International Journal of Engineering and TechnologyVol. 1, No.4 ,October, 2009
ISSN: 1793-8236

Extracellular ribonuclease from Aspergillus niger : process optimization for production

Gundampati R.K, Debnath M*.

Abstract— **Ribonucleases (RNase) are important analytical enzymes and widely distributed in nature. RNase from microorganisms widely used in molecular biology study, food, and pharmaceutical industry. As an important analytical tool, they have played a major role in study on the structure and function of RNA. In single cell protein production, they are used to remove RNA in cell. RNases have also been applied commercially to produce nucleotides for clinical use or for seasoning nucleotides. Many ribonucleases are highly cytotoxic. They have several important biological roles such as antitumor and antiviral activity.The selected strain Aspergillus niger ATCC 26550 is chosen for extra cellular RNase enzyme production by maintaining the culture medium at 30°C, pH 5.5 where the enzyme production was found to be growth associated. Beef extract, peptone and ammonium sulfate are the superior nitrogen source. Addition of Mg2+, Ca2+ to the growth medium significantly enhanced the enzyme production. Glucose found to be the excellent carbon source for RNase production. This method of process optimization is the classical approach of one factor at a time.**

Key words— **Aspergillus niger, enzyme activity, medium optimization, ribonuclease.**

I. INTRODUCTION

Ribonuclease(RNase) catalyzes the digestion of RNA and are widely existing in organisms. RNases are nucleases produced by a diversity of organisms including fungi, plants, submammalian vertebrates, and mammalian liver, kidney, brain, placenta, pancreas, milk and semen [1]. Ribonucleases may exhibit activities other than ribonucleolytic activity, such as anti mitogenic [2], antibacterial, antifungal [3], antiproliferative, antiviral activity[1,4], HIV-I reverse transcriptase inhibitory[5], translation inhibitory[6] and angiogenic[7] activities. As the demand for DNA vaccines and biological drugs increases, usage of ribonuclease attached to a solid support (immobilized) is better way which has many advantages over free enzymes [8]. They are widely used in molecular biological study, food and pharmaceutical industry. As an important analytical tool, they have played a major role in study on the structure and function of RNA. In single cell protein production they are used to remove RNA from cell [9]. These enzymes are produced by numerous microorganisms among which the fungi are the most potent producers. RNases have also applied commercially to produce nucleotides for clinical use or for the food industry. Many RNases are highly cytotoxic. Recently, a series of scientific studies showed that RNases had important biological functions, in controlling the tumor formation [10]. In recent years, the RNase functions related to the control of gene expression, cell growth and differentiation, cell protection from pathogens, and apoptosis induction have received special attention [11].RNases has been produced by many other Aspergillus species such as Aspergillus clavatus and Aspergillus nidulans. Aspergillus is a kind of good producers for RNases [12]. At present, the contradiction between low production and high demands is becoming acute, giving rise to the urgency of addressing the problem of increasing the production of RNase. In this higher production by using biochemical mutants resistant to metabolic inhibitors was a very efficient method for improvement of ribonuclease production by Aspergillus niger [13]. Aside from genetic alteration, culture components also affect the yield of RNase production. However, conventional methods of optimization changing variable-to-variable while maintaining all others at a fixed level; these often do not yield reliable results because interactions between different components are neglected. Besides, they are laborious, time-consuming, and impractical. Several strategies have been widely used to enhance RNase production, such as introducing superproductive strains, optimizing fermentation operating conditions, and building mathematical models.

II. MATERIALS AND METHODS

The source material for ribonuclease Aspergillus niger ATCC26550 was obtained from NCL, Pune, India. Organism was maintained in complex medium. The medium composition was potato (20g), dextrose (20g), agar (15g) and Distilled water (1 liter). 20 g of potatoes were sliced and boiled in 500 ml of water until thoroughly cooked, filtered through cheese cloth and water was added to filtrate to make the final volume to one liter. Agar and dextrose were added to the filtrate and dissolved by boiling and pH adjusted to 5.0.

Strains from the slant culture were then grown in 250 ml shake flask. Flask contain´s 50ml sterile medium glucose (3.0%), beef extract (0.5%), peptone (1.0%), MgSO4 .2H2O (0.05%), CaCl2.2H2O (0.01%). Seed culture was prepared incubating the flask, at 30°C for 24hrs at pH 5.5.

Spores suspension (1ml, about 107 spores) was inoculated into each flask with inoculum medium. The fermentation medium inoculated with 10% (v/v) seed culture. The flasks were placed on a rotary shaker at 180 rpm with 5 cm amplitude at 30°C for 120h to produce RNase. After fermentation, the fungal biomass was separated from the culture fluid by filtration and then the filter was used to determine the enzyme activity.

A. Enzyme And Protein Assay

RNase activity was measured in a reaction mixture containing 200µl citrate buffer (100mM, pH 3.5), 200µl yeast RNA (substrate, 5mg/ml in the same buffer) 200µl of suitably diluted enzyme solution. After incubation at 30°C for 15min, the reaction was terminated by 200µl of 25% (v/v) perchloric acid solution containing 0.75% (w/v) phosophotungstic acid and 0.6% (w/v) bovine serum albumin. The undigested RNA

was precipitated by centrifugation at (10,000rpm for 10min) and the acid soluble nucleotides were estimated spectrophotometrically at 260nm (14). The amount of acid-soluble ribonucleotides was calculated by assuming a molar absorption coefficient of 10,600 L.mol-1 cm-1(4)

One unit of enzyme activity is defined as the activity to release acid soluble oligonucleotides per minute to increase one unit of A260 value in the reaction mixture.

III. RESULTS AND DISCUSSION:

A.niger ATCC26550 was used for ribonuclease production in submerged fermentation. Optimization of the fermentation medium was studied with a view to develop economically viable fermentation technology for ribonuclease production. The *A.niger* ATCC26550 grown in production medium (complex) containing 3% glucose as a carbon source, showed maximum enzyme production at pH range of 3.0 – 6.0. Optimum pH and temperature for enzyme production was found to be 5.5 and 30°C respectively (Fig.3). Cell protein reached a maximum at around 5days (120hrs) of cultivation.

A. Effect of different carbon and nitrogen sources for RNase production:

Carbohydrates, which play key roles as structural and energy compounds in cells, are distinguished as monosaccharide and disaccharide. Various carbon sources such as glucose, fructose, maltose, sucrose, and lactose, were tested, respectively, to find the optimal medium for ribonuclease production. For the maximal production of ribonuclease from *Aspergillus niger* ATCC26550, glucose was found to be the most effective carbon source of all the different carbon sources used glucose was found to be the most suitable to induce the ribonuclease production. The carbon content was maintained constant in all experiments. Fig. 1 shows the different carbon sources (3%) studies in the production medium of ribonuclease enzyme. To optimize the nitrogen content various sources of nitrogen were used as shown in Fig. 2. The nitrogen level was maintained the same in all cases as 88.75mg of nitrogen/50ml of media. It was found that beef extract and peptone gave the best results and was chosen as the suitable nitrogen source for ribonuclease production. The maximum enzyme production was studied at pH range of 3.0-6.0. Optimum pH and temperature for enzyme production was found to be 5.5 and 30°C respectively(Fig.3). Maximum production was at around 5 days (120hr) of cultivation.

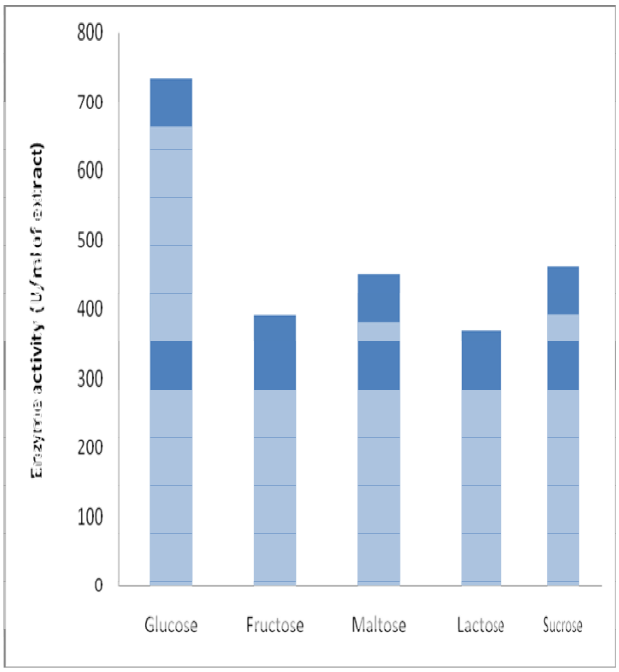

Figure.1: Effect of different carbon sources on production by *Aspergillus niger*.

The rapidly metabolizable complex nitrogen sources are widely used for studying the growth and product formation characteristics of microorganisms in the laboratory as they provide a good source of various amino acids, vitamins, minerals and other unknown growth factors to sustain good growth of microorganism. *Aspergillus niger* ATCC 2655O shows a good growth and considerable amount of ribonuclease production when grown on beef extract, peptone. Inorganic nitrogen sources like ammonium sulfate, ammonium nitrate, ammonium chloride, and potassium nitrate showed their positive effect on cell growth as well as on ribonuclease production. The complex nitrogen sources such as beef extract and peptone produce ribonuclease 515.7 U/ml. These optimal conditions pH and temperature, carbon source and nitrogen source concentration will be used in our subsequent investigations for ribonuclease production by *Aspergillus niger* ATCC26550. Beef extract is a complex nitrogen source; it provides a good source of various minerals and other unknown growth factor for production of enzyme. The strain produces significant amount of enzyme on beef extract, and peptone individually. Combination of beef extract with peptone also produced higher amount of enzyme (515.7 U/ml). It was found that the amount produced by peptone and beef extract were 488U/ml and 344.6 U/ml respectively.

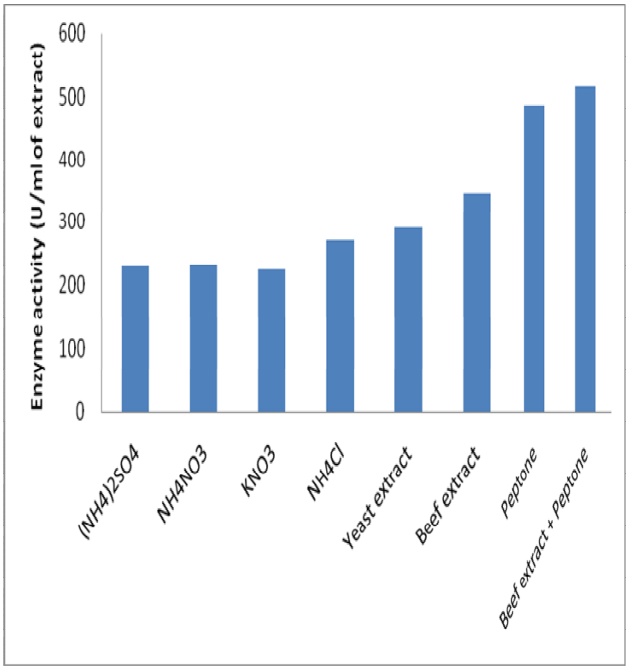

Figure.2: Effect of different nitrogen sources on production by *Aspergillus niger*.

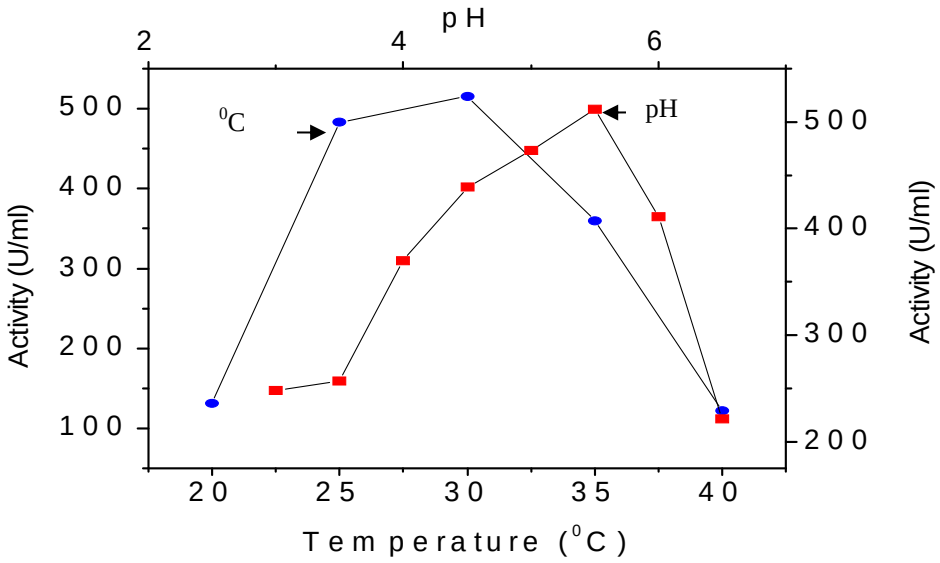

Figure.3: Effect of pH and Temperature on RNase production by *Aspergillus nig*

IV. CONCLUSION

In the present study, Aspergillus niger ATCC26550 used for RNase production by using self directing optimization which is a high-efficient method for parameters optimization in small scale production. Optimal conditions for RNase production was obtained by determining media composition (as given above) along with temperature and pH. Higher amount of RNase production was observed using our optimization conditions. The data can provide method for enhanced production of RNase

ACKNOWLEDGMENT

The authors with to thank the University Grants Commission (UGC) for financial supports and to the School of Biochemical Engineering, Institute of Technology, BHU, Varanasi, India for providing laboratory and technical support.

REFERENCES

[1] G.P. Guan, H.X. Wang, and T.B. Ng, " A novel ribonuclease with antiproliferative activity from fresh fruiting bodies of the edible mushroom Hypsizigus marmoreus", Biochimica et Biophysica Acta , 1770, pp. 1593-1597, 2007.

[2] P.H.K. Ngai, and T.B. Ng, "A ribonuclease with antimicrobial, antimitogenic and antiproliferative activities from the edible mushroom Pleurotus sajor-caju", Peptides, pp. 11-17, 2004.

[3] S.K. Lam, and T.B. Ng, "Isolation of a novel thermolabile heterodimeric ribonuclease with antiproliferative activities from roots of the sachi ginseng Panaxnotoginseng", Bioche. Biophys. Res. Commun, 285, pp. 419-423, 2001.

[4] Wen-Wen Zhou, and Tain –Gui Niu, "Purification and some properties of an extracellular ribonuclease with antiviral activity against tobacco mosaic virus from Bacillus cereus", Biotech-Lett , 31, pp. 101-105, 2009.

[5] H.X. Wang, and T.B. Ng, Quinqueginsin, "A novel protein with anti-human immunodeficiency virus, antifungal, ribonuclease and cell-free translation-inhibitory activities from American ginseng roots", Biochem. Biophys. Res. Commun, 269, pp. 155-159, 2000.

[6] T.B. Ng, and H.X. Wang, Panaxagin, "A new protein from Chinese ginseng possesses antifungal, antiviral, translation-inhibiting and ribonuclease activities", Life Sci, 68: pp. 739-749, 2001.

[7] R. Shapiro, and B.L. Valle, "Human placental ribonuclease inhibitor abolishes both angiogenic and ribonucleolytic activities of angiogenin", Proc. Natl. Acad. Sci. U.S.A, 84: pp. 2238-2241, 1987.

[8] Mojca Bencina, Janja Babic, and Ales Podgornik, "Preparation and characterization of ribonuclease monolithic bioreactor", Journal of Chromatography A, 1144: pp. 135-142, 2007.

[9] G.D. Reddy, and V. Shankar, "Immobilized nucleases" CRC Critical Review of Biotechnology , 13: pp. 255-273, 1993.

[10] R.J. Youle, Y.N. Wu, S.M. Mikulski, K. Shogen, R.S. Hamitton, D. Newton, G. Dalessio, and M. Gravell, "RNase inhibition of human immunodeficiency virus infection of H9 cells", Proceedings of the National Academy of Sciences of the United States of America, 91: pp. 6012-6016, 1994.

[11] O.N. Ilinskaya, and A.A. Makarov, "Whey Ribonucleases Induce Tumor Cell Death. Molecular Biology, 39: pp. 1-10, 2005.

[12] H. Horitsu, Y. Higashi, and M. Tomoyeda, "Production, purification and properties of ribonuclease from Aspergillus niger", Agr.Bio. Chem, 38: pp. 933-940, 1974.

[13] Y.H. Xiong, J.Z. Liu, H.Y. Song, L.N. Ji, "Selection of biochemical mutants of Aspergillus niger with enhanced extracellular ribonuclease production",World Journal of Microbiology and Biotechnology, 20: pp. 203-206, 2004.

[14] S. Barindra, G. Debashish, S. Malay, and M. Joydeep, "Purification and characterization of an extracellular, uracil specific ribonuclease from a Bizionia species isolated from the marine environment of the sundarbans", Microbiological Research, 163: pp. 31-38, 2006.