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Fructooligosaccharide production using fructosyl transferase obtained from recycling culture of *Aspergillus oryzae* CFR 202

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Abstract

Fructooligosaccharide (FOS) production was carried out using fructosyl transferase (FTase) produced by *Aspergillus oryzae* CFR 202 under submerged fermentation conditions. The pellets of *A. oryzae* CFR 202 obtained after 48 h of fermentation were supplemented with fresh media after every 24 h and fermentation was carried out to produce FTase. FTase so obtained was used to produce FOS using 60% sucrose as substrate at 55 °C at pH 5.15. FTase activity was maintained in the range of 15 ± 2 U/ml/min up to six recycles. FOS yields were maintained at 53% up to 6th cycle. Recycling of pellets could not be carried out after 6th cycle due to disintegration. The system is advantageous and economical in that it does not require supplementation of any additional nutrients nor it requires the development of fresh inoculum.

Keywords: Fructooligosaccharides; Fructosyl transferase; *Aspergillus oryzae*; Fungal pellets; Cell recycling

1. Introduction

Fructooligosaccharides (FOS) are found in trace amounts as natural components in fruits, vegetables and honey. They are produced by the action of fructosyl transferase (FTase) from many plants and microorganisms. FOS formed in this process contain two to four β (1-2)-linked fructosyl units to a terminal α D-glucose residue. FOS derived from sucrose using microbial enzymes have attracted special attention due to their sweet taste being very similar to that of sucrose, a traditional sweetener [1].

The safety and health benefits of FOS have been reviewed [2,3]. FOS has low intensity of sweetness since they are only about one-third as sweet as sucrose. It is scarcely hydrolyzed by the digestive enzymes and not utilized as an energy source in the body. They are non-cariogenic, encourage the growth of beneficial bifidobacteria, and decrease the levels of serum cholesterol, phospholipids, and triglycerides [4]. Production of FOS was observed during the growth of several fungi in sucrose medium. Microbial production of oligosaccharides has been extensively reviewed by many authors [5,1,6].

Preliminary studies on the screening of a few fungal strains for FTase activity resulted in the selection of *Aspergillus oryzae* CFR 202 as a potent strain based on high Ut/Uh ratio [7] (Ut stands for transfructosylating activity and Uh for hydrolytic activity). Important parameters influencing the production of FTase and FOS using this strain were studied using Plackett Burmann design [8] and their levels were optimized using response surface methodology. Using the optimized media, *A. oryzae* CFR 202 produced compact, round pellets which were stable throughout the fermentation period of 90 h. This is a common phenomenon observed when filamentous fungi are grown in submerged culture. These pellets consisting of compact masses of hyphae may change their morphology as growth proceeds. In an industrial fermentation, the formation of pellets is advantageous, since the filamentous form of fungus may wrap around the impeller and damage the agitator blades, and is often prone to block the spargers [9]. Also, pellet formation makes downstream processing easier in industrial fermentation.

FTase is produced as an extracellular enzyme by *A. oryzae* CFR 202. The biomass is not being used for any purpose and is discarded after filtration of the broth. However, the pellets were so compact that they could be used for the next cycle of fermentation, provided they are capable of

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producing extracellular FTase. Thus, a cell recycling system can be designed by recycling the cells every 24 h to produce FTase and thereby FOS. The objective of this study is to find an efficient use for the pellets grown during fermentation, which will save the development of fresh inoculum.

2. Materials and methods

2.1. Chemicals

All chemicals used were of analytical grade. Fructooligosaccharide standards 1-kestose (GF_2), 1-nystose (GF_3), and 1-fructofuranosyl nystose (GF_4) were from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

2.2. Microorganisms and culture conditions

Aspergillus oryzae CFR 202 is from the type culture collection of CFTRI, Mysore. The strains are maintained on potato dextrose agar slants at 4 °C.

2.3. Inoculum development

The inoculum was prepared by transferring a loopful of spores from a 5-day-old slant to 100 ml medium containing 1% sucrose and 0.2% yeast extract (pH 5.5) in 500 ml flasks autoclaved at 121 °C for 15 min. The flasks were then incubated at 30 ± 1 °C on a rotary shaker (Emenvee Rotary Shaker, 48N3, Pune, India) at 250 rpm for 24 h.

2.4. Production of fructosyl transferase enzyme by recycling cell culture

A 24-h-old inoculum (20% v/v) was transferred to 100 ml of fermentation medium containing 10% sucrose, 0.8% yeast extract, 1% $NaNO_3$, 0.03% $MgSO_4{\cdot}7H_2O$, 0.4% K_2HPO_4, 0.9% KH_2PO_4, 1% NH_4Cl and 0.6% NaCl with an initial pH of 5 in 500-ml flasks. The flasks were incubated at 30 ± 1 °C on the rotary shaker at 250 rpm. At the end of 48 h of fermentation, the pellets were separated by decanting the culture broth in to a container under aseptic conditions and fresh culture media was added to the pellets. At the end of every 24 h, the broth was decanted and fresh culture media was added. Fermentation was continued up to 144 h. The decanted broth was checked for its pH, FTase activity, and production of FOS.

2.5. Assay for fructosyl transferase

The reaction mixture for the assay of FTase activity consisted of 1.5 ml of 60% (w/v) sucrose in 0.1 M citrate buffer (pH 5.5) and 0.5 ml crude enzyme-culture fluid. The reaction was carried out at 55 ± 1 °C for 1 h using a water bath (Inlab Equipments (M) Private Limited, Chennai, India). The reaction was terminated by keeping the reaction mixture in boiling water bath for 15 min. Glucose released at the end of the reaction was estimated using Glucose kit (GOD/POD method) (Dr. Reddy's Laboratories, Hyderabad, India). One unit of FTase activity was defined as the amount of enzyme required to release 1 μmol of glucose released per ml per minute under the above mentioned reaction conditions.

2.6. FOS production

FOS production was carried out by incubating the reaction mixture (1.5 ml of 60% (w/v) sucrose in 0.1 M citrate buffer (pH 5.5) and 0.5 ml crude enzyme-culture fluid) for 18 h under the above mentioned reaction conditions. Quantitative analysis of the products was done using HPLC (LC –6 A, Shimadzu, Japan) with a refractive index detector (RID-6A) using the polar bonded phase column (Exsil-NH_2, 4.6 mm × 250 mm, 5 μ) with acetonitrile:water (75:25) as mobile phase at a flow rate of 1.0 ml/min. The samples were diluted appropriately and filtered through a membrane filter with a pore size of 0.45 μm (Millipore) before injection. The retention times of the individual FOS were compared with that of standards for identification. The final FOS was expressed as yield (% w/w) based on the initial sucrose concentration.

3. Results and discussion

Fructosyl transferase production was carried out using a recycling culture of *A. oryzae* CFR 202 in sucrose containing medium. After 48 h of fermentation, the pellets were recycled every 24 h and used for FTase production in fresh medium. FTase activity and pH were monitored in the culture fluid obtained at the end of each cycle of fermentation. As shown in Fig. 1, the pH of the broth increased from 5.0 to 6.03 at the end of 48 h of fermentation. Afterwards, the pH increased to 6.18 at the end of 96 h and remained same throughout the fermentation time of 7 days. FTase activity was 7.8 U/ml/min at the end of 48 h of fermentation. It reached a maximum value of 16.5 U/ml/min after the next two consecutive cycles and then decreased to 11.9 U/ml/min at the end of the sixth recycle (Fig. 2). However, FOS yield was maximum at the end of the second recycle (53% w/w) and was maintained at the same level even at the end of the sixth recycle (Fig. 2). The pellets could not be recycled further since it lost the compactness and started disintegrating after the sixth recycle.

Since the production and application of FOS have gained commercial importance because of their favourable functional properties, there is always scope to search for newer and potential sources of FTase. The present study describes a system wherein FTase activities were maintained high for six cycles of fermentation. Using this FTase, FOS yields were also found to be maintained high for six cycles. The advantage of this system is that the pellets were available for repeated production of FTase and FOS and there was no need for further seed culture.