AJAB

Asian J Agric & Biol. 2019;7(2):210-213.

Original Article

Optimization of culture media for protease production by *Aspergillus* fungi

Raushan Blieva*[1], Nurlan Akhmetsadykov[1], Aigerim Zhakipbekova[1], Aigul Kalieva[2], Zhanar Rakhmetova[1]

[1] Department of Biochemistry, RPE Antigen, Almaty, Kazakhstan

[2]Aktobe Regional State University named after K. Zhubanov, Aktobe, Kazakhstan

Received:
May 21, 2018

Accepted:
April 07, 2019

Published:
June 30, 2019

**Corresponding author email:*
raubil@mail.ru

Abstract

Proteases are among the most important hydrolytic enzymes which have great potential in various industrial processes such as leather, detergent, textile, food, feed industries. Although many microorganisms produce these enzymes, in the recent period *Aspergillus* fungi have most widely been used for proteases production. The production of protease enzymes has been affected by a variety of physical and chemical factors, such as inoculum concentration, time of incubation, pH, temperature, carbon, nitrogen and mineral sources etc. However, composition of the cultivation medium (carbon and nitrogen sources) play significant role in enzymes production. The aim of the present study was the selection of suitable carbon and nitrogen sources of *Aspergillus awamori 16* and *Aspergillus awamori 22* mixed cultures for maximal production of extracellular protease. Sucrose (4.2 U/ml) and peptone (4.8 U/ml) were found as the best carbon and nitrogen sources, respectively.

Keywords: Protease, *Aspergillus* fungi, Carbon sources, Nitrogen sources

How to cite this:

Blieva R, Akhmetsadykov N, Zhakipbekova A, Kalieva A and Rakhmetova Z, 2019. Optimization of culture media for protease production by *Aspergillus* fungi. Asian J. Agric. Biol. 7(2):210-213.

Introduction

Proteolytic enzymes have potential application in a wide number of industrial processes such as food, feed, detergent and pharmaceutical. Proteases from microbial sources have dominated applications in industrial sectors. In spite of the fact that proteases occur in animals, plants and microorganism microbial proteases are directly secreted into the fermentation broth, thus simplifying downstream processing of the enzyme production compared to plant and animal proteases (Savitha et al., 2011). The producers of these enzymes were found among *Actinomyces rimosus*, *Streptomyces griseus*, *Actinomyces fradiae*, *Aspergillus niger*, *Aspergillu foetidus*, *Bacillus subtilis*, *Bacillus licheniformis* etc. (Sharma et al., 2017; Homaei et al., 2016; Li et al., 2013; Siala et al., 2009; Qureshi et al., 2011; Maghsoodi et al., 2013). Despite the fact that among microorganisms that produce proteases there are bacteria, fungi, and actinomycetes, in the recent period micromycetes got wide application. Among the many advantages offered by fungi are low material costs coupled with high productivity, faster production, and the ease of enzymes modification. Besides, fungal enzymes are commonly used in industries due to the feasibility of obtaining enzymes at high concentration in the fermentation medium (Monteiro et al., 2015). In

enzyme production processes, even small improvements have been significant for commercial success (Reddy et al., 2008). On industrial scale, cultivation conditions such as carbon and nitrogen sources, temperature, pH, incubation time, agitation, and inoculum density are essential in successful enzyme production. Among them medium composition (e.g. carbon and nitrogen sources) is one of the most important parameters for industrial enzymes production (Suganthi et al., 2013; Pant et al., 2015; Salihi et al., 2017; Souza et al., 2017).

On the other hand, in natural environments microorganisms live in mixed populations, while in laboratory conditions monocultures are mainly used (Benoit-Gelber et al., 2017). Mixed cultures of *Aspergillus fungi* have shown interesting features for enzyme production. In this research optimization of media components (e.g. carbon and nitrogen sources) for growth and production of extracellular protease by *Aspergillus awamori 16* and *Aspergillus awamori 22* in mixed cultures was investigated.

Material and Methods

Inoculum preparation

Association of mixed fungi *Aspergillus awamori 16* and *Aspergillus awamori 22* (own collection) was used in this study. The microorganisms were maintained on potato dextrose agar at 4 ºC. For inoculum preparation, 25 ml of sterile distilled water was added to the 5-day-old culture grown on potato dextrose agar plate and scraped aseptically with inoculating loop. This suspension with spore concentration of $1.3x10^7$ cells/ml, was used as inoculum for the fungal cultivation.

Effect of carbon sources

Protease production was quantified using a standard Czapek-Dox medium. This basic medium already contained balanced levels of nutrients for the enzyme production. The standard Czapek-Dox production medium containing (g/l): $NaN0_3$ – 5.0, KH_2PO_4 – 1.0; $MgSO_4$ – 0.5; KCL – 0.5; $FeSO_4$ – 0.01 was supplemented with different carbon sources like sucrose, glucose, fructose, galactose, maltose, lactose and starch. Each source was used at concentration of 10 g/l. Liquid broth was inoculated with fungal suspension at concentration of $1.3x10^7$ spores/ml in 250 ml Erlenmeyer flasks with a working volume of 50 ml and incubated in a rotary shaker (210 rpm) for 72 h at 30°C.

Effect of nitrogen sources

The production medium containing (g/l): KH_2PO_4 – 1.0; $MgSO_4$ – 0.5; KCL – 0.5; $FeSO_4$ – 0.01; sucrose – 10.0 was supplemented with different organic and inorganic nitrogen sources like $(NH_4)_2SO_4$, $(NH_4)_2HPO_4$, NH_4NO_3, KNO_3 KNO_3, yeast extract, peptone, casein hydrolysate, gelatin in concentration of 5.0 g/l. The 250 ml Erlenmeyer flasks (50 ml per flask) were inoculated with fungal suspension at concentration of $1.3x10^7$ spores/ml and incubated at 30 °C on a rotary shaker at 210 rpm for 72 h.

Protease enzyme assay

Protease activity was determined by spectrophotometric method (State Standard of Russian Federation, 1988). One unit of protease activity was defined as the amount of enzyme which catalyzes the hydrolysis of 1g of protein (casein) in 30 minutes under standard conditions. The experiments were carried out in triplicates and standard deviation was determined. To determine the significance, the data was analyzed using Microsoft excel software 2010.

Results and Discussion

Various carbon sources were supplemented in the production medium to study their effect on extracellular protease production. The protease activity ranged from 1.1 U/ml to 4.2 U/ml. Among the carbon sources, sucrose supported moderate growth and protease production in *Aspergillus awamori 16* and *Aspergillus awamori 22* mixed cultures with enzymatic activity of 4.2 U/ml (Table 1).

Table 1: Effect of carbon sources on protease production in *A. awamori 16* and *A. awamori 22* mixed cultures

Carbon source	pH	Protease activity, U/ml
Sucrose	6,2	4.2±0.6
Glucose	5,9	1.4±0.3
Fructose	6,0	1.1±0.3
Galactose	6,5	1.3±0.5
Maltose	6,1	2.8±0.6
Lactose	6,0	1.3±0.4
Starch	6,5	3.4±1.0

As can be seen from data presented in Table 1 all other carbon sources used had less effect on protease

production. When the culture broth was supplemented with glucose, decreased protease production was observed. It seems that "catabolite repression" phenomenon, is the best possible explanation for reduction of protease production in the presence of glucose. All data obtained indicate that sucrose is the most efficient source for protease production by fungal association of *Aspergillus awamori 16* and *Aspergillus awamori 22* mixed culture. Screening of nitrogen sources allowing the maximum protease production was performed in nutrient medium with 10 g/l sucrose as a carbon source. Nitrogen is a very important nutrient in metabolism of microorganisms especially in the synthesis of enzymes and other proteins. So nitrogen has been always one of the most important components of fermentation substrate/media. Sodium nitrate was replaced with various inorganic (ammonium sulfate, ammonium hydrogen phosphate, ammonium nitrate, potassium nitrate) and organic (yeast extract, peptone, casein hydrolysate, gelatin) nitrogen sources at equivalent nitrogen concentration.

Table 2 – Effect of nitrogen sources on protease production in *A. awamori 16* и *A. awamori 22* mixed culture

Nitrogen source	pH	Protease activity, U/ml
$(NH_4)_2SO_4$	5,8	2.4±0.6
$(NH_4)_2HPO_4$	5,5	2.7±0.3
NH_4NO_3	4,9	2.9±0.8
KNO_3	5,8	1.8±0.4
Yeast extract	5,0	2.9±0.6
Peptone	5,6	4.8±1.1
Casein hydrolysate	5,1	3.14±1.3
Gelatin	4,8	3.25±0.9

Peptone was found to be the best nitrogen source giving maximum enzyme activity (4.8 U/ml), while other nitrogen sources had less effect on protease production. The results were in accordance with those made by N. Akhter who reported that peptone are a best nitrogen source for the protease production by *Aspergillus niger* (Akhter et al., 2011).

Conclusion

Among various carbon and nitrogen sources examined, sucrose and peptone were found to show maximal protease activity. The optimal fermentation medium for the production of protease by *A. awamori 16* and *A. awamori 22* mixed cultures in submerged cultivation was as a follow (g/l): sucrose – 10.0; KH_2PO_4 – 1.0; $MgSO_4$ – 0.5; KCL – 0.5; $FeSO_4$ – 0.01; peptone – 5.0. The results presented in this work therefore, suggests the possibility of secretion of protease by *A. awamori 16* и *A. awamori 22* mixed culture using locally available substrates as carbon and nitrogen sources and its subsequent application in industries.

Acknowledgment

This research was supported by Ministry of Education and Sciences of the Republic of Kazakhstan (Grant # AP05132402). The authors are grateful to the Laboratory of Microbiology, RPE Antigen for providing laboratory facilities to perform experiments.

Contribution of Authors

Blieva R: Conceived Idea, Data Analysis, Manuscript Writing
Akhmetsadykov N: Data Collection, Manuscript Writing
Zhakipbekova A: Statistical Analysis, Data Collection, Literature Review
Kalieva A: Data Analysis, Manuscript Writing, Literature Review
Rakhmetova ZH: Data Interpretation, Literature Review, Designed Research Methodology

Disclaimer: None.
Conflict of Interest: None.
Source of Funding: This study was conducted with financial support from Ministry of Education and Sciences of the Republic of Kazakhstan (Grant # AP05132402).

References

Akhter N, Morshed MA, Uddin A, Begum F, Sultan T and Azad AK, 2011. Production of protease by *Aspergillus niger* cultured in solid state media. Int. J. Biosci. 1(1): 33–42.

Benoit-Gelber I, Gruntjes T, Vinck A, van Veluw JG, Wösten HAB, Boeren S, Vervoort JJM and de Vries RP, 2017. Mixed colonies of *Aspergillus niger* and *Aspergillus oryzae* cooperatively

degrading wheat bran. Fungal Genet. Biol. 102: 31-37.

Homaei A, Lavajoo F and Reyhaneh S, 2016. Development of marine biotechnology as a resource for novel proteases and their role in modern biotechnology. Int. J. Biol. Macromol. 88: 542-555.

Li Q, Yi L, Marek P and Iverson BL, 2013. Iverson Commercial proteases: Present and future. FEBS Lett. 587(8): 1155-1163.

Maghsoodi V, Kazemi A, Nahid P, Yaghmaei S and Sabzevari MA, 2013. Alkaline protease production by immobilized cells using *B. licheniformis*. Sci. Iran. 20: 607-610.

Monteiro PS, Mona LAB, Carolina CC, Marcela F, Renata PCA, Dâmaris S, Yris MF, Edivaldo XFF, Adalberto PJ and Pérola OM, 2015. A biotechnology perspective of fungal protease. Braz. J. Microbiol. 46(2): 337–346.

Pant G, Prakash A and Bera S, 2015. Production, optimization and partial purification of protease from *Bacillus subtilis*. J. Taibah. Univ. Sci. 9(1): 50-55.

Qureshi AS, Bhutto MA, Khushk I and Dahot MU, 2011. Optimization of cultural conditions for protease production by *Bacillus subtilis* EFRL 01. Afr. J. Biotechnol. 10: 5173-5181.

Reddy LV, Wee YJ, Yun JS and Ryu HW, 2008. Optimization of alkaline protease production by batch culture of *Bacillus sp.* RKY3 through Plackett-Burman and response surface methodological approaches. Bioresour. Technol. 99: 2242-2249.

Salihi A, Asoodeh A and Aliabadian M, 2017. Production and biochemical characterization of an alkaline protease from *Aspergillus oryzae CH93*. Int. J. Biol. Macromol. 94: 827-835.

Savitha S, Sadhasivam S, Swaminathan K and Lin FH, 2011. Fungal protease: Production, purification and compatibility with laundry detergents and their wash performance. J. Taiwan Inst. Chem. Eng. 42: 298–304.

Sharma KM, Kumar R, Panwar S and Kumar A, 2017. Microbial alkaline proteases: Optimization of production parameters and their properties. J. Genet. Eng. Biotechnol. 15(1): 115-126.

Siala R, Sellami-Kamoun A, Hajji M, Abid I, Gharsallah N and Nasri M, 2009. Extracellular acid protease from *Aspergillus niger* I1: purification and characterization. Afr. J. Biotechnol. 8(18): 4582–4589.

Souza PM, Werneck G and Aliakbarian B, 2017. Production, purification and characterization of an aspartic protease from *Aspergillus foetidus*. Food Chem. Toxicol. 109: 1103-1110.

State Standard of Russian Federation, 1988. Enzymatic Preparations. Methods for determining of proteolytic activity. Moscow, Russia.

Suganthi C, Mageswari A, Karthikeyan S, Anbalagan M and Sivakumar A, 2013. Screening and optimization of protease production from a halotolerant *Bacillus licheniformis* isolated from saltern sediments. J. Genet. Eng. Biotechnol. 11(1): 47-52.