 conical flask having 250 ml volume. Take 2g of fish scale powder in flask. Then Add 8 ml of TCA. And add 2 ml of spore suspension after that Incubate for 5 days after incubation; add 50 ml of distilled water then Content shaken for 30 min Solution filtered through Whatman filter paper Supernatant was collected. Centrifuge at 4,400 rpm at 4°C for 20 min. Use supernatant as enzyme assay .(Bacteriology, Analytical Manual, 8th edition,1998)

Protease assay:-

Table no .1 protease assay protocol

Test	Blank
3ml casein	3ml casein
0.5 ml enzyme	----------
Incubate for 10 min at 30°C in water bath	
3.2ml TCA	3.2 ml TCA
----------	0.5 ml distilled water
Incubate for 20 min at 37°C	
Sample centrifuged at 4000 rpm for 15 min	

Absorbance value of sample were measured using colorimeter at 660 nm

Note: - same protocol for protease assay were followed for mix culture

IV. CALCULATIONS :-

$$\text{Protease activity} = \frac{\text{Absorbance value of test – blank X 67}}{\text{10 X Volume of enzymes}}$$

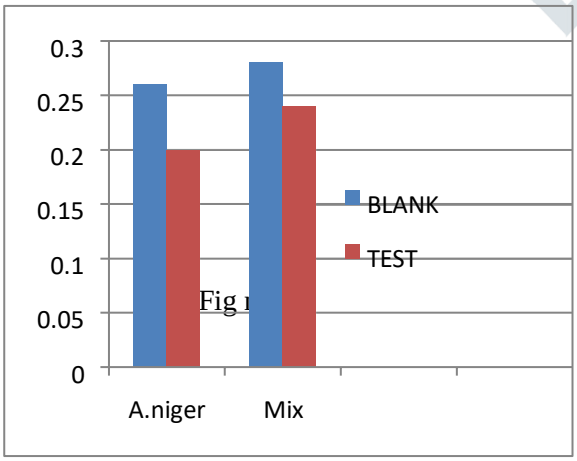
67 = dilution factor.
10 = time of assay (in min) as per unit definition.

V. RESULTS AND DISCUSSION :-

In this study, solid state fermentation was performed for the production of protease. The mucor formation of A. Niger was formed on the surface of the medium during fermentation.(Anson .ML,1938) The optical densities of the enzyme sample were measured on colorimeter at 660nm. And the colorimeter readings are tabulated in Table 1. The results are also represented in the form of bar diagram as shown in fig1. (Ramya.G., journal of chemical and pharmaceutical research,2015).

Based on the calculation that is tabulated in Table 2.and also the bar diagram shown in fig 2

We can deduced that mix culture has shown high enzyme activity which attributed to its increased growth rate. As Aspergillus Niger has lower growth rate as well as least enzyme activity Microbial proteases have a number of commercial applications in industries like food, leather, meat processing and cheese making (sidra.A .etal 2006). A major commercial use is the addition of microbial proteases to domestic detergents for the digestion of pertinacious stains of fabrics (Sharma et al., 1980). It has been reported that the production of extracellular proteases by different microorganisms can be strongly influenced by the culture conditions. (Ravi.G , Research Gate, 2015). So, it becomes necessary to understand the nature of proteases and their catalytic potentiality under different conditions.(Michael.O.etal, Advance in enzyme research, ,2019).Protease production by microbial strains strongly depends on the extra-cellular pH because culture pH strongly influences many enzymatic processes and transport of various components across the cell membranes, which in turn support the cell growth and product production (Elliah et al., 2002). Aspergillus flavus showed maximal protease production at pH - 4 (Fig. 2). Identical observations were earlier recorded in A. flavus, A.oryzae and A. candidus at pH 4.0 (Nasuno and Onara, 1972; Dworschack et al., 1952) (Jordan journal biological science,ISSN: 1995).



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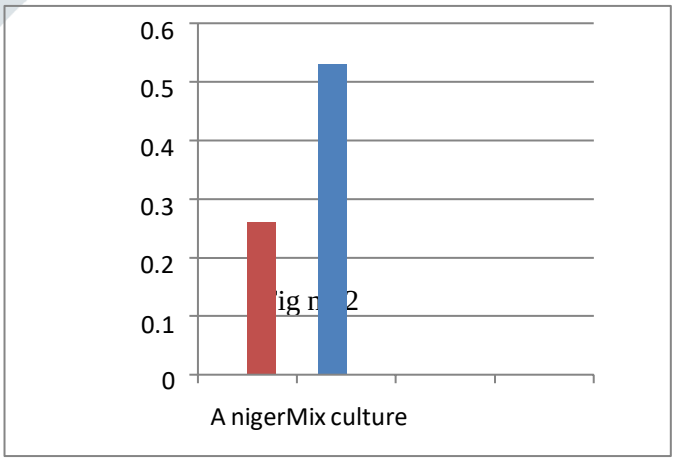

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VI .CONCLUSION:-

Proteases break peptide bonds. It is often necessary to measure and/or compare the activity of proteases. This nonspecific protease activity assay may be used as a standardized procedure to determine the activity of proteases for quality control purposes. Chee .K.O et al ,2021).In this assay, casein acts as a substrate. When the protease we are testing digests casein, the amino acid tyrosine is liberated along with other amino acids and peptide fragments. Industrial enzymes are produced by various organisms using a wide variety of substrates.(Journal of chemical and pharmaceutical research, October 2012) 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. This study found out that the enzyme solution produced from Aspergillus Niger under SSF was slightly alkaline. The enzyme produced was also tested for enzyme activity. The results indicate that it possessed protease activity.(Marguerite kamdem simo et al. Biomed Res Int,2023)

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