African Journal of Biotechnology Vol. 8 (17), pp. 4197-4198, 1 September, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Short Communication

Production of protease by *Aspergillus* sp. using solid-state fermentation

Shirish Rajmalwar* and P. S. Dabholkar

G.H. Raisoni Institute of interdisciplinary sciences, Department of Microbiology and Biotechnology, Nagpur - 440016, India.

Accepted 17 August, 2007

Assessment of six oil seed cakes as substrate for production of protease by solid state fermentation by *Aspergillus sp.* isolated from leather. The agro-industrial by product (oilseed cakes) namely soybean, groundnut, sesame, linseed, mustard and cotton were evaluated as inducer for production of protease using solid state fermentation (SSF) in the presence of defined diluent as moistening agent was investigated. Enzyme synthesis was maximum (107.66 U/ml) when soybean oil seed cakes was used as substrate followed by sesame oilseed cake (76.04 U/ml). Protease production using such low cost substrate provide economical efficacy.

Key words: Protease, *Aspergillus*, substrate, moistening agent, fungi.

INTRODUCTION

Protease is essential constituents of all forms of life on earth including prokaryotes, fungi, plant and animals. Proteases are highly exploited enzymes in food industry, detergent, pharmaceutical, diagnostic and waste management. Proteolytic enzymes are the most important industrial enzymes, representing worldwide sale at about 60% of total enzyme market (Woods et al., 2001).

Extracellular enzymes are usually capable of digesting insoluble nutrient material such as cellulose, proteins and starch (Aniflousa et al., 2002). Proteases of fungal origin have an advantage over bacterial protease as mycellium can be easily removed by filtration. Protease produced by *Aspergillus* sp. is of greater importance due to its higher protease producing ability. Solid-state fermentation (SSF) has many advantages including superior volumetric productivity, use of inexpensive substrate, simpler downstream processing, lower energy requirement and low wastewater output (Malathi and Chakraborty, 1990).

The present study was undertaken to produce protease under laboratory conditions by solid-state fermentation of *Aspergillus* sp. using oil seed cakes as substrate, which are by product of oil industries. This procedure of protease production with the use of low cost substrate and cost effective method allows possible production and Application of crude enzyme for various industrial processes (Ikram-ul-Haq et al., 2003).

*Corresponding author. E-mail: shirish_ar@yahoo.co.in, Tel: 07152 (241399). Mobile: +919901310280.

MATERIALS AND METHODS

Growth conditions and extraction

The strain of *Aspergillus* sp. was isolated from leather source and maintained on potato dextrose agar (PDA) slants. The slants of 5 – 7 days old culture was wetted by adding 10 ml of distilled water; a homogeneous suspension was obtained by shaking for approximately 1 min. 1 ml spore suspension was used for inoculation. A 250 ml of conical flasks containing 10 g of substrates with 15 ml of moistening agent were sterilized at 121°C (15 1bs/inch pressure), cooled, inoculated and incubated at 30°C. After incubation, 80 ml of distilled water was added to the culture flask, flask was shaken for 14 h at 200 rpm. The content of flask was filtered and filtrate was analyzed for enzymatic activity.

Substrates and moistening agents

Different agro-industrial by products (oil seed cakes) of soybean, groundnut sesame, linseed, mustard and cotton seed were evaluated for protease production using the following moistening agents:

i) Diluent (% w/v): Glucose (7.0), peptone (2.0), KH_2PO_4 (0.4), $MgSO_4.7H_2O$ (0.05), $CaCl_2.2H_2O$ (0.05) and $ZnSO4.7H_2O$ (0.01)
ii) Distilled water.

The protease activity was measured by Folin -Ciocalteau Method in the terms of its action on casein and optical densities were read at 660 nm (see Ikram-ul-Haq et al., 2003).

Table 1. Activity of enzyme produced by using types of substrates.

Substrates (oil seed cakes)	Diluent (enzyme activity = units/ml)	Distilled water (enzyme activity = units/ml)
Soybean meal	36.03	5.90
Groundnut meal	8.22	3.93
Sesame meal	7.86	23.65
Linseed meal	10.93	5.46
Mustard meal	2.56	3.93
Cottonseed meal	0.079	3.83

Table 2. Best activity showing substrates were analyzed for the enzyme activities of crude extract by varying incubation period for maximum enzyme production.

Incubation Period	Soybean meal diluent (enzyme activity= units/ml)	Sesame meal distilled water (enzyme activity= units/ml)
48 h	13.76	13.76
72h	107.66	76.02
96h	21.63	20.73
120h	39.48	29.51
144h	36.18	23.18

RESULTS AND DISCUSSION

Types of substrates, moistening agents and varying incubation period are the key factors which influenced the out come of a solid-state fermentation (SSF) system. The fungal strain isolated from leather source was identified as *Aspergillus* sp. and used in further studies for production of protease using solid state fermentation. Different substrates (oil seed cakes) such as soybean, groundnut, sesame, linseed, mustard and cotton were evaluated for synthesis of protease (Table 1). During enzyme production, effect of varying incubation periods on enzyme production was analyzed. Soybean oil seed Cake gave maximum enzyme activity of 107.66 U/ml with defined diluent as moistening agent followed by sesame oil seed cake 76.04 U/ml with distilled water as moistening agent after 72 h of incubation period (Table 2).

The results indicate that protease produced by *Aspergillus* sp. using low cost substrate might be applied in future as depilatory agent in leather industry thereby providing eco-friendly substitute for conventional methods.

REFERENCES

Aniflousa A, Leonie AS, Kasutaka F, Makoto M, Kozako N, Galba MCT (2002). Production of extracellular protease by *M. circinelloides* using D. Glucose as carbon source / substrate, Braz. J. Microbiol. 23: 112-114.

Ikram-ul-Haq, Hamid M, Sikandar, Auador MA (2003). Production of protease by locally located mold culture under lab conditions, J. Biotechnol. 21: 30-36.

Malathi S, Chakraborty R (1990). Production of alkaline protease by *A. flavous* isolated under solid substrate fermentation conditions and its use as depilation agent, Am. Soc. Microbial. (22)18: 246-249.

Woods RG, Burger M, Beven C-A, Beacham IR (2001). The aprX-lipA operon of Pseudomonas fluorescens B52: a molecular analysis of metalloprotease and lipase production. Microbiology 147: 345-354.