View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Journal of Drug Delivery and Therapeutics (JDDT)

Available online on 15.05.2016 at http://jddtonline.info
Journal of Drug Delivery and Therapeutics
An International Peer Reviewed Journal
Open access to Pharmaceutical and Medical research
© 2016, publisher and licensee JDDT, This is an Open Access article which permits unrestricted noncommercial use, provided the original work is properly cited

RESEARCH ARTICLE

EFFECT OF FERMENTATION KINETICS FOR THE BIOSYNTHESIS OF PROTEASE FROM *ASPERGILLUS AWAMORI*

Radhika Pilli[1], *Siddalingeshwara K G[2]

[1]Research & Development Centre, Bharathiar University, Coimbatore, India

[2]Scientific & Industrial Research Centre, Bangalore, India

Received 19 April 2016; Review Completed 12 May 2016; Accepted 12 May 2016, Available online 15 May 2016

ABSTRACT

Among the various groups of microorganisms filamentous fungi are most widely exploited because of their ability to grow on complex medium and production of wide range of extracellular enzymes. This study highlights the production of extracellular protease biosynthesis were carried out by using *Aspergillus awamori* was evaluated under different fermentation kinetics by employing submerged fermentation method. The protease producers detected by the clear zone (casein hydrolysis) around the colony by simple plate assay method. *Aspergillus awamori* KGSR 12 was the potential strain among the fungal isolates. The Protease biosynthesis was increased their yield after the optimization of fermentation parameters. The optimum pH 6.0 (1.96 IU), temperature 35^{0}C (2.12 IU) and inoculum size 0.5 ml showed 2.23 IU.

Key words: Fermentation Kinetics, *Aspergillus awamori,* Protease, Plate assay.

INTRODUCTION

Microorganisms have been endowed with vast potentials of new biocatalysts (enzymes). They produce an array of enzymes, which have been exploited commercially over the years. In recent years, the potentials of using microorganisms as biotechnological sources of industrially relevant enzymes has stimulated interest in the exploration of extracellular enzymatic activity in several microorganisms (Jayani *et al*., 2005; Alva *et al*., 2007).

Proteases (EC 3.4.21-24) are enzymes that hydrolyze proteins and form a bulky cluster of enzymes which are ubiquitous in nature and most central category of enzymes from an industrial point of view. An extensive range of microorganisms has great potential to produce alkaline proteases under suitable growth conditions (Arulmani et al., 2007).

The industrial demand for highly active preparations of proteolytic enzymes with appropriate specificity and stability to extreme pH and temperature continues to stimulate the search for new enzyme sources. Although the majority commercial proteases originated from bacteria belonging to the genus *Bacillus*, fungi exhibit a wider variety of proteases than bacteria. In addition, fungi are normally GRAS (generally regarded as safe) strains and they produce extracellular enzymes, which can be recovered easily from the fermentation broth. Certain genera such as *Aspergillus, Penicillium* and *Fusarium* have been reported to produce neutral proteases (Sandhya et al., 2005).

Among the various groups of microorganisms filamentous fungi are most widely exploited because of their ability to grow on complex solid substrates and production of wide range of extracellular enzymes.

The aim of the present study was to evaluate *Aspergillus awamori* as a producer of protease under submerged fermentation by using designed medium with different fermentation kinetic parameter.

MATERIALS AND METHODS

Microorganism and maintenance

The fungi used in this study were collected from stressed environmental soils. The soils were taken from different regions of Bangalore (12^{0}59′ N latitude and 77^{0}35′ E longitude) of Karnataka. Fungal strains were isolated from soil samples through serial dilution method (Waksman, 1927). The stock cultures of the isolates were maintained by sub-culturing themon CzapekDox Agar slants and 120h to 168 h incubated cultures were stored at 4^{0}C.

Protease screening-Plate assay

The isolated strains were screened for their protease production by plate assay where protease producers were detected by the clear zone (casein hydrolysis) around the colony and were used for further studies. Totally thirty five fungal strains were isolated and the best protease producer fungal strain was identified as *Aspergillus awamori KGSR 12*. Tentatively identified in the laboratory and was confirmed by molecular level. The screening medium is as follows. Glucose,2; Skim

milk,0.5; KH_2PO_4,1.52; KCL,0.52; $MgSO_4.7H_2O$,0.52; $CuNO_3.3H_20$, trace; $ZnSO_4.7H_20$, trace $FeSO_4$, trace; agar, 20.0 and pH-5.0 (g/L distilled water) (Plate-1).

Inoculum preparation

The fungal inoculum was prepared by adding 10ml of 0.01% Tween 80 solution to 168 h culture slant and was suspended well with the sterile loop to obtain homogeneous spore suspension (Lingappa and VivekBabu, 2005). Each mL of spore suspension of inoculum containsa final concentration of 1x 10^7 spores/ml.

Fermentation Medium

The selected *Aspergillus awamori* KGSR 12 were cultured on production medium. The production medium consists (g/L of distilled water) Sucrose-30.0; Sodium nitrate-2.0; K_2HPO_4-1.0, $MgSO_4$. $7H_2O$-0.5; KCl-0.5; $FeSO_4$-0.01; pH 6.8 for 96 -120h.

Fermentation Kinetic studies for the biosynthesis of Protease

Initial pH

The 250 ml Erlenmeyer flasks containing 100 ml of production medium were prepared by mixed with acid/alkali solution to obtain required intital pH. The pH was adjusted in the range of 3-7 with increments of 1.0. Thus prepared flasks were cotton plugged and autoclaved at 121^0C for 15 min. The flasks were inoculated and incubated.

Temperature

The 100ml of the production medium was separately taken in 250 ml Erlenmeyer flasks and prepared for submerged fermentation. Thus prepared flasks were incubated at different temperatures like 25-40^0C with in increments of 5^0 C.

Inoculum size

The inoculum was prepared separately by reviving the 168h old culture of *AspergillusawamoriKGSR12* at different levels i.e., 0.25, 0.50, 0.75, 1.0 and 1.25 ml and then fermentation studies were carried out.

Extraction of protease from production medium

The culture broth samples of volume approx. 5 mL were withdrawn periodically at 24 hrs in aseptic condition. The extract was filtered through Whatman filter No.1. The clear extract was centrifuged at 2000-3000 rpm for 15 min, supernatant were used for enzyme preparation. Thus prepared crude enzyme was used for assay of protease.

Assay of Protease

The protease activity was determined by the modified method proposed by Keay et al.(1970). 0.5 ml of suitably diluted enzyme is added to 1.0 ml of 1% casein and 0.5 ml of glycine-NaOH buffer (25 mM, pH 10.0) whole mixture was incubated at 35^0C for 10 min. The reaction was terminated by the addition of 3 ml of 10% TCA solution. The solution was allowed to stand for 10 min in cool and was filtered. To the clear filtrate, 5 ml 0.4 M Na_2CO_3 and 0.5 ml of Folin Ciocalteau reagent (FCR) was added, mixed thoroughly and incubated at 75^0C for 30 min, in dark. The absorbance was measured at 660 nm.

International units (IU)

One protease unit was defined as the amount of enzyme that released 1 µg of tyrosine per ml per minute under the above assay conditions.

RESULTS AND DISCUSSION:

The Fungal isolates were identified tentatively as *Aspergillus awamori* in the laboratory which produced clear zone around colony in casein (0.5%) plate medium; those were selected from the soil samples. Of the thirty isolates, *Aspergillus awamori* KGSR 12 was considered to be the best and high protease producing strain. It showed 1.12 cm of cleared zone around the colony and 41.3% of hydrolysis. The results obtained in the present study on the effect of pH and temperature on submerged fermentation is represented in (Fig. 1 and 2) which reveals that the production of protease increased with the increase in pH of the medium up to pH 6.0 (1.96 IU), with increase in temperature 35^0C (2.12 IU) at 72nd hours of fermentation period and thereafter the decrease of protease was observed.

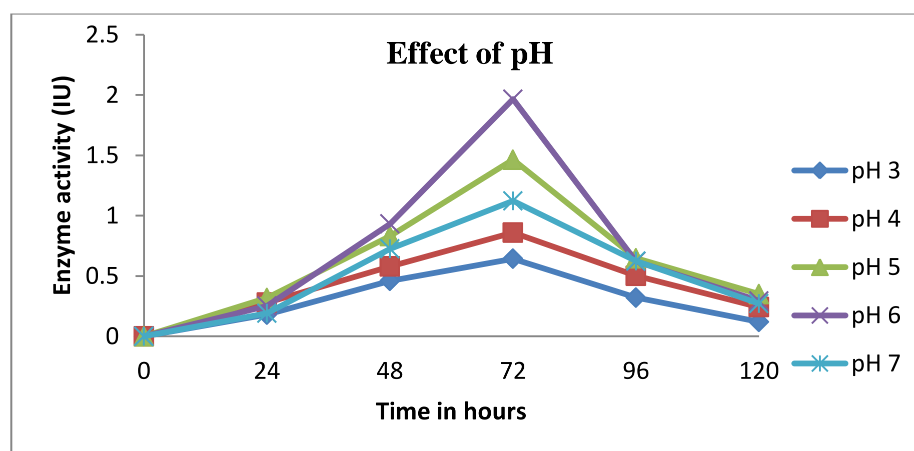

Figure 1: Effect of pH on Protease production

© 2011-16, JDDT. All Rights Reserved

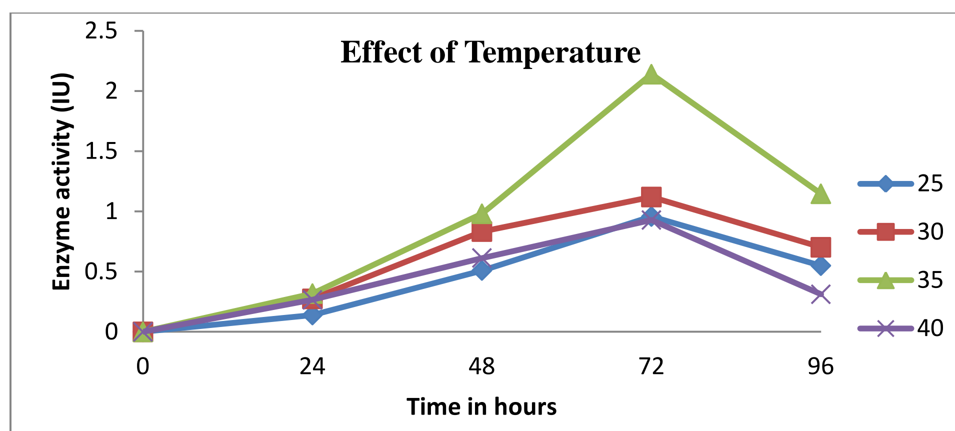

Figure 2: Effect of temperature on Protease production

The results reviewed that Francois *et al*. (2011), they showed that pH 6 was the suitable for maximum protease production by using *Aspergillus carbonarius* and it shows 0.14 IU. Karthic *et al.* (2014) were reported screening and production were carried out by using *Aspergillus oryzae* and they reported 1.7 IU at 72 hrs of fermentation period. Vishwantha and Appu (2010), who reported pH 5.0 as the best initial pH for the production of protease from *Aspergillus oryzae* MTCC 5341. Our results are good agreement with Karthic et al., (2014).

The temperature is an important environmental factor for the production of proteases by microorganisms because it affects growth rates of microorganisms, regulates the synthesis of the enzyme and also the enzyme production by changing the properties of the cell wall (Satynarayana et al., 1994). Similar observation were observed by Karthic et al., (2014) were reported that the production were carried out by using *Aspergillus oryzae* and they reported 1.36 IU at 72 hrs of fermentation period. Similar observations were reported by Francois et al., (2013), reported that the maximum production of protease was observed at temperature 37^0 C by using *Aspergillus terreus* and it showed approximately7 IU/ ml. Our results are coincides with Karthic et al., (2014).

The importance of inoculum size on microbial fermentation process is widely accepted. Out of five inoculum size tested (0.25, 0.50, 0.75, 1.0 and 1.25 ml) and 0.5 ml inoculum was found to be the most suitable for high production of protease by *Aspergillus awamori* KGSR 12 in submerged fermentation at 72 hrs of fermentation and it showed 2.23 IU. From Fig. 3, it is clear that the protease production steadily increased with the increasing in the size of the inoculum until it reaches to the magnitude when enzyme productivity became maximum, thereafter no appreciable change in production of protease with high inoculum size could be observed.

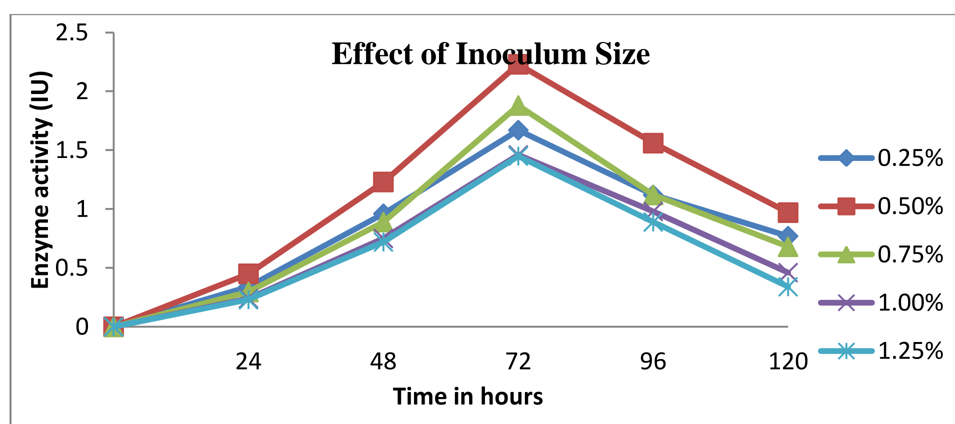

Figure 3: Effect of inoculums size on Protease production

Francois et al (2013) reported that 2% of fungal spores as an inoculum. The maximal protease production (9.021 PU/ml) was observed when an inoculum concentration of 2% fungal culture was added. At lower and higher inoculum levels, poor protease production was observed. Karthic et al., (2014) reported that the maximum enzyme production (1.7 IU/ ml) was observed in 1.0% of initial inoculum supplemented conditions. Our results are best agreements with the results of Karthic et al., (1994).

© 2011-16, JDDT. All Rights Reserved

REFERENCES:

1. Jayani RS., Saxena S. and Gupta R. "Microbial Pectinolytic Enzymes: A review." *Process Biochem.* 2005; 40 (9): 2931-2944.
2. Alva S., Anupama J., Savla J., Chiu, Y. Y. and Vyshali, P., *et al*. Production and characterization of fungal amylase enzyme isolated from *Aspergillus* sp. JGI 12 in solid state culture. *Afr. J. Biotechnol.* 2007; 6: 576-581.
3. Arulmani M, Aparanjini K, Vasanthi K, Arumugam P, Arivuchelvi M. and Kalaichelvan, P. T. Purification and partial characterization of serine protease from thermostable alkalophilic *Bacillus laterosporus*-AK1. World J. Microbiol. Biotechnol. 2007; 23:475-481.
4. Sandhya, C, Sumantha A, Szakacs G, Pandey A. Comparative evaluation of neutral protease production by A*spergillus oryzae* in submerged and solid state fermentation. *Process Biochem.* 2005; 40: 2689–94.
5. Waksman SA. Principles of soil microbiology, Batimore; William & Wilkins co. 1927.
6. Lingappa, K, Vivek Babu CS. Production of lovastatin by solid state fermentation of carob (Ceratoniasiliqua) pods using *Aspergillus terreus* KLVB28. *Ind. J. Microbiol.* 2005; 45:283-286.
7. Keay K, Moser PW and Wildi BS. Proteases of the genus *Bacillus*. II. Alkaline proteases. *Biotechnol. Bioeng.* 1970; 12: 213.
8. Niyonzima FN. and More SS. Screening and optimization of cultural parameters for an alkaline protease production by *Aspergillusterreus* gr. under submerged fermentation. *International journal of pharma and bio sciences.* 2013; 4(1): (B) 1016 – 1028.
9. Karthic J, Siddalingeshwara K.G, Sunil Dutt P.L.N.S.N, Pramod T, Vishwanatha T. An approach on isolation, screening and production of protease from *Aspergillus oryzae*. Journal of Drug Delivery & Therapeutics, 2014; 4(2):86-89.
10. Vishwantha K.S. and Appu Rao AG. Acid protease production by solid-state fermentation using Aspergillus *oryzae*MTCC 5341: optimization of process parameters. *J. Ind. Microbiol. Biotechnol.* 2010; 37: 129-138.
11. Satyanarayana T. (1994). Production of bacterial extra-cellular enzymes by solid-state fermentation, 1st Edn, Wiley Eastern Ltd.

For Correspondence

Siddalingeshwara K G

Scientific & Industrial Research Centre, Bangalore, India, Email: siddha_lingeshwar@rediffmail.com

How to cite this article:
Pilli Radhika, Siddalingeshwara K G, Effect of fermentation kinetics for the biosynthesis of protease from Aspergillus tamari, Journal of Drug Delivery & Therapeutics. 2016; 6(3):115-118