Carbohydrate Polymers 110 (2014) 253–258

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Recycling of cell culture and efficient release of intracellular fructosyltransferase by ultrasonication for the production of fructooligosaccharides

Mohd Anis Ganaie*, Uma Shanker Gupta

Microbial Technology Laboratory, Department of Zoology, Dr. Harisingh Gour University Sagar, 470003 MP, India

ARTICLE INFO

Article history:
Received 6 April 2013
Received in revised form 11 January 2014
Accepted 20 March 2014
Available online 3 April 2014

Keywords:
Ultrasonication
Fructooligosaccharides
Fructosyltransferase
Recycling

ABSTRACT

Production of fructooligosaccharide (FOS) through efficient cultivation of biotransformation process by fructosyltransferase (FTase) was evaluated by two new isolates, *Aspergillus niger* and *Aspergillus flavus* NFCCI 2364. The saccharide consumption revealed lag phase of *A. niger* in 10 h which were smaller extent than *A. flavus* of 14 h. For the recycling of cell culture, the pellet cells were continuously reused after 24 h of submerged fermentation by these microorganisms in which FTase activity remains stable in four consecutive cycles in *A. niger* and six cycles in *A. flavus*. When freshly prepared pellets were sonicated for efficient release of intracellular FTase, the best transformation reaction was performed at 20 W acoustic power giving conversion yield of FOS 61.43% (w/w) by *A. niger* and 70.44% (w/w) by *A. flavus respectively*. This study was shown that the two fungal isolates can serve as veritable source of intracellular FTase for industrial production of FOS.

1. Introduction

Fructooligosaccharide an important food candidate with several functional benefits exposes new perspectives in nutrition and food sciences from the last two decades. Fructooligosaccharides (FOS) are oligosaccharides of fructose consisting of one to three fructose units bound by β 2→1 position of sucrose (Maugeri & Hernalsteens, 2007). FOS represent major group of fructan oligomers and are mainly composed of 1-kestose (GF2), nystose (GF3) and 1-fructofuranosylnystose (GF4). They are considered both alimentary additives and nutraceutical that are not susceptible to decomposition by human or animal digestive enzymes (Mussatto, Rodrigues, & Teixeira, 2009; Yun, 1996). Due to its various interesting functionalities the market potential of FOS further includes applications in pharmaceutical and diagnostic sector underlying great industrial value (Fernandez, Ottoni, Silva, Matsubra, & Carter, 2007; Maiorano, Piccoli, da Silva, & de Andrade, 2008; Zuccaro, Gotze, Kneip, & Dersch Seibel, 2008; Ganaie et al., 2014). The important health benefits include bifidogenic property, bioavailability of minerals, non-cariogenic property, safe for diabetes and prevention of colonic carcinogenesis (Dominguez et al., 2012; Ganaie, Lateef, & Gupta 2014; Sangeetha, Ramesh, & Prapulla, 2004). The synthetic process of FOS is carried out by transfructosylating process of fructosyltransferase (FTase) or β-fructofuranosidase (FFase) from many plants, bacteria and fungi (Ganaie, Gupta, & Kango, 2013; Mussatto et al., 2012). The FOS producing enzymes from microorganisms are excreted either outside of cell as extracellular enzyme or retained within the cell as an intracellular enzyme. A variety of methods have been used to break up cells and each one having its advantage and disadvantage (Chisti & Young, 1986). Ultrasonication a liquid shear method has received much attention for disruption of microbial cells in suspension at laboratory scale. This method is suitably used for industrial purpose, since it provides low operational cost, neither sophisticated device nor extensive technical skills (Barton, Bullock, & Weir, 1996; Feliu, Cubarsi, & Villaverde, 1998; Ozbek & Ulgen, 2000). Ultrasound in enzyme solutions leads positive impact on enzyme activity. It increases growth of microorganisms but high frequency ultrasound of 15–20 kHz is capable of causing disruption of cell wall thereby releasing intracellular enzymes (Matsuura, Hirotsune, Nunokawa, Satoh, & Honda, 1994; Swamy, Sukla, Narayama, Kar, & Panchanadikar, 1993), while too high intensity ultrasound (>20 kHz) can cause denaturation of enzyme (Mason, Paniwynk, & Lorimer, 1996). Ultrasonication had been widely used for obtaining various intracellular enzymes from microbial cells in order to release enzymes like glucose oxidase from *Aspergillus niger* CCT 7415 (Ishimori, Karube, & Suzuki, 1982), β-galactosidase of *E. coli*

* Corresponding author. Tel.: +91 7582 265056; fax: +91 7582 265994.
E-mail addresses: mohd.anis90@yahoo.com, anisurehman.2011@rediffmail.com (M.A. Ganaie).

http://dx.doi.org/10.1016/j.carbpol.2014.03.066
0144-8617/

and *Lactobacillus* strain (Feliu et al., 1998; Wang & Sakakibara, 1997), invertase from *Aspergillus niger* (Vargas, Piao, Domingos, & Carmona, 2004) and fructosyltransferase from *Aureobasidium pullulans* CFR 77 (Lateef, Oloke, & Prapulla, 2006) respectively. The mechanism of cell disruption by ultrasound is due to cavitation phenomenon in which microbubbles form at various nucleation sites in fluid. These bubbles grow during refraction phase of sound waves, and then in compression phase, these bubbles collapse releasing violent shock wave which propagates though the medium. Through this phenomenon, large quantity of sonic energy is converted into mechanical energy in form of elastic waves (Chisti & Young, 1986).

In our previous study, *A. flavus* (NFCCI 2364) and *A. niger* (SI) proved to be potential producers of FOS (Ganaie, Gupta et al., 2013). These strains also produced compact fungal pellets when grown under submerged fermentation. The main objective of present work is to investigate effect of ultrasound on release of intracellular FTase from these strains and its impact on FTase activity for FOS production. For successful performance of FOS yield, recycling of cell culture was also investigated so as to avoid the need for the development of fresh inoculum.

2. Materials and method

2.1. Microorganisms and culture conditions

A. niger (SI), a soil isolate microorganism obtained from culture collection Department of Applied Microbiology and Biotechnology, Dr. H.S. Gour University Sagar and *A. flavus* NFCCI 2364 obtained from Culture Collection ARI, Pune, India were used in this study. Both these microorganisms were grown on potato dextrose agar (PDA) at 28 °C and were kept on slants at 4 °C until further use (Supplementary Figs. S1 and S2). Cultivation of FTase was performed in 250 ml shake flask containing sucrose 20%, yeast extract 0.5%, $NaNO_3$ 1%, $MgSO_4{\cdot}7H_2O$ 0.05%, $KH_2PO_4{\cdot}0.25\%$, NH_4Cl 0.5%, and NaCl 0.25% in 100 ml of distilled water with an initial pH of 6.0. The medium was sterilized at 110 °C for 15 min and incubated on rotary shaker (Lark Germany) at 28 °C for 72 h. At the end of respective fermentation periods, flasks were withdrawn at regular time intervals and their contents were filtered through Whatman filter paper No. 2, 110 mm diameter. The cell free culture filtrate was used as a source of extracellular enzyme without further purification. All the experiments were carried out in triplicate for determining FOS formation comparing with its standard components 1-kestose, 1-nystose, 1-β fructofuranosylnystose and sugars such as sucrose, glucose, fructose from Wako Chemical Laboratories (Japan) and Sigma–Aldrich (USA) respectively (Supplementary Figs. S3 and S4).

2.2. Recycling of cell culture

For recycling, the 24 h pellet cells were separated by decanting the culture broth into container in aseptic conditions and fresh cultivation media was added. At the end of every next 24 h, broth was decanted and fresh media was added consecutively up to seven cycles (168 h). The fermented decanted broth was investigated for change in pH, transfructosylating activity and FOS formation.

2.3. Extraction of FTase enzyme by ultrasonication

After 72 h of fermentation, the freshly prepared harvested media of both microorganism *A. niger*, and *A. flavus* were centrifuged at 4 °C in cooling centrifuge (Eltek RC 8100 SF, India) and compact pellet cells were resuspended in 100 ml of cold distilled water for ultrasonication to obtain intracellular FTase. Ultrasonication of cells was carried out using Ultrasonic processor 200 W (Soniweld, Imeco, Ultrasonics, Mumbai, India). A probe type wave guide 2 mm diameter was immersed at a depth of 3 cm into 20 ml of cell suspension and two level of acoustic power 20 W and 40 W were investigated for irradiation period of 2–12 min. The sonication was done at 10 °C and cell suspension was kept in salt ice bath during disruption to prevent over heating. The ultrasonic energy was pulsed 0.5 s active and passive intervals for reduction of free radical formation (Lateef et al., 2007). At the end of ultrasonication, cell free lysate was centrifuged at 4 °C and crude FTase was used as source of intracellular enzyme for FOS production. The release of FTase enzyme was monitored by determining OD at 275 nm and protein content of the fraction by the Bradford method.

2.4. Enzyme assay

The FTase activity was determined by incubating 250 μl of enzyme with 750 μl of sucrose 60% (w/v) in 0.1 M citrate buffer (pH 6.0) at 55 °C for 1 h in water bath. At the end, reaction was stopped by keeping the reaction mixture in hot boiling water bath up to 10 min. Transfructosylating activity (Ut) was done by diluting 20 μl of reaction mixture with 980 μl of distilled water in order to make 50-fold dilution. Then, 10 μl from 1 ml reaction mixture was allowed to react with 1 ml test reagent using glucose oxidase peroxidise kit (Sigma) for 30 min until pink colour develops. The absorbance of glucose released was read at 505 nm on UV/visible spectrophotometer (Hitachi Techcom India). The units of fructosyltransferase (Ut) and hydrolytic (Uh) activities were defined as the amount of enzyme that required to release 1 μmol of glucose per ml per minute under the chosen experimental conditions.

2.5. Production and analysis of fructooligosaccharides

FOS production was carried out using reaction mixture of FTase and sucrose of (1:3) ratio in shaker incubator (Lark Innovata Germany) at 55 °C for 24 h. At the end of incubation reaction was arrested in boiling water bath at 100 °C for 10 min. The samples were diluted and filtered though membrane filter millipore 0.45 μm pore size and analysis was done by HPLC at room temperature 30 °C. The HPLC instrument was from Waters (USA) with RI detector 2414 and injection valve of 20 μl. Sugar pak column (6.5 × 300 mm) was used for identification and quantification of FOS derivatives kestose (GF_2), nystose (GF_3), 1-β-fructofuranosyl nystose (GF_4) and sugars such as sucrose (GF), glucose (G), fructose (F) respectively. The column temperature was kept 70 °C and RI detector temperature at 30 °C. The solvent system used was water as mobile phase at flow rate of 0.2 ml/min. Calculation of analysis was done by Empower 2 software Build 2154 Waters (USA).

2.6. Statistical analyses

The data in the tables and figures were expressed in triplicates as mean ± standard deviation. Using one-way analysis of variance (ANOVA), a difference was considered statistically significant if the p value was less than 0.05.

3. Results and discussion

3.1. Consumption and production of saccharides in cultivation media

After spore inoculation in cultivation media the selected microorganisms showed disparate growth pattern. The concentration of saccharides formation during course of fermentation periods was analyzed by HPLC. The results showed that the lag phase of *A. niger* was 10 h which was comparatively less as compared to *A. flavus* whose lag phase stayed up to 14 h before FOS formation commence. The lag phase of these microorganisms were quite