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Alpha 1, 5- L endo-arabinanase production media formulation and optimization using cost effective substrate

Sindhu Shetty K[1], Ajith Madhavan[1], Sreejith Ben C S[1], Lekshmi R[1], Binal S. Kumar[1], Govind K[1], Aswathy S[1], Shanmugham S[2]

[1]Amrita School of Biotechnology Amrita Viswa Vidyapeethom, Amritapuri, Kollam, Kerala, 690525,India
[2]LRG Government Arts and Science, Tirupur, Tamilnadu, India

Abstract: A new, cost effective media was formulated with soy chunk powder (SCP) as the nutrient source. Growth parameters such as temperature, pH and substrate concentration were optimized. Temperature of 30^0C, pH of 4 and 1.5% (w/v) of substrate concentration, constitute the optimal condition for the maximal production of the enzyme. The enzyme production peaked in the 5th day of incubation with a maximal enzyme activity of 1.218 U/mg which is 42% as efficient as production in media employing pure substrate. *Aspergillus niger* isolated from the soil samples collected from the precincts of the campus was employed in the current study.
Keywords: Alpha1, 5- L endo-arabinase, debranched arabinan, Azurin cross linked debranched arabinan, Soy chunk powder.

Introduction

Alphas 1, 5- L endo-arabinanase (endo-arabinase) [EC.3.2.1.99] are endo acting glycosidases belonging to the arabinase group of enzymes. Endo-arabinases [GH43] principally hydrolyse α-1,5-arabinofuranosidic linkages (Fig.1) that constitute the backbone of pectin polysaccharide- arabinan found in the cell walls of seeds, fruits, vegetables and in some organisms[1]. the hydrolysis results in the formation of arabinotriose, arabinobiose and L-arabinose[2].

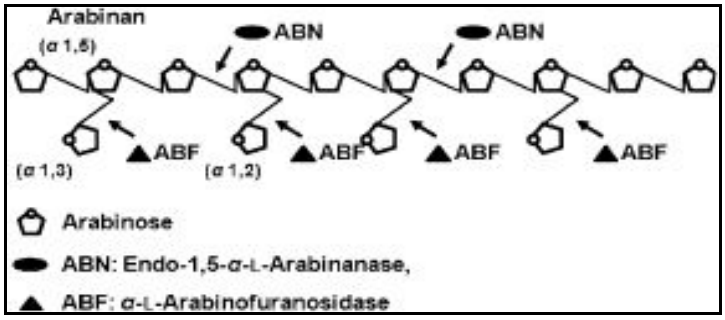

Fig.1. Hydrolysis of linear arabinan by endo-arabinase [3]

Endo-arabinase has gained much attention from the enzyme industry owing to its application potential in diverse fields such as food technology, nutrition research, organic synthesis and in various agro-industrial

processes. Specifically the applications involves the conversion of hemi cellulosic biomass to biofuels/ethanol, delignification of paper pulp, digestibility enhancement of animal feed stock, clarification of juices, consistency improvement of beer and so on. Moreover, its potential to work synergistically with other enzymes to remove biofilm is also being exploited as an analytical tool for the determination of cell wall polysaccharide structure[3][4][5][6] [7][8]. The end products of enzyme hydrolysis are also valuable from pharmacological perspective owing to its immunological, anti tumor, anti inflammatory, anti coagulant and fibrinogenic activities [9].

Viable sources of endo-arabinases are primarily natural isolates of bacteria and fungi. Use of few recombinant strains of bacteria are also reported [3]. Overall cost of enzyme production is exorbitant due to many contributing factors, most important amongst which is the use of pure substrates during enzyme production. So development of a cost effective production media would be the key in reducing cost of production of endo-arabinase. This study proposes a formulation of a production media using SCP as the nutrient source. SCP is a byproduct of extraction of oil from beans. It is rich in cellulose (40-45%) with arabinogalactan and 1,5 linked L-arabinofuranose residues[10]. Locally isolated fungal culture of *Aspergillus niger* is used in the study for the production of endo-L-Arabinase. The study involved the optimization of the enzyme production with regard to temperature, pH, and incubation time and substrate concentration. Enzyme activity was also deduced and compared with the production involving pure substrate.

Experimental Set Up

Materials

Gum arabic, AZCL debranched arabinan was purchased from Megazyme, Trizma buffer (pH 12) was purchased from Sigma and Bovine serum albumin was purchased from Himedia

Primary screening of fungal strains elaborating Endo-L-arabinase.

Fruits and vegetables (Orange, melon, apple, ginger) which contain arabinan were selected and superficially inoculated with soil and incubated at ambient temperature in humid condition. Different fungal colonies developed after 48 to 72 hrs of incubation and were segregated based on differential colony characteristics and sub cultured and maintained. Primary screening was done using Gum arabic media containing $NaNO_3$:0.2%, KH_2PO_4:0.1%, KCl:0.05%, $MgSO_4.7H_2O$: 0.05% and 1% of gum arabic[11] [12]. To prime the growth of fungi 0.01% galactose was incorporated in the media. To inhibit the growth of bacteria chloramphenicol was included in the media at a concentration of 35µg/mL. Plates were incubated at ambient temperature for four days.

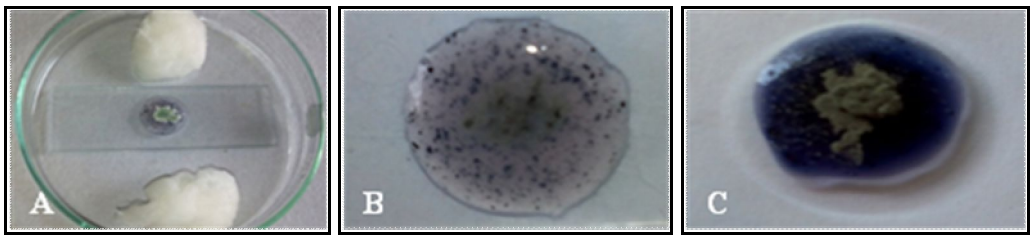

Fig.2. A) Experimental setup, B) control and C) Test

Fungal cultures grown on gum arabic media were subjected to further screening for elaboration of endo-arabinase on a cavity slide containing 0.1% AZCL-debranched arabinan. 0.1% galactose, 1% agar-agar chloramphenicol (35µg/mL) by providing a moist condition. The fungal cultures which can elaborate produces a colloidal blue color (Fig.2.) [2][13].

Selection of substrate

Locally purchased Soya chunks were powdered and filtered through a sieve and precooled to 4^0 temperatures for 15 minutes. The soya chunk powder (SCP) then subjected to a heat treatment at 100^0C for 50

seconds repeatedly for 3 times in hot air oven to sterilize them. The SCP was then cooled and used for further study.

Determination of endo-arabinase activity

Test samples were prepared with 400µL of debranched arabinan (1% concentration) and 600µL crude enzyme. Suitable controls to check the reducing sugar contributions from the debranched arabinan substrate and enzyme preparation were also set up. The test and control samples were incubated at 50^{o}C for 5 minutes. The reactions were arrested by adding Trizma buffer (pH 12). The amount of arabinose sugar liberated was determined by salicylic acid assay (DNS assay) [14]. Enzyme activity is expressed in µmol/mL/min of arabinose liberated. Standard graph was plotted with pure arabinose (0-4mg)

Optimization of the initial pH for the production of enzyme

Production media was prepared and dispensed in different Erlenmeyer flask (250mL) with 1% substrate. And the pH in different flasks were adjusted to a pH from 3-7 using 1N HCl and 1N NaOH.

Optimization of incubation temperature

Media was prepared with 1% substrate and the organism was inoculated and incubated in a shaking incubator for 200rpm keeping pH constant at 4. The temperatures of incubation were varied from 30^{0}C- 70^{0}C with a 10^{0}C increment.

Optimization of the concentration of soya chunk for the production of enzyme

Minimal media was prepared and was supplemented with different concentration (0.5%, 1%, 1.5% and 2%) of substrate keeping other parameters at optimum. Galactose (0.01%) was also included in the media in order to induce the growth of fungi. *Aspergillus niger* (Locally isolated and identified by sequence analysis from Bioserve Biotechnologies (India) Pvt. Ltd., Hyderabad) was inoculated (with an agar chunk containing the spores and mycelium) and incubated in a orbital shaking incubator for 8 days at a speed of 200rpm at ambient temperature and at pH 4. Enzyme activity was checked daily using DNS method.

Optimization of the time of incubation

Media was prepared in 250mL Erlenmeyer flask with 1.5% substrate. *Aspergillus niger* was inoculated and incubated in a orbital shaking incubator for a speed of 200rpm at room temperature at pH 4. Enzyme activity was checked on a daily basis till the 8th day of incubation using DNS assay.

Estimation of specific activity of endo-arabinase

Protein concentration was determined by Bradford assay with bovine serum albumin as standard [15]. The protein concentration was expressed in milligram protein per milliliter (mg/mL) and the specific activity was expressed as enzyme units per milligram (U/mg).

Results and Discussion

Influence of initial pH on enzyme production

Initial pH of the media exhibited an influence on the production of the enzyme. Intial pH of the media was varied from 3-7. Maximal endo-arabinase activity of 0.96U/mg was observed in the production media which was adjusted to pH4 as the initial and the trend showed a sharper decrease when the pH increased then onwards (Fig.3.).

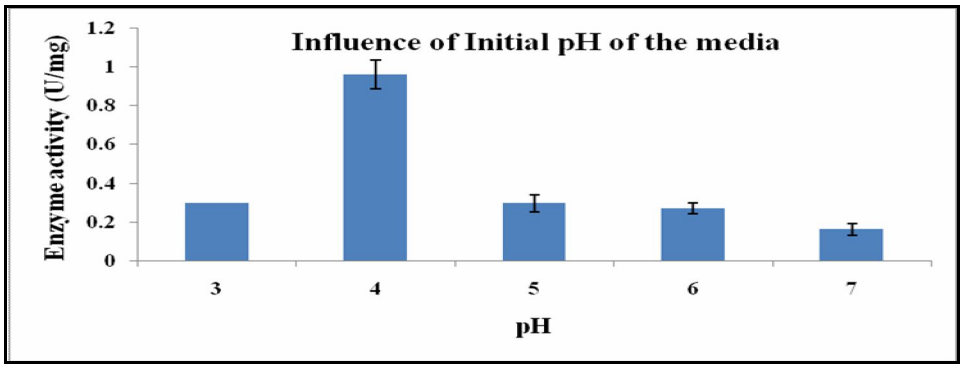

Fig.3. Effect of initial pH of the production media on enzyme production

Influence of incubation temperature on endo-arabinase production

The influence of temperature on enzyme production depends on the temperature requirement of the *A.niger*. Maximal enzyme activity (0.64U/mg) was observed at 30^0C and the enzyme activity found to be decreased from 40^0C to 50^0C. The result also shows absence of enzyme activity after 50^0C (Fig.4.).

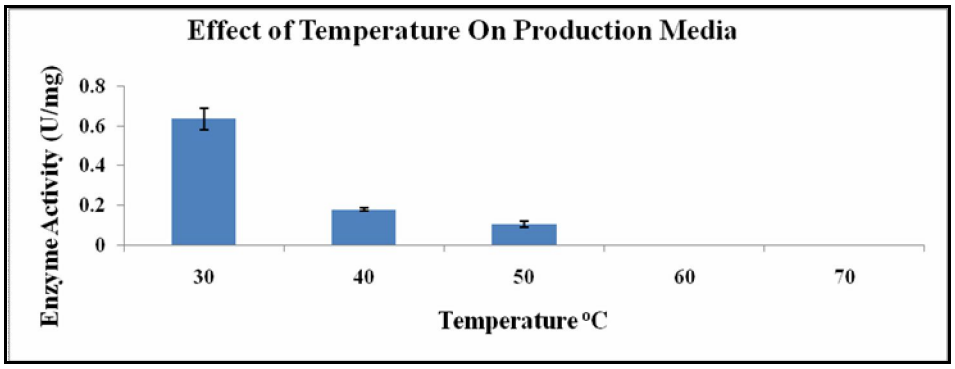

Fig.4. Effect of temperature of the production media on enzyme production

Influence of incubation time and substrate concentration on enzyme production

Enzyme activity gradually increased and peaked at fifth day of incubation (1.218U/mg). After that it showed decline in the enzyme activity decreases with an increase in the incubation time. According to B.Johnvesly the decrease in enzyme activity is due to the interaction of other components present in the production media at the later stage [24]. The optimum substrate concentration for maximal production was found to be 1.5% (Fig.5.)

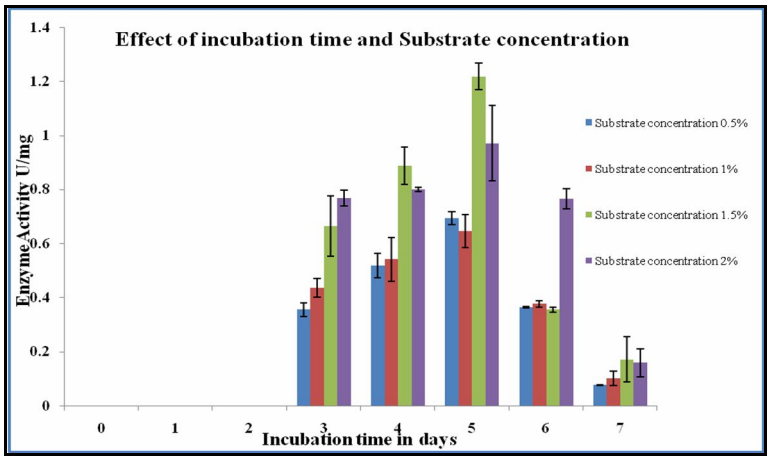

Fig.5. Effect of incubation time and substrate concentration on endo-arabinase production

Comparitive study of the formulated media with pure substrate

On comparison with the media formulated with debranched arabinan, the SCP media showed a 42% efficiency with regard to enzyme activity (Fig.6.)

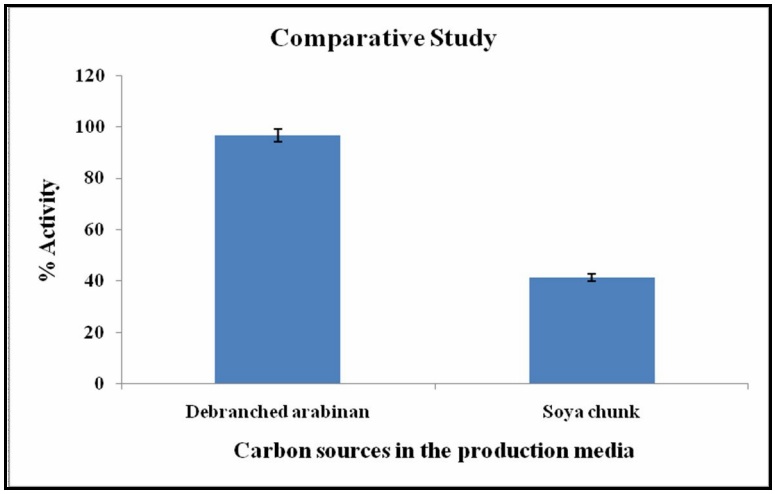

Fig.6. A comparison of the percentage between media with pure substrate and SCP

Conclusion

The present study describes the formulation of a cost effective substrate for the production of endo-arabinase. SCP has never been used for the production of endo-arbinase and hence can open up avenues for the use of other arabinan containing agro-wastes in the formulation of media with higher production potential. Incidentally, the development can also be a value addition to soya chunk and other waste materials which has arabinan as a major component in it.

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