DEMERARA: A COST-EFFECTIVE CARBON SOURCE FOR ENHANCED FTASE PRODUCTION IN *Aspergillus oryzae* IPT-301

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ABSTRACT

Fructo-oligosaccharides (FOS) are prebiotic substances that have been extensively incorporated in various food industry products, mostly for their bifidogenic properties and economic value. The production of FOS is largely dependent on the enzyme Fructosyltransferase (FTase). The activity of this enzyme can differ based on the type of microorganism and the composition of the culture medium, particularly the carbon source, which can act as an inducer. In particular, the strain *Aspergillus oryzae* IPT-301 was found to produce both extracellular and intracellular FTase when using Demerara sugar, a cost-effective carbon source. The peak production of extracellular and intracellular FTase was observed within 40 and 48 hours of cultivation, respectively. The results are promising for scaling up process in a bioreactor, aiming for much higher FTase production levels desirable for industrial applications.

Keywords: Demerara sugar. Fructosyltransferase. *Aspergillus oryzae* IPT-301.

1 INTRODUCTION

The FOS fructooligosaccharides market is projected to reach US$5.22 billion by the year 2028. To meet the demand generated by this consumer market, microbial enzymes have been used for industrial scale production[1]. These enzymes have greater specificity and selectivity and can be found in a wide range of filamentous fungi[2].

Fructosyltransferases (FTase, EC 2.4.1.9) are considered the most efficient enzymes for the synthesis of FOS[3]. FTase synthesize FOS preferentially using sucrose as a carbon source[4]. The use of cheap and readily available carbon sources for FTase synthesis has been the subject of intense research as a cost-effective alternative[5].

Considering its production cost in Brazil, demerara (DM) sugar can be considered as an alternative carbon source for the production of FTase due to it is an abundant, low-cost and little-processed product. Furthermore, there are no studies reporting and describing the use of DM sugar in FTase synthesis. This raw sugar appears to be a promising source since its chemical composition displays adequate nutritional requirements for a fermentative medium, allowing it to be an excellent substrate alternative[6]. In this context, the aim of the present study was to examine the effect of DM carbon source on cell growth and intracellular and extracellular FTase production by filamentous fungi *Aspergillus oryzae* IPT-301.

2 MATERIAL & METHODS

The microorganism used in this study was the fungal strain *Aspergillus oryzae* IPT-301, obtained from the Industrial Biotechnology Laboratory at the São Paulo Institute of Technological Research (LBI/IPT-SP).

The composition of the culture medium used in submerged fermentation included the following components: Demerara sugar (150.0 g, Native®), yeast extract (5.0 g, Synth®), $NaNO_3$ (5.0 g, Dinâmica®), KH_2PO_4 (2.0 g, Synth®), $Mg_2SO_4.7H_2O$ (0.5 g, Dinâmica®), $MnCl_2.4H_2O$ (0.3 g, Synth®), and $FeSO_4.7H_2O$ (0.01 g, Synth®), with a pH adjusted to 5.5 using a NaOH (Synth®) solution. Fifty milliliters of the culture medium were added to 250 mL Erlenmeyer flasks and sterilized at 120 °C for 15 minutes. After sterilization, 0.5 mL of a spore suspension with a concentration of 1 x 10^7 spores mL^{-1} was inoculated into each flask, followed by incubation in an orbital shaker (Tecnal®, modelo TE-4200) at 30 °C and 200 rpm for 72 hours [7,8]. Samples were collected in triplicate at 8-hours intervals. The total content of each Erlenmeyer flask was vacuum-filtered using a pump TE-058 (Tecnal®) and Whatman No. 1 filter paper (90 mm diameter). The retained biomass (cake) was thoroughly washed with distilled water and dried in an oven, at 55 °C for 24 hours. Biomass concentration was determined by dry cell mass per volume (g L^{-1}). The average biomass concentration values were plotted on a graph of biomass concentration versus fermentation time. pH values of the samples were measured using a digital pH meter (DigiLab®, Modelo 82588).

An enzymatic activity unit of transfructosylation was defined as the amount of enzyme that produces one micromole (1 µmol) of FOS per minute. To determine the extracellular and mycelial transfructosylation activities, a Falcon tube containing 1.2 mL of tris-

acetate buffer solution (0.2 mol.L^{-1}, pH 5.5, Synth®) and 3.7 mL of sucrose solution (63.6 % m/v, Synth®) was used. The enzymatic reaction was initiated by adding 0.1 mL of filtered fermented broth containing extracellular FTase or 0.05 g of wet mycelium retained on filter paper. This reaction was conducted in a Dubnoff water bath (Bunker®, modelo NI 1232) at 190 rpm and 50 °C, for 60 minutes, interrupted by boiling in a water bath for 10 minutes, followed by an ice bath [9,10].

3 RESULTS & DISCUSSION

The impact of different carbon sources on cellular growth and FTase production by *Aspergillus oryzae* IPT-301 is illustrated in Figure 1 (A, B). The enzyme synthesis was significantly influenced by the carbon source Demerara (DM) sugar. Experimental results revealed that DM in the culture medium affected both cell growth and extracellular and intracellular FTase activities. At 40 and 48 hours, the maximum extracellular activity reached 192.98 U/mL, while the intracellular activity reached 506 U/g for transfructosylation (A_T). Interestingly, the extracellular A_T values exceeded those reported in our previous study that used sucrose as the carbon source, and the intracellular A_T values were similar. In comparison, another study[11] employed sucrose as the carbon source for FTase synthesis by filamentous fungi. The FTase levels detected in culture filtrates of *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus terreus*, and *Penicillium islandicum* were lower than those described in our current study. Additionally, *Aspergillus tamarii* Kita UCP 1279 achieved a maximum FTase activity of 42.71 U/mL using 3.0 g of wheat bran, 70 % moisture, and 20 % sucrose at 30 °C during 96 hours of incubation[14]. We therefore emphasized the importance of using agroindustry residues to obtain FTase with high specific activity.

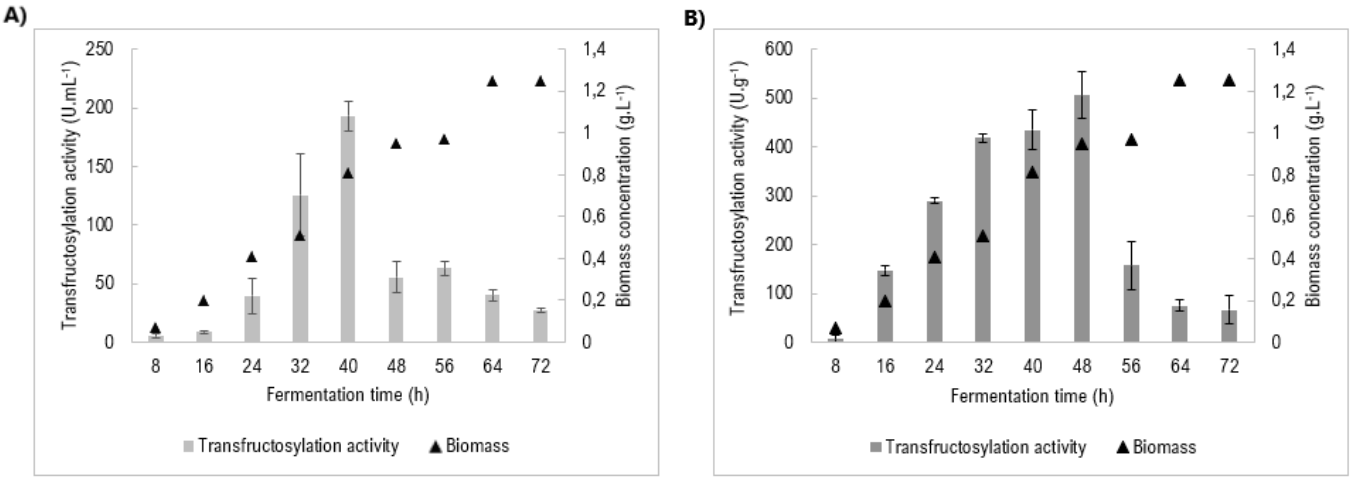

Figure 1. Transfructosylating activity (A_T) (A) extracellular and (B) intracellular of *Aspergillus oryzae* IPT-301 grown on medium containing Demerara sugar, as carbon source, over a period of 72 hours.

4 CONCLUSION

The use of accessible carbon sources, such as DM sugar, in a synthetic culture medium for the production of the enzyme FTase by the fungus *Aspergillus oryzae* IPT-301 shows promise. The results obtained reveal a high effectiveness of sugar in producing extracellular FTases, as evidenced by the significant outcome observed at the 40-hour mark. When compared to previous studies, this process demonstrates robust production of extracellular enzyme activity, with mycelial transfructosylation activity aligning with the averages reported in earlier research. This consistency reinforces the robustness and promising applicability of DM sugar as an efficient and economically viable alternative for FTase and, consequently, FOS production.

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