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Production of Xylooligosaccharide from Corn Cob Xylan by Xylanase Obtained from Aureobasidium Pullulans

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ABSTRACT

The work is focussed on the production of xylooligosaccharide (XOS), a widely acknowledged prebiotic, by utilizing the xylan extract which was obtained by autohydrolysis of Corn cob which yielded 62.5±0.7 g total sugar/kg. Partially purified xylanase obtained from an indigenous strain of Aureobasidium pullulans was found to be efficient in the production of XOS. One factor at a time (OFAT) approach was used for optimizing the production of XOS upon which the resultant yield obtained was 6.2±1.5 mg/ml of XOS in 50 minutes. The prebiotic was then studied for its stability in acidic pH. Furthermore, the produced prebiotic was then infused in MRS medium and evaluated for its stimulatory effect on the growth of Lactobacillus.

Keywords: Xylanase, Aureobasidium pullulans, Corn Cob, Xylooligosaccharide

I. INTRODUCTION

Food industry is growing at a prolific rate and the market for functional foods has gained momentum especially in the recent years [1]. Functional food is a component of human diet which provides additional health benefits than traditional foods. As these claim to enhance health and provide additional benefits, the quest continues to comprehend the effect of various components of food on human health [2]. One such category of functional food is prebiotics. Prebiotics are majorly fibers that are short-chain carbohydrate which are non-digestible food ingredients and are beneficial for human health as it supports the proliferation of beneficial gut microflora, generally lactobacilli and bifidobacteria [3]. There are many types of prebiotics depending upon its components, such as fructooligosaccharides (FOS), xylooligosaccharides (XOS), galactooligosaccharides (GOS), raffinose oligosaccharides, soybean oligosaccharide etc [4].

Xylooligosaccharide (XOS) are short chained oligomers of xylose. Xylanase has been used to break down xylan extracted from xylan rich sources and to produce XOS. For XOS to be used as a prebiotic it must possess some crucial characteristics, like, it must elicit some beneficial health effect, should have high stability over acidic pH, and, should promote the growth of prebiotics [5].

XOS can be synthesized from numerous xylan-rich substances by various processes. XOS can be produced from materials such as wheat straw, corncobs, tobacco stalk, sunflower stalk etc. by various methods such as chemical, autohydrolysis, direct enzymatic hydrolysis of susceptible portion, acid hydrolysis, or a combination of the other methods [5, 6]. To extract xylan, or so to say any hemicellulosic component, breaking of the interactive bonds is a prerequisite. The reason why such treatments are necessary is because xylan does not occur in a free form but in a complex with lignin in the lignocellulosic biomass. As a consequence, XOS synthesis has to be carried out in two steps, where first step deals with the extraction of

xylan from the biomass while second step includes enzymatic hydrolysis of the extract [6-8]. Xylan extraction have been reported from variety of lignocellulosic biomass but the most frequently used materials are beech wood [9], birch wood [10], and corncob [6, 10. 11].

XOS (alone or as active components of pharmaceutical preparations) exhibit a range of biological activities different from the prebiotic effects related to gut modulation. The other effects for XOS include antioxidantactivity (conferred by phenolic substituents)[12], blood- and skin-related effects, antiallergy, anti-infection and anti-inflammatory properties, immunomodulatory action, anti-hyperlipidemic effects [13] and cosmetic and a variety of other properties.

II. METHODS AND MATERIAL

A. Production and partial purification of xylanase

Xylanase was obtained from an indigenous strain of Aureobasidium pullulans by submerged fermentation carried out in medium containing 10 g/L glucose, 10 g/L urea, and 1 mM Tween 20. The fermentation was carried out at 28° C for 48 hours under shaking conditions. The enzyme was extracted by centrifuging at 8500 rpm for 10 min at 4°C to separate the biomass, and the supernatant thus obtained was used as crude enzyme for determining the enzymatic activity. The enzyme obtained was partially purified by addition of ammonium sulfate at different saturations to obtain extract with highest protein content and xylanolytic activity. It was then centrifuged at 10000 rpm for 15 minutes and pellet was re-suspended in 5 ml of 0.05 M sodium citrate buffer (pH 5). It was dialyzed overnight against the buffer at 4°C and then checked for enzyme activity. Xylanase and protein assay was carried out at each step.

B. Enzyme assay and protein estimation

Xylanase activity was determined by following a standard procedure by Bailey [14] by using 0.75% beechwood xylan solution as substrate. The assay was done by following the procedure specified by Bailey et al., 1992. One International unit of xylanase activity (IU) is defined as the amount of µmol of xylose which is liberated by 1 ml enzyme per minute under the standard assay conditions. Protein content was estimated of the appropriately diluted sample [15].

C. Selection of source of xylan

In order to select the best source of xylan, different raw materials including agro-residues (barley husk, corn cob, corn stover, and sunflower stalk) and the Eucalyptus bark were screened. The extraction of xylan from these sources was carried out by auto hydrolysis. For this, the raw materials were first soaked in distilled water in 1:8 ratio and autoclaved at 121˚C at 15 lbs for 1 hour. The xylan was obtained by filtering the extract (obtained after autoclaving) with muslin cloth. The pH of this filtrate was set to xylanase's optimal pH. The raw material giving the maximum xylan was selected for further studies.

D. Selection of method of aqueous extraction of xylan

Four different methods of extraction, as described by Chapla et al [16], were used for obtaining the xylan from the selected raw material.

1) Dilute acid treatment: The selected raw material was soaked in 0.01 M sulphuric acid and was incubated at 60˚C for 12 hours. It was then filtered and washed with distilled water till pH of 7.0 was attained and dried in oven. Thereafter, distilled water was added to raw material in the ratio of 1:3 and was autoclaved for 1 hour at 121˚C and 15 lbs. The material obtained after autoclave was filtered and mashed, dried and ground.

2) Treatment with 1% sodium hypochlorite solution: The selected raw material was soaked in 250 ml of 1% sodium hypochlorite solution at room temperature for an hour. It was then washed with water and was filtered with muslin cloth. Next, the raw material was soaked in 15% of NaOH at room temperature for 24 hours to extract xylan. After

filtration by muslin cloth, filtrate was neutralized by 3M sulphuric acid. The filtrate was collected by centrifugation at 8500 rpm for 30 minutes and was stored at 4˚C.

3) Treatment with dilute alkali: For the treatment of raw material with alkali, 5 grams of raw material was blended with 80 ml of 1.25 M NaOH for 10-15 minutes. Mixture was then kept under shaking (at 150 rpm) at 37˚C for 3 hours. The mixture was then centrifuged at 8500 rpm for 20 minutes. Supernatant was acidified to pH 5.0 with concentrated hydrochloric acid and was stored at 4˚C.

4) Auto hydrolysis: For the purpose of auto hydrolysing the sample, Raw material was soaked in distilled water in ratio 1:8 and slurry was autoclaved at 121˚C at 15 lbs for 1 hour. The obtained extract was filtered by muslin cloth and pH of the filtrate was adjusted to 5.0.

E. Quantifying amount of sugar released

The total sugar released after every treatment indicates their respective efficiency, a factor which will determine the selection of the best method for processing of the raw material selected. Total sugar in the sample was estimated by method described by DuBois [17]. To 1 ml sample, that was appropriately diluted, we added 1 ml of 30% (v/v) Phenol solution and 5 ml of concentrated sulphuric acid. This mix was mixed well with the help of vortex and was incubated at 30 ˚C for 20 minutes. Next, the absorbance was measured at 490 nm. The obtained absorbance can be used to obtain the quantity of the extracted sugar.

F. Treatment of aqueous extract of xylan with xylanase produced by A. pullulans

After completion of respective treatments, the extracts were exposed to xylanase produced by A. pullulans (test culture). Enzymatic hydrolysis was carried out using the screw cap tubes containing extracted solution with 20 U/ml of partially purified xylanase. It was then incubated in water bath at 45˚C under shaking (50 rpm). Controls were kept for each reaction in which the active enzyme was replaced with heat inactivated enzyme. Amount of reducing sugar released was checked to estimate the amount of sugar released from each process. Method which showed maximum release of sugar was selected.

G. Optimization of production of XOS

Various parameters involved in XOS production were varied within a range in order to optimize its production. The dose of enzyme (10, 20, 30 and 40 U/ml), time of incubation of extract with enzyme (10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, and 160 min), and temperature (40˚C, 50˚C and 60˚C) were optimized to ensure maximum yield of the product.

H. Estimating the production of XOS (reducing sugars)

The amount of XOS released was estimated to observe the increase in levels of reducing sugar by using DNSA method [18].

I. Evaluation of stability of XOS

The evaluation of the stability of XOS was done by heating at different pH. Buffers having pH in the range of 2-7 were mixed with 10% (v/v) XOS solution. Each of the sample solutions were maintained for 30 min in water bath at appropriate temperature (37 ˚C). Following the termination of thermal processing, the samples under investigation were autoclaved at 121 ˚C, at 15 lbs for 30 minutes. The treated samples were used for quantification of residual XOS.

J. Incorporation of prebiotic in the in vitro growth medium for probiotics

Media was prepared according to the method suggested by Hernandez-Hernandez et al [19]. Prebiotic was incorporated into carbohydrate free, MRS basal medium which consisted of peptone (10g/L), beef extract (5g/L), yeast extract (10g/L), ammonium citrate (2g/L), sodium acetate (5g/L), magnesium sulphate (0.1g/L), manganese sulphate (0.05g/L), dipotassium phosphate (2g/L) and polysorbate 80 (1g/L). The prebiotic samples were cooked with minimal amounts of water and filtered

with a muslin cloth. The filtrate was obtained and 2g/L of the filtrate was added to the media in place of dextrose to provide a carbon source.

K. Evaluation of prebiotics utilization by different strains of Lactobacillus

To study the effect of the synthesized prebiotics on the growth of different strain of Lactobacillus, which is a common constitutive of probiotics, the MRS media was modified by supplementing prebiotic samples as the sole carbon source for utilization by Lactobacillus. Three different strains of Lactobacillus, namely, L. acidophillus, L. plantarum, and L. casei, were studied and incubated at 37 ˚C for 48 hours, after which the viable count was determined by plating onto MRS agar.

III. RESULTS AND DISCUSSION

A. Selection of source of xylan

Upon autohyrolysis of the studied lignocellulosic residues, it was revealed that corn cob yielded the highest amount of 62.5±0.7 g total sugar/kg of corncob (Fig 1). Although there are multiple sources of xylan, the most widely used source is corn cob because the hemicellulose fraction of corncobs has a comparatively high content of acetylated xylan [20]. Another fact which makes corn cob a suitable raw material is their availability as maize, the source of corn cob, is the third most widely grown cereal crop in the world [21]. Hence, corn cob, which is a residue after agricultural, commercial and industrial use of maize, can be used easily and readily for the production of XOS.

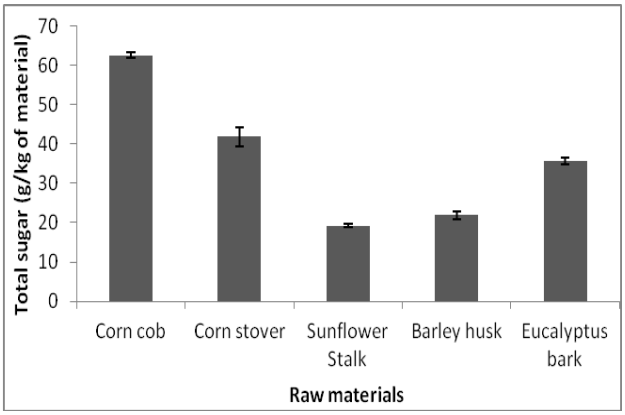

Figure 1. Total sugar obtained from different raw materials upon auto hydrolysis, Values are mean of triplicates ± SD.

B. Selection of method of aqueous extraction of xylan

The selected raw material, corn cob, was exposed four conventional treatment methods and it was found that the yield of sugar was the highest (62.5±0.7 g/kg of raw material) when autohydrolysis was used as a treatment for extraction of xylan from corn cob, as seen in Fig 2. The obtained result is in corroboration with the report which suggests that when corncobs when immersed in water and is heated, water autoionisation causes release of hydronium ions which cause both xylan depolymerisation and cleavage of acetyl groups from xylan residues, which is an effective way to obtain xylan from complex lignocellulosic residues [20].

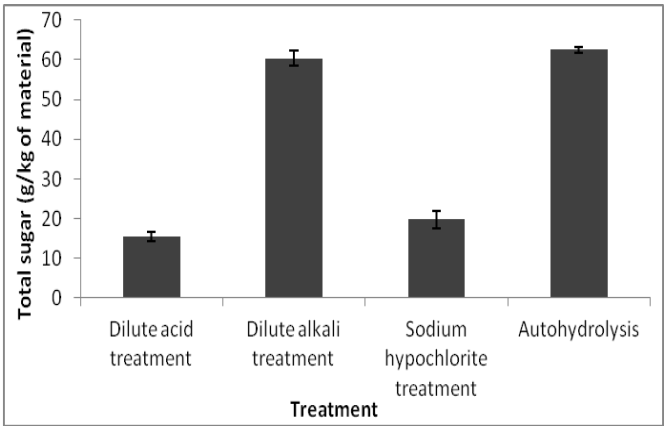

Fig ure 2. Yield of total sugar from corncob after treatment by different methods, Values are mean of triplicates ± SD.

C. Treatment of aqueous extract of xylan with xylanase and its optimization

After the extraction of xylan, the next step involved hydrolysis by xylanase to produce XOS. Two different enzyme concentrations (30 and 40 U) were studied for their effect on extracted xylan for the period of 160 minutes. It was found that exposure of the xylan extract to 40 U of xylanase resulted in production of 6.2±1.5 mg/ml of XOS in 50 minutes (Fig 3). The reports for this amount of yield of XOS in such a period of time by xylanase were not encountered in the existing literature. The reported yields of XOS from corncob were 7.89 mg/ml in 120 minutes by xylanase from Aspergillus foetidus MTCC 4898 and 10.09 mg/ml of XOS in 16 hours by xylanase extracted from Aspergillus oryzae MTCC 5154 [10, 16].

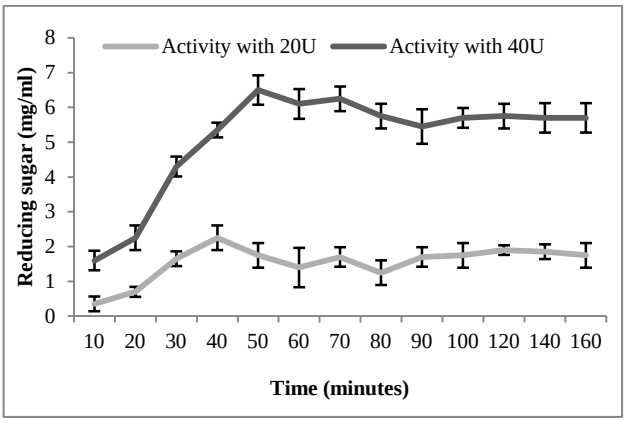

Fig ure 3. Yield of xylooligosaccharide from xylan extract. Values are mean of triplicates ± SD.

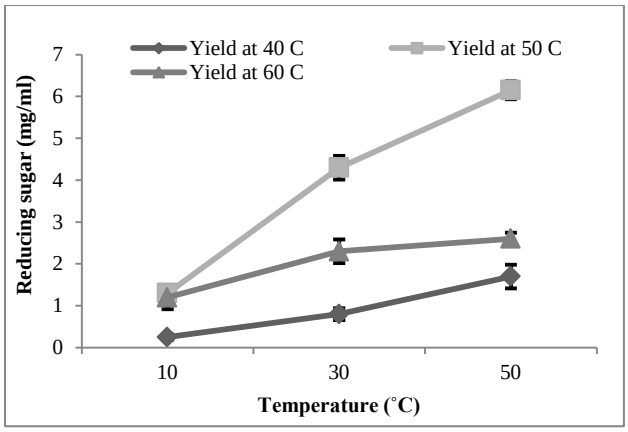

Figure 4. Effect of temperature on the production of XOS, Values are mean of triplicates ± SD.

Upon study of effect of temperature on the production of XOS, theresults obtained indicated the maximum production of XOS at the enzyme's optimal temperature, i.e. 55 ˚C. The yield declined with any change in temperature, for instance, at 40 ˚C and 50 ˚C the yield was 80% and 32% of the original yield respectively (Fig 4). Moreover, multiple studies made on XOS have been encountered which stares the maximum yield of XOS when the treatment is carried out at 50-55 ˚C [21-23].

D. Evaluation of stability of XOS

Since the pH of intestine lies in the range of 6-7, i.e. around neutrality. But it has to pass through acidic pH in stomach. Hence, the stability in the pH range of 2.0 -7.0 was examined and it was found that 92-96% stable at pH 2 and 3. The stability at pH 4 to 7 was found to be the maximum (98-100%) as shown in Fig 5. These results indicate its possible applicability of supplementation of XOS in heat processed and acidic foods.

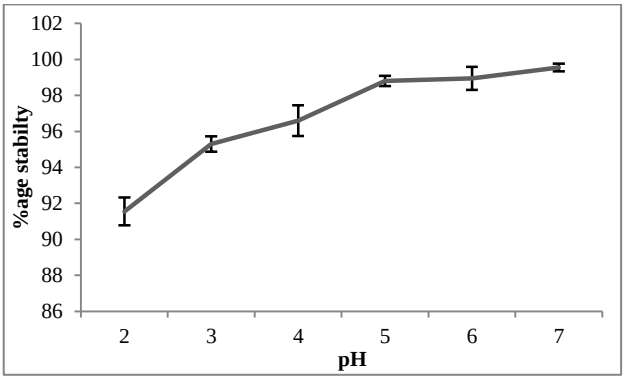

Fig ure 5. Percentage stability of xylooligosaccharide at different pH, Values are mean of triplicates ± SD.

E. Evaluation of prebiotics utilization by different strains of Lactobacillus

For the purpose of evaluating the utilization of prebiotics by standard probiotic strains, the cultures obtained of different strains of Lactobacillus, (L. acidophilus, L. casei, and L. plantarum) were inoculated in MRS Medium and then for determining the viable count, colonies grown on MRS agar were counted. It was found that MRS medium having XOS as carbon source showed more growth of organisms in 48 hours, than the MRS media, where dextrose is the carbon source. These results were indicative of the

fact that the prebiotics synthesized was able to stimulate the growth of all three Lactobacillus strains under study.

IV. CONCLUSION

The present study was done to establish the potential of partially purified xylanase from an indigenous strain of A. pullulans to produce XOS from different agricultural residues. After conducting screening experiments, it was revealed that corncobs was providing with highest yield of sugar and hence was used for further studies. Further, upon testing the method to be used for the production of xylan extract, it was observed that autohydrolysis was the best suitable for the stated purpose. The XOS was produced with exemplary efficiency, i.e., 6.2±1.5 mg/ml, when xylan extract was exposed to 40U of xylanase for 50 minutes at 55 ˚C. Moreover, the stability of the produced XOS was checked under acidic conditions and it exhibited 98-100% stability in the pH range of 2.0-7.0. Finally, the stimulatory effect of the XOS was checked on the three prebiotic strains, namely L. acidophilus, L. casei, and L. plantarum and the results were encouraging.

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