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Screening of low cost agricultural wastes to maximize the fructosyltransferase production and its applicability in generation of fructooligosaccharides by solid state fermentation

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ABSTRACT

Production of fructosyltransferase by *Aspergillus flavus* NFCCI 2364 was exploited in solid state fermentation using low cost agricultural wastes for their ability to generate fructooligosaccharides. Among sixteen screened agro wastes, sugar cane bagasse was remarked as the most promising substrate suited for excellent growth and adequate production of fructosyltransferase (FTase) in 96 h of fermentation. The fermentation attempted in one variable executed yeast extract 0.2 g/gds (gram per gram dry substrate) as the most applicable nitrogen source and inoculum size 1×10^8 spores/gds resulted FTase activity of 197.10 U/gds (unit per gram dry substrate). The recuperated FTase activity of 423.18 U/gds was executed by rotable central composite design RCCD) approach of response surface methodology by validated process variables viz. pH 5.0, substrate particle size 2.5 mm, substrate moisture ratio 1:1.4 and substrate quantity of 7.0 g respectively. The study signifies excellent stability and compatibility of *A. flavus* NFCCI 2364 using sugarcane baggase as an economical substrate for industrial production of fructosyltransferase.

1. Introduction

The structural diversity of carbohydrates by interaction with microbial enzymes led the formation of new essential oligosaccharides which are beneficial for human health. From the past three decades, prebiotic fructooligosaccharides (FOS) documented well both in pharmaceutical and biotechnological industries due to its wide scope of health applications (Sangeetha et al., 2005; Hernalsteens and Maugeri, 2010; Tian et al., 2014). FOS also renamed as oligofructose or oligofructan are exploited as an artificial or alternative sweetener which is regarded as safe for peoples with diabetes (Ganaie et al., 2013). The usefulness of FOS also comprises non carcinogenicity, assistance of calcium and magnesium absorption, decrease level of cholesterol, phospholipids and triglycerides (Yun, 1996; Linde et al., 2012; Ganaie and Gupta, 2014a). Moreover its functional properties invigorate growth of bifidobacteria which serves as substrate for enhancement of microflora in large intestine and discourage growth of potentially putrefactive microorganisms that have capability of causing diarrhea (Dominguez et al., 2013; Rawat et al., 2015). The production of FOS by most researchers led their involvement to comprise new investigation processes in submerged fermentation and fewer researchers focused on solid state fermentation (SSF). The exploitation of low cost agro-industrial residues for the cultivation of microbial enzymes is relatively an economical aspect than submerged fermentation due to mild recovery products, absence of foam formation, reduces risk in contamination and releasing of some essential metabolic compounds in fermented media (Mukherjee et al., 2008; Soni et al., 2015; Aruldass et al., 2016). For cultivation of these enzymes, the cost induced with SSF technique is simpler due to modest substrate medium, less requirement of instrumentation, low energy and easy downstream process and

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yield output is preferentially higher than submerged fermentation (Pandey et al., 1999; Sandhya et al., 2005; Mazutti et al., 2006). Optimization of Physio-chemical parameters by stastical experimental designs is practically applicable for helping to comprehend the interactions amid certain process parameters at varying levels and calculating each optimal level of parameter for efficient production of FOS (Xiong et al., 2007). RSM model is a mathematical and stastical established technique to examine the effect of distinct variables and to strive for the optimum conditions in a multivariable system (Abd et al., 2014; Gomez and Sartaj, 2014). The estimation of certain fermentation conditions for FTase production is pertinent as many fermentation parameters significantly influence the cultivation of biocatalysts.

In our previous experiments, *A. flavus* NFCCI 2364 was corroborated to be the most credible strain for high FTase titers in submerged fermentation. The intimate relationship between sucrose and fructosyltransferase specify admirable transfructosylating activity which configured considerable amount of FOS (Ganaie et al., 2014b). Brazil is regarded as one of the leading producer of agriculture and commodities of its economy depends one-quarter on the agricultural products. In this context the main objective of the present investigation was to optimize the process variables for cultivation of FTase with low cost agriculture wastes under solid state fermentation. The comparative evaluation performed by response surface methodology (RSM) using rotable central composite statistical design (RCCD) was employed for optimizing the fermentation parameters for increased FTase production and its applicability in generation of fructooligosaccharides.

2. Materials and methods

All chemicals used were of analytical grade. FOS standards kestose (GF2), nystose (GF3) and fructofuranosyl nystose (GF4) were purchased from Wako Pure Chemicals USA and sucrose, glucose and fructose were obtained from Sigma Aldrich, USA, respectively.

2.1. Microorganisms and inoculum preparation

Aspergillus flavus NFCCI 2364 strain was obtained from National Fungal Culture Collection, Pune, India. Considering its excellent performance in submerged conditions (Ganaie et al., 2013, 2014b), this fungus was assayed in SSF studies for FTase production. The fungal strain was cultured on Potato dextrose Agar (PDA) medium at 28 °C for 6–7 days and was maintained in a slant at 4 °C for further use. The spore suspension was prepared by scrapping the full loop of spores from PDA plates and suspended in double distilled water containing 0.1% Tween 80 by counting in neubers chamber.

2.2. Screening of substrates for fructosyltransferase production

Different agro wastes including wheat bran, corn straw, sugar cane bagasse, cassava peels, apple pomace, banana peels, beet root peels, orange peels, guava peels, guava seed powder, pine apple peels, papaya peels, mango peels, passion fruit peels, jabuticaba peels and cashew peels were evaluated for FTase production. The waste materials were first washed with distilled water to remove any dust present on their adhered surface and then blenching operation was performed by immersing them in hot water at 70–80 °C for 10 min followed by drying overnight at 45 °C. The dried material was ground in grinder (Remi) and stored at 4 °C in wide mouth rounded bottles. Solid state fermentation was employed in 250 ml conical flask containing 5 g of substrate supplemented with 0.1 g/gds of yeast extract, moistened with 5 ml of distilled water. The components in the flask were blended thoroughly and autoclaved at 121 °C, 15 lbs pressure for 15 min. The flasks were cooled at room temperature and then inoculated 1×10^7 spores in asceptic condition and assisted for incubation at 28 °C under motionless condition. After 72 h of fermentation, 50 ml of distilled water was added to fermented medium and flasks were shaken sporadically for 24 h at 150 rpm. For enzyme extraction, the complete content of flask was squeezed through muslin cloth and extract was centrifuged at 10000g for 15 min at 4 °C in a refrigerated centrifuge. The clear supernatant was decanted for FTase activity and FOS production.

2.3. Nitrogen and carbon supplements on FTase production

Various components of carbon and nitrogen sources were supplemented in the media at concentration of 0.1 g/gds at 28 °C for 72 h. The flask without any nitrogen source was served as ANS (Absence of any nitrogen source). The best nitrogen source was selected and its concentration was monitored from 0.1 to 0.5 g/gds to evaluate the maximum production of FTase enzyme.

2.4. Initial spore concentration on FTase production

It is important to examine the optimum spore concentration so as to maximize the possible yield of FTase enzyme. Different spore concentration levels varying at 1×10^6, 1×10^7, 1×10^8, 2×10^6, 2×10^7, 2×10^8 spores/gds were inoculated in the flasks containing 5 g of substrate and incubated at 28 °C for 72 h.

2.5. Incubation and fermentation time

Solid state fermentation was employed in 250 ml conical flask containing 5 g of substrate supplemented with optimum value of yeast extract and spore suspension with 5 ml of distilled water at different temperatures from 25 to 35 °C. The optimum incubated flask harvested good amount of FTase was further investigated from 24 to 144 h for prominent fermentation time so as to get cumulative amount of FTase.

2.6. Experimental design for optimum FTase production

Initial pH, particle size, substrate concentration and moisture content are crucial factors affecting enzyme production in SSF. All these conspicuous factors were optimized and parameters were selected to find their optimum values for FTase production using RCCD. The ranges and levels of the variables evaluated through RSM are listed in Supplementary Table 1. According to RCCD, the total number of experimental combinations is $2^k + 2k + n_0$, where k is the number of independent variables and n_o is the number of repetitions of the experiments at the center point. A total of 30 set experiments including six center points were conducted along with different combinations of physical parameters. Each numeric factor was varied over 5 levels, that is, plus and minus alpha (axial point), plus and minus one (factorial points), and zero (center point).

2.7. Statistical analysis and validation of experimental model

The data obtained from RSM was subjected to analysis of variance (ANOVA) for analysis of regression coefficient, prediction equations, and case statistics. Analysis of data was performed using Design-Expert software (Version 6.0). The experimental results of RSM were fitted using the second order polynomial equation: