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INFLUENCE OF MEDIA COMPOSITION ON ACID PROTEASE PRODUCTION BY *Aspergillus niger* (NRRL 1785)

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ABSTRACT

Aspergillus niger (NRRL 1785) has been reported as a good producer of acid protease with optimum pH of 4.0 using glucose as the sole carbon source and peptone as sole nitrogen source. The production of acid protease by *A. niger* (NRRL 1785) using other carbon sources (lactose, sucrose, starch, maltose and glycerol) to substitute for glucose and other nitrogen sources (urea, casein, beef extract, groundnut meal and corn steep liquor) to substitute for peptone were studied. The effects of media composition on acid protease production were determined. All the fermentation media with different carbon and nitrogen sources tested supported acid protease production. Peak of enzyme production for all media composition was at the 96th hour of fermentation though at varying yields. Lactose supported the maximum production of 23.80 Uml^{-1} amongst the tested carbon source (glucose substitutes) while beef extract supported maximum protease production amongst peptone substitutes with protease activity of 19.95 Uml^{-1}. None of the media tested measured up to the protease production in the control medium containing glucose as carbon source and peptone as nitrogen source, which gave protease activity of 26.05 Uml^{-1}. The result of this study gives a strong indication that *A. niger* (NRRL 1785) is capable of utilizing different carbon and nitrogen sources for acid protease production. Economically, this strain seems to be promising as an alternative microorganism for acid protease production.

KEYWORDS: Acid protease, *Aspergillus niger* (NRRL 1785), fermentation media.

INTRODUCTION

Microorganisms produce a large variety of enzymes, most of which are made in only small amounts and are involved in cellular processes. 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 (Oh *et. al.*, 2000). Proteases are one of the most important groups of industrial enzymes and account for nearly 60% of these enzymes are obtained from animal and plant as well as microbial sources (Rao et. al., 1998). Acid proteases have been isolated and characterized from mammals, plants, fungi, yeast, retrovirus and bacteria (Wu and Hang, 1998).

The major applications of proteases are in the food, diary and pharmaceutical industries (Nout and Rombouts, 1990, Sutar et al., 1986). Although bacterial proteases have long been used in industry, the main drawback in their use is that they require cost- intensive filtration methodologies to obtain a microbe-free enzyme preparation. On the other hand, the proteases of fungal origin offer an advantage in that the mycelium can be easily removed by filtration (Phadatare et al., 1993).

Several reports describe the efficient protease biosynthesis by fungi belonging to the general *Aspergillus* (Olajuyigbe, 2002; Fan-Ching and Lin, 1998; Boing, 1982), *Penicillum* (Chrzanowska *et. al.*, 1995), and *Rhizopus* (Farley and Ikasari, 1992). Investigations for production by many fungal cultures have shown that the production of proteases varies greatly with the media used and regulatory effects exerted by the carbon sources have been described (Oh *et. al.*, 2000). Many experiments have been carried out with microorganisms cultivated with glucose, as a substrate but there is little information on the use of other carbon sources as well as different nitrogen sources.

In our previous work, *Aspergillus niger* (NRRL 1785) was found to be a good producer of acid protease with optimum pH of 4.0 using glucose as the sole carbon source and peptone as sole nitrogen source (Olajuyigbe *et. al.*, 2003). The aim of this present work was to investigate acid protease production from *A. niger* (NRRL 1785) using other carbon sources such as lactose, sucrose, starch, glycerol and maltose to substitute for glucose. Acid protease production was also investigated using different nitrogen sources to substitute for peptone.

MATERIALS AND METHODS

Microorganisms

Aspegillus niger (NRRL 1785) was obtained from the culture collection from The Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria (FIIRO). The cultures were obtained on potato dextrose agar slants at 4°C.

Chemicals and reagents were products of Sigma Chemical Company, USA and Merck, Darmstadt, Germany. All reagents were of analytical grade.

Inoculum preparation

A. niger (NRRL 1785) spores were harvested from 5d old slants containing potato dextrose agar at 30°C. A stock suspension was prepared and adjusted to 1.0×10^5 spores ml^{-1} using a haemocytometer for counting. The solution was then stored at 4°C.

Fungal culture and growth conditions

The cultures were grown in fermentation media composed of the following per 100ml: 2% carbon source, 1% nitrogen source, 0.1% KH_2PO_4 and 0.3% yeast extract. The fermentation medium containing glucose as carbon source and peptone as nitrogen source was used as control media. For the different carbon sources (sucrose, lactose, maltose, starch and glycerol) tested, peptone was used as nitrogen source. Also, for different nitrogen sources (beef extract, casein, corn steep liquor, groundnut meal and urea) tested, glucose was used as carbon source. The pH of the fermentation media was adjusted to pH 4.0 with 0.05 M citrate buffer before autoclaving. The sterilized media were cooled at room temperature. 5ml inoculum was transferred into 250ml Erlenmeyer flasks containing 100ml of desired medium. The flasks were incubated at 30°C on a rotary shaker at 200 $revmin^{-1}$ for a period ranging from 24 hours to 120 hours.

The mycelia were harvested from the fermentation broth by filtration through Whatman No. 1 filter paper at 24hours interval over the 120 hour fermentation period and

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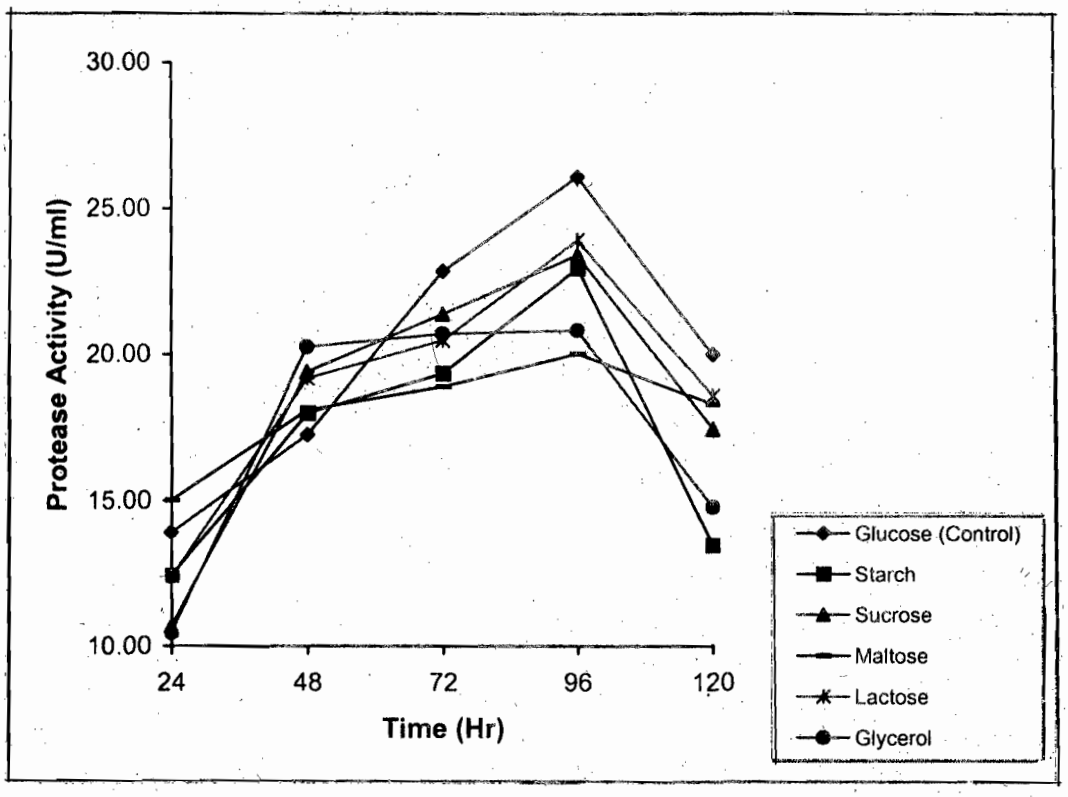

Figure 1: Influence of Media Carbon Sources on Acid Protease Production

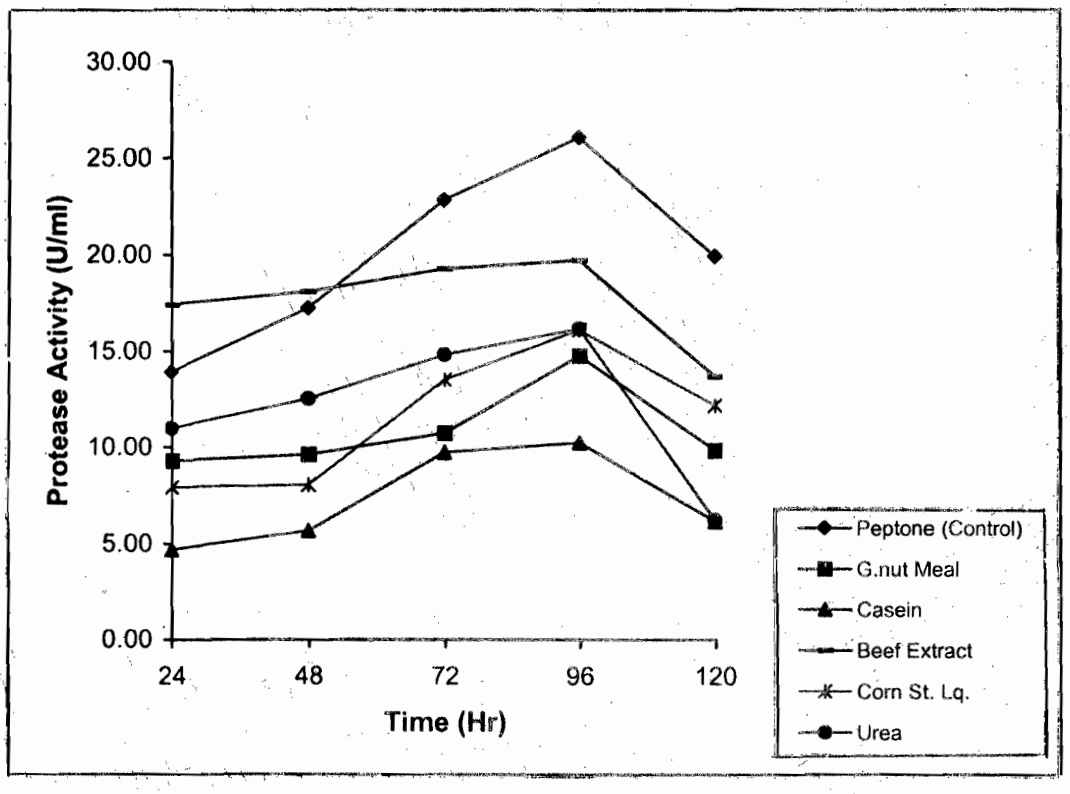

Figure 2: Effects of Media Nitrogen Sources on Acid Protease Production by *Aspergillus niger* (NRRL 1785)

the filtrate collected was the source of crude protease whose activity was monitored at 24 hours intervals to determine acid protease production by *A. niger* (NRRL 1785) over the entire fermentation period.

Analytical Procedure

Assay of Acid Protease Activity

The enzyme assay was based on a modified procedure of Wang and Hesseltine (1965). The reaction mixture contained 1ml of 1% casein, 0.4 ml of 0.05 M citrate buffer, pH 4.0 and 1.0 ml enzyme solution. 100 μgml^{-1} tyrosine served as standard. This was incubated at 50^0C for 15 minutes. The reaction was stopped by adding 3.0 ml of 5% trichloroacetic acid and the mixture was centrifuged at 5000 rpm for 15 minutes and the supernatant was collected. 4.0 ml of 0.4 M sodium carbonate was added to 1.0 ml of supernatant. Folin-Ciocalteau reagent (0.5ml) was added to the mixture and left for 10minutes for colour development. The absobance at 660 nm was measured with Biochrom 4060 uv/visible spectrophotometer (Pharmacia LKB). One unit of enzyme activity was defined as micrograms of tyrosine equivalents per minute per ml under assay conditions.

RESULTS AND DISCUSSION

The results obtained in this work revealed the ability of *A. niger* (NRRL 1785) to produce acid protease in the culture medium of different carbon sources tested in combination with peptone as illustrated in Figure 1. The peak of enzyme production was at the 96th hour of fermentation. All the carbon sources tested supported protease production from *A. niger* (NRRL 1785) in varying yields. Lactose supported the maximum protease activity of 23.80Uml^{-1} while maltose gave the lowest protease activity of 19.95Uml^{-1} at the 96th hour of fermentation. According to our observation, Yang *et. al.* (1999) studied the effect of carbon sources on the production of protease by *Bacillus subtilis* growing in shrimp and crab shell powder medium containing one of the additional carbon sources: glucose, lactose, carboxymethyl cellulose, D (-) arabinose, D (+) xylose and rice bran. They found that protease production was greatly enhanced by the addition of lactose or arabinose into the medium.

On the other hand, Phadatare *et. al.* (1993) evaluated various sugars such as glucose, fructose, lactose, maltose, sucrose, xylose and sugar alcohols, glycerol, mannitol and sorbitol for their effect on enzyme production. The results obtained revealed that sucrose gave the maximum protease activity. Glucose has been reported to suppress protease production (Sen *et. al.*, 1993; Sonnleitner, 1983), but in the present study, it was found that substituting glucose with other carbon sources resulted in decrease in enzyme production. Our observation showed glucose to be a substrate for enzyme production as observed in the control medium containing glucose as carbon source (Figure 1). The protease production in the control medium was 26.05 Uml^{-1}, this was higher than that observed with lactose (26.05 Uml^{-1}) which exhibited maximum enzyme production amongst the test media. Other workers have also reported better protease production in the presence of glucose as substrate (Gajju *et. al.*, 1996).

Also, results revealed that all the nitrogen sources tested in combination with glucose as sole carbon source supported acid protease production from *A. niger* (NRRL 1785) (Figure 2). The peak of enzyme production in all media investigated was at the 96th hour of fermentation. Beef extract gave the highest protease activity of 19.35Uml^{-1} while casein gave the lowest protease activity of 10.00Uml^{-1}. The reason for this might be due to the beef extract which is a complex media providing nitrogen, vitamins, amino acids and other growth factors aiding the production of protease enzyme by *A. niger* (NRRL 1785) while casein is a protein which had to be broken down into simpler peptides and consequently amino acids before it could be taken up by the fungus for growth and protease production. These results agree with reports by Phadatare *et. al.*, (1993) and Aleksieva *et. al.* (1981) that organic nitrogenous compounds are good nitrogen sources for growth and protease production in organisms. Similarly, Griffin, (1981) reported that organic nitrogen sources induce the production of acid protease.

The production of acid protease, which was higher in the control medium containing peptone as nitrogen source and glucose as carbon source suggests that *A. niger* (NRRL 1785) utilized amino acids for biosynthesis and production of acid protease. Peptones are pepsin hydrolysates thus, mixture of amino acids. Deacon (1988) reported that in terms of nitrogen nutrition, the generalization can be made that all fungi utilize amino acids, most can utilize ammonium ion and fewer can utilize nitrate. The fact that *A. niger* (NRRL 1785) utilize urea for acid protease production shows that this fungus which made use of amino acids for biosynthesis also had the ability of utilizing ammonium ion for its biosynthesis and enzyme production.

Considering the interest of this study in investigating the influence of media composition on acid protease production from *A. niger* NRRL 1785, it is not advantageous for economic application to utilize maltose and sugar alcohol such as glycerol as carbon sources or casein and groundnut meal as nitrogen sources for protease production from *A. niger* (NRRL 1785).

Various microorganisms and cultivation media have been studied for protease production (Gibb *et. al.* 1987). The results presented here show that *A. niger* (NRRL 1785) is capable of utilizing different carbon and nitrogen sources tested for acid protease production. This is a strong indication that *A. niger* (NRRL 1785) is a promising alternative microorganism for acid protease production.

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