RESEARCH ARTICLE

OPTIMIZATION OF SOLID STATE FERMENTATION FOR THE PRODUCTION OF GLUCOAMYLASE FROM ASPERGILLUS SPECIES USING AGRO WASTESUBSTRATE

1, Shantha, 2Revathi chitra, 3Rega and 4Arockia Badsheeba

Department of Biotechnology, Kumararani Meena Muthiah College of Arts and Science, Adyar, Chennai-600020, Tamil Nadu, India

Received 24th February, 2018; Accepted 28th March, 2018; Published Online 06th April, 2018

ABSTRACT

Aspergillus species is one of the most suitable species to grow in the solid substrate for maximum glucoamylase production. The enzyme titres varied considerably in different substrates. The particle size and the chemical composition of the substrate influence the fungal growth and enzyme production. The aim of our study is to analyze the biochemical contents present in the selected substrates (Rice bran, wheat bran and tea waste) for glucoamylase production and to optimize the moisture content in the selected substrates to enhance glucoamylase production and also to find out the effect of various physical and biochemical parameters to maximize glucoamylase production. From the study it was found that good production of glucoamylase from Aspergillus niger and Aspergillus flavus species was found in 65 to 75% of moisture content were the pH 3 and pH 9 with the optimum temperature of 45°C. Among the different solid state substrates used nitrogen source of 1% peptone and 1% yeast extract accelerated the enzyme production and it was also noted that 1% maltose and 1% starch showed higher enzyme production. The produced glucoamylase enzyme was finally eluted using the column chromatography. The eluted glucoamylase can be used for industrial product production like fermented foods.

Key words: Glucoamylase, Solid State Fermentation, Aspergillus niger, Aspergillus flavus and column chromatography.

Copyright © 2018, Shantha et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Citation: Shantha, Revathi chitra, Rega et al., 2018. “Optimization of solid state fermentation for the Production of glucoamylase from Aspergillus species using agro wastesubstrate” International Journal of Current Research in Life Sciences, 7, (04), 1457-1461.

INTRODUCTION

Solid State Fermentation involves the growth and fermentation by microorganisms, especially fungi, on moist, water in soluble Solid substrate in the absence of free flowing water. The water content is quite low with the required moisture existing in complex form with in the Solid material so that microorganisms are almost not in constant contact with gaseous oxygen but also serves as an anchorage for the microbial cells. The ideal solid substrate is one that provides all necessary nutrients for the growth of microorganisms. Production of concentrated enzyme preparations could be obtained more by Solid State Fermentation than by submerged fermentation. New applications are currently gaining momentum such as production of enzymes and feed additives. Some of the salient features of solid state fermentation in enzyme production are less requirements of space with simple equipments, utilization of cheap raw materials and less pollution hazards. Glucoamylase is a glyco protein containing mannose, glucose, galactose and uronic acid and has a molecular weight of 60X10³ to 100X10³.

*Corresponding author: Shantha
Department of Biotechnology, Kumararani Meena Muthiah College of Arts and science, Adyar, Chennai-600020, Tamil Nadu, India.
conditions for glucoamylase production using Aspergillus species by solid state fermentation.

MATERIALS AND METHODS

Collection of Substrates

The agro wastes such as rice bran, wheat bran, tea waste were collected and dried.

Cultures Used

Aspergillus species such as Aspergillus niger and Aspergillus flavus was maintained through successive transfer on czeppekdox medium and 1mL of a spore suspension (10^7 spores/mL) prepared was used as the inoculum, Raw starch activity was measured to this selected cultures. 2% agar in phosphate buffer was prepared to the sterile plate and informed diameter of well was made. To this well 1ml of culture filtrate was added and incubated at 37°C for overnight and hydrolysis was visualized by the addition of 0.01N iodine solution.

Parameters Analyzed In Substrates Before Inoculation

The raw substrates used for the study were analyzed for carbohydrate, reducing sugars and protein.

Estimation of Carbohydrate: (Anthrone Method)

Carbohydrates were dehydrated with concentrated H2SO4 to form furfural. This furfural condenses with anthrone to form blue colour complex which was measured spectrophotometrically. Total Carbohydrate was estimated by the anthrone Method by Sadasivam and Manickam (1992). 100mg of the sample was taken and to this 5mL of cold anthrone reagent was vortexed rapidly. The tubes were covered with aluminum foil and kept in a boiling water bath for 10 minutes and then cooled to room temperature. The absorbance was read at 625nm using spectrophotometer and standard graph was made using glucose as standard and total carbohydrate of the sample was calculated from the standard graph.

Protein Estimation: (Lowry’s Method)

Protein content was analysed by Lowry Method described by Lowry et al., (1951). 0.2g of sample was mixed with water and centrifuged at 2000rpm for 20m. 1mL of the supernatant was taken and to this 5mL of freshly prepared alkaline copper sulphate solution (reagent – C) was added and incubated for 10m. And then 0.5mL of Folin Phenol reagent was added and incubated for 30m. The absorbance was read at 660nm using spectrophotometer. A blank containing 0.1mL of NaOH and all the other reagent were added to adjust the absorbance to zero and the protein content of the sample was calculated from the standard graph.

Estimation Of Reducing Sugar: (Dns Method)

100mg of substrates was mixed with 80% of ethanol and allowed it to centrifuge at 3000rpm for 15m. To 2mL of the supernatant 3mL of DNS reagent was added and kept the tubes in a boiling water bath for 15m. The reaction was stopped by the addition of 4mL of distilled water and OD was measured at 540nm.

Determination of Optimization of Moisture Content For The Production Glucoamylase Using Aspergillus Niger And Aspergillus Flavus

Commercially available Rice bran, Wheat bran, Tea waste was used as solid substrates. The substrates of varying moisture levels (45%, 55%, 65%, 75%) for the optimization glucoamylase production, was tested and the optimum moisture content for Aspergillus niger and Aspergillus flavus was determined in all the substrates. The moisture content was fixed for both the organisms namely Aspergillus niger (65%) and Aspergillus flavus (75%) and further analysis were done having this concentration of moisture content.

Effect of Carbon Sources on Glucoamylase Production Using Aspergillus Niger And Aspergillus Flavus

Using fixed moisture content, the carbon sources such as dextrose, fructose, maltose, and starch at 1% concentration were applied individually in the place of sucrose in the czeppekdox medium. After adjusting the moisture content to fixed level, the substrates were autoclaved at 15lb for 20m. A loopful of culture were inoculated and incubated at 37°C for 48hrs and the enzyme production was analyzed.

Effect of Nitrogen Source on Glucoamylase Production Using Aspergillus Niger And Aspergillus Flavus

Using fixed moisture content, the nitrogen source namely Ammonium nitrate, Peptone, Tryptone and yeast extract at 1% concentration were supplemented in each treatments. After adjusting the moisture content to fixed level, the substrates were autoclaved at 15lb for 20m. A loopful of culture were inoculated & incubated for 48hrs and enzyme production was analyzed.

Effect of Ph on Glucoamylase Production

The substrates at different range of pH (such as pH3, 5.7 and 9) were used. Using the fixed moisture content after adjusting the pH and moisture content to a fixed level, the substrates were autoclaved at 15lb for 20m. A loopful of culture was inoculated to the substrates & incubated at 37°C for 48hrs and the production was analysed.

Effect of Temperature on Glucoamylase Production

Using fixed moisture content of the substrates, the substrates were autoclaved at 15lb for 20m and a loopful of culture were inoculated to the substrates and incubated at different temperature such as 35°C, 45°C, 55°C, and 65°C.

Effect of Inoculum Size on Glucoamylase Production

0.1mL of culture was taken and dropped into a clean haemocytometer, and the total number of spores in the A.niger and Aspergillus flavus cultures was counted, and the values were noted respectively. After adjusting the moisture content to a fixed level, the substrates were autoclaved at 15lb for 20m of the culture. The Aspergillus niger suspension was inoculated as 3x10^6, 6x, 9x10^6, 12x10^6 the Aspergillus flavus
suspension was inoculated as $4 \times 10^8$, $8 \times 10^8$, $12 \times 10^8$ respectively.

**Enzyme assay**

To 1mL of culture filtrate, 32mg of soluble starch in 0.1M citrate buffer was added and incubated at 50-60°C for 30m. 0.5mL of enzyme substrate mixture was taken and 2mL of DNS was added and kept the tubes in a boiling water bath for 5mins and cooled. The volume was made to 5mL of distilled water, and the enzyme was analysed spectrophotometrically.

**Elution of glucoamylase using column chromatography**

Two volume of cold acetone (-10°C) was added to the culture filtrate and the pellet was removed by centrifugation and dissolved with 150ml of 0.02M Phosphate buffer (pH 7.0). This solution was applied to a DEAE cellulose column (diethyl amino ethyl cellulose column). Washing of DNAE cellulose was done by 10g of DEAE cellulose containing 200mL of 0.1 M NaCl and it was stirred vigorously for a few minutes and filtered through a WHATMAN No.1 filter paper and was suspended the materials in 200mL of phosphate buffer. The column tube (2.5x2.5cm) was set on a burette stand vertically. The suspension DEAE cellulose was powdered into the column gently through the sides, avoiding trapping of air bubbles. Simultaneously; the column outlet was opened so that the adsorbent settle down. Then the column was equilibrated with phosphate buffer and the column was eluted with a linear gradient of NaCl (0.1M to 0.5M) with a flow rate of 0.7mL/min. From this, 5mL of fraction was collected and the fraction containing glucoamylase activity was pooled together and used for various propose.

**Immobilization of the enzyme**

was done using 7mL of 2.5% (W/V) sodium alginate added to the enzyme solution and the solution was allowed to stand for 30mins. Then 7mL of 2.5% glutaraldehyde was added and kept it for 11/12hrs and this solution was added drop wise into 0.05M CaCl$_2$ solution and the beads were collected and stored at 4°C.

**RESULT AND DISCUSSION**

**Starch hydrolyzing activity in Aspergillus niger and Aspergillus flavus**

Glucoamylase production was carried out by selected agro wastes using fungal organisms such as Aspergillus niger, and Aspergillus flavus. It was noted that better results was observed in Aspergillus niger culture than in Aspergillus flavus.

**Biochemical analysis of raw substrates**

The proteins and reducing sugar content was found to be higher in rice bran (0.36mg/mL, 0.099 mg/mL) whereas the Carbohydrate content was more in wheat bran (2.632 mg/mL) than the other two substrates. The production of glucoamylase was high when wheat bran and rice bran used as a substrate than the tea waste. Hence rice bran and wheat bran are considered to be ideal substrates for glucoamylase production.

**Effect of temperature on glucoamylase production in solid state fermentation using Aspergillus niger and Aspergillus flavus**

High glucoamylase production was observed at pH of 3 and pH9 in Aspergillus niger and Aspergillus flavus respectively. Temperature 45°C and 55°C yields maximum glucoamylase activity in both the organisms. The augmentation of inoculums size $3 \times 10^8$ and $16 \times 10^8$ (spores/mL) influence the glucoamylase activity both in Aspergillus niger and Aspergillus flavus.

**Effect of inoculums size on the production of Glucoamylase using Aspergillus niger and Aspergillus flavus**

With regard to the effect of inoculums size on the enzyme production, it was observed that S3 treated with $3 \times 10^8$ and $9 \times 10^8$ amount of inoculums produced maximum amount of enzyme when Aspergillus niger was used. Similarly Aspergillus flavus was used, it was found that in S3 and S1 with $8 \times 10^8$ & $16 \times 10^8$ amount of inoculums enhanced the production of glucoamylase.

**Effect of Fructose and Maltose on glucoamylase production in rice bran and wheat bran using Aspergillus niger and Aspergillus flavus**

The present study rice bran (S1) and tea waste (S3) also produced high enzyme production when starch was used as the carbon source. Same results were observed in Aspergillus flavus. High enzyme activity exhibited when dextrose, maltose and starch were used as the carbon source in which, maximum production was noticed in S3 and in S1. Poor enzyme activity was observed in the fructose treated substrate.
Effect of yeast extract and peptone on glucoamylase production in rice bran and Tea waste using *Aspergillus niger* and *Aspergillus flavus*

High glucoamylase production was found in the substrate supplementation of 1% fructose and 1% of yeast extract.

**Elution of Glucoamylase using 0.1-0.5 m CaCl₂ column chromatography**

It was found elution of enzyme production was high when 0.3M and 0.5M CaCl₂ were used in *A.niger* treatments. In contrast, only low enzyme was eluted in *A.flavus* treatments using 0.3M and 0.4M CaCl₂. Experiments on elution of glucoamylase enzyme with 0.1M concentration of CaCl₂ in column chromatography also showed higher glucoamylase activity.

**Immobilization of Glucoamylase using CaCl₂**

It was found that 65% and 75% of moisture content was found optimum for *Aspergillus niger* and *Aspergillus flavus* for glucoamylase production.

**REFERENCE**


*Production by Aspergillus awamori in SSF.* 58: 708-712.

Ramesh M.V. and Lonsane b.K., 1990, Production of bacterial thermostable alpha amylase by SSF. A potential tool for achieving economy in enzymes production and starch

******