International Food Research Journal 23(1): 425-428 (2016)
Journal homepage: *http://www.ifrj.upm.edu.my*

Short Communication

Prebiotic fructooligosaccharide production from yeast strain ML1

Kaenpanao, P., Piwpan, P. and *Jaturapiree, P.

Department of Biotechnology, Faculty of Engineering and Industrial Technology, Silpakorn University, Muang, Nakorn Pathom, 73000 Thailand

Article history

Received: 1 June 2015
Received in revised form: 22 September 2015
Accepted: 14 October 2015

Keywords

Fructooligosaccharide
Optimization
Prebiotics
Sucrose

Abstract

Fructooligosaccharides (FOS) are non-digestible oligosaccharides, mainly composed of 1-kestose (GF_2), nystose (GF_3) and 1^F-β-fructofuranosyl nystose (GF4), which are recognized as prebiotics because they can stimulate the bifidobacteria growth in the human colon. The aim of this work was to evaluate the factors affecting the production of fructooligosaccharides using sucrose as the substrate by a yeast strain ML1 isolated from sugarcane juice. The effects of sucrose concentrations (50-150 g/L), yeast extract concentrations (10-30 g/L) and agitation speeds (75-300 rpm) were studied in a 3L fermenter. It was found that sucrose concentration was the most effective parameter. This factor showed a positive influence on FOS yields. The optimized conditions for FOS production were 150 g/L sucrose, 20 g/L yeast extract with the agitation speed of 150 rpm. Under these controlled conditions, the maximum specific growth rate and the FOS concentration were obtained approximately 0.27 h^{-1} and 65 g/L, respectively.

Introduction

Fructooligosaccharides (FOS) are mixture of one to three fructosyl units bound to the β-2, 1 position of sucrose (GF) such as 1-kestose (GF_2), nystose (GF_3) and 1^F-β-fructofuranosyl nystose (GF_4). They are recognized as prebiotics defined as a non-digestible food component that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of colonic bacteria (Gibsan and Roberfroid, 1995; Rao, 2001). Besides this, FOS are non-cariogenic and non-caloric but their sweet taste are much similar to that of sucrose (Yun, 1996). They are widely used in food and pharmaceutical industries as a functional sweetener (Sangeetha *et al*., 2005a; Spiegel *et al*., 1994; Crittenden and Playne, 1996; Yun, 1996). FOS are principally formed from sucrose by the action of transfructosylation activity from plants and vegetables like garlic, banana, plum, onion, shallot, chicory, Jerusalem artichoke, etc. and bacteria and fungi such as *Aspergillus oryzae* (Sangeetha *et al*., 2005b), *Aspergillus japonicas* (Mussatto *et al*., 2009a), *Aspergillus niger* (Hidaka *et al*., 1988; Hirayama *et al*., 1989), *Aureobasidium pullulans* (Vandáková *et al*., 2004; Silva *et al*., 2011), *Arthrobacter* sp. (Fujita *et al*., 1994) and *Furarium* sp. (Gupta and Bhatia, 1980). The enzymes used industrially for FOS synthesis are mainly based on microbial enzymes. During the reaction of sucrose hydrolysis, a frutosyltransferase possesses the ability to bind the acceptor, fructosyl moiety, and to exclude H_2O and the various oligosaccharides were formed through transfructosylation. Typically, the production of FOS is a two-stage process; the fructosyltransferase production by microorganisms and the reaction of the extracted enzyme with sucrose to produce FOS (Mussatto *et al*., 2009b). In the present work, the factors affecting the production of fructooligosaccharides from sucrose by a yeast strain ML1, which isolated from sugarcane juice was investigated in a 3L jar fermenter.

Materials and Methods

Microorganism and inoculum preparation

A yeast strain ML1 isolated from sugarcane juice was maintained at 4°C on the standard medium agar (SMA) containing (per L): sucrose 20 g, yeast extract 20 g, $NaNO_3$ 10 g, $MgSO_4 \cdot 7H_2O$ 0.1 g, K_2HPO_4 1 g and agar 15 g. The initial pH was adjusted to 5.5. Cells from the stock cultures were transferred into a test tube containing 5 mL of standard medium broth (SMB) and grown at 37°C for 24 h. This culture was transferred into 250 mL Erlenmeyer flasks containing 50 mL of the standard medium broth (SMB) and incubated at 37°C on a rotary shaker (150 rpm) for 24 h. This broth was used for further inoculation in a fermenter culture.

*Corresponding author.
Email: *phimchanok@hotmail.com*
Tel: +66-3421-9360; Fax: +66-3421-9360

Culture conditions

In order to study the effects of sucrose concentrations (50-150 g/L), yeast extract concentrations (10-30 g/L) and agitation speeds (75-300 rpm) on the yeast growth and the FOS production by a yeast strain ML1, batch fermentation were carried out in a 3L (EYELA, Japan) fermenter with a working volume of 1.5 L. Samples were withdrawn at time interval and were used for analyses.

Analytical methods

The cell growth was assessed spectrophotometrically by measuring the optical density (OD) of the culture broth at 600 nm. The cell concentration was calculated from the standard curve between the optical density against the cell concentration; Y = 1.689X, where Y and X are OD600 and cell concentration (g/L), respectively. The maximum specific growth rate was obtained by; , where μ is the specific growth rate (h^{-1}) and t is the culture time (h).

Sugar mixtures (sucrose, glucose, fructose, 1-kestose and nystose) were determined and quantified by high pressure liquid chromatography with refractive index as a detector (HPLC-RID). A Rezex (Phenomenex) Oligosaccharide column (7.8 x 300 mm) was maintained at 45°C with Milli Q water as the mobile phase at a flow rate of 0.4 mL/min. The samples were diluted appropriately and filtered through a 0.22 μm filter before injection. The reaction time of each individual FOS was compared with that of each standard.

Results and Discussion

The production of FOS depends on several parameters including the composition of culture medium and operating conditions. In this study, the initial concentrations of sucrose and yeast extract, and the agitation speeds were varied in the range of 50-150 g/L, 10-30 g/L and 75-300 rpm, respectively.

Effect of sucrose concentration

To study the effect of sucrose concentrations on cell concentration, experiments were conducted varying the initial sucrose concentrations from 50 to 150 g/L. The results showed that the maximum specific growth rates obtained from 50, 100 and 150 g/L sucrose were 0.429, 0.443 and 0.27 h^{-1}, respectively (Figure 1). The FOS production was highest at 65 g/L obtained from 150 g/L sucrose. Likely, this indicated that the production of FOS depended on the sucrose concentration. Similarly, the higher FOS produced by *Aureobasidium pullulans* from higher sucrose concentration was reported previously (Dominguez *et al*., 2012). It described an availability of fructosyl acceptors increased at higher sucrose concentration, while decreased the water availability (Vega-Paulino and Zúniga-Hansen, 2012).

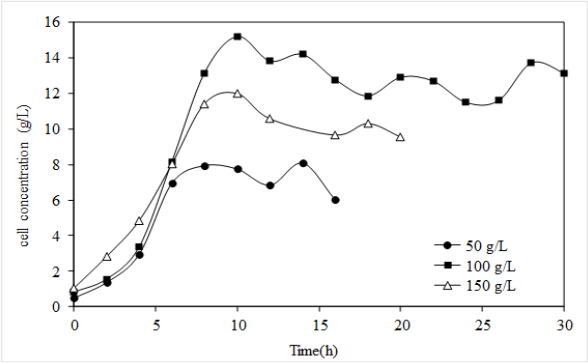

Figure 1. The effect of sucrose concentrations on cell growth

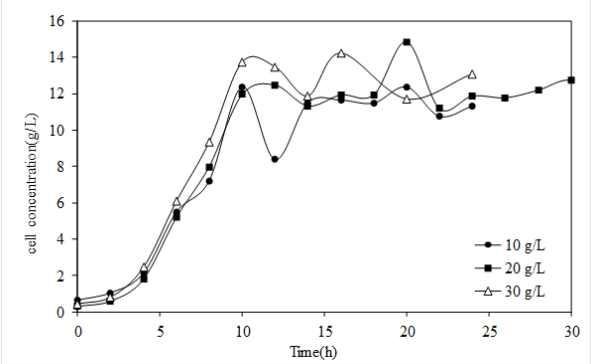

Figure 2. The effect of yeast extract on cell growth

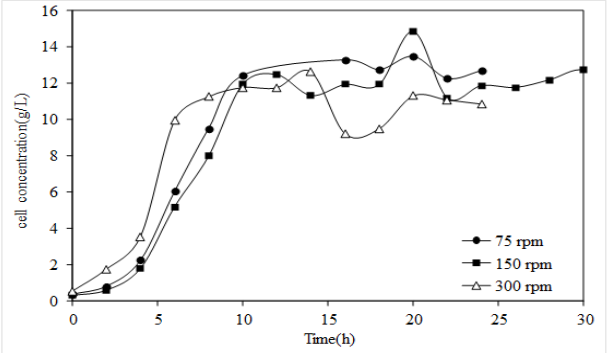

Figure 3. The effect of agitation speeds on cell growth

Effect of yeast extract concentration

The cell growth was also influenced by the nitrogen source. Yeast extract is known as a source of vitamins and growth factors, and provides nitrogenous compounds, which are essential for the growth of diverse microorganisms (Vandáková *et al.*, 2004). Figure 2 presented the effect of yeast extract concentrations on the cell growth, however, no significant difference in cell growth was observed. The maximum FOS production of 65 g/L was attained

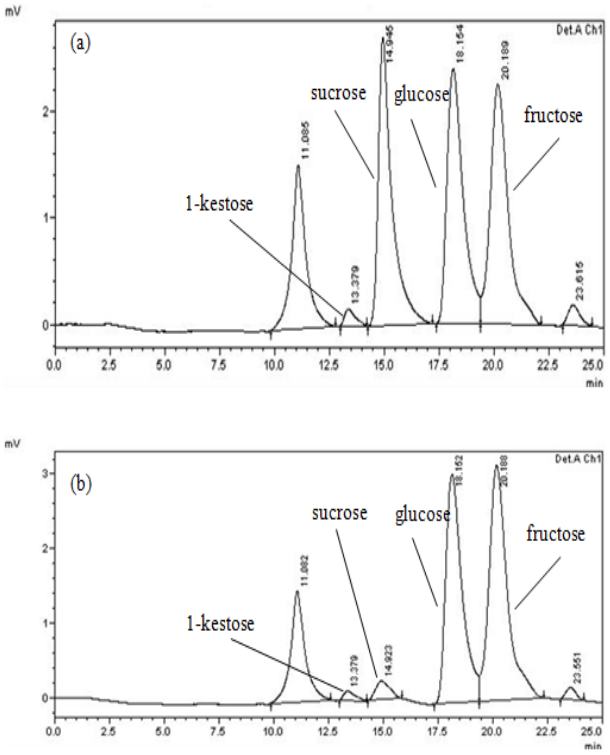

Figure 4. The HPLC chromatogram of the fermentation broths taken at (a) 2 and (b) 4 h of cultivation time

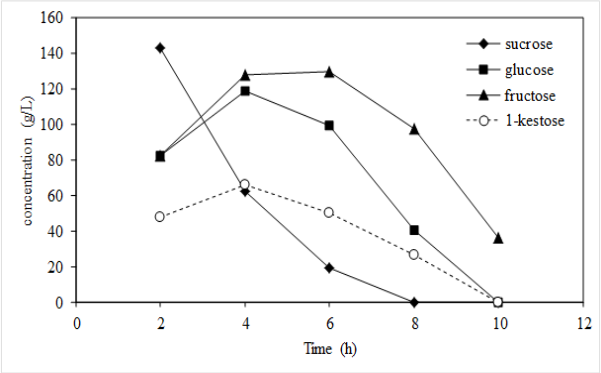

Figure 5. The profiles of sugars and FOS produced by the yeast strain ML1 in a 3L fermenter

at 20 g/L of yeast extract, as previously reported that yeast extract gave positive effect on neo-FOS rather than sucrose (Lim *et al.*, 2005).

Effect of agitation speed

The agitation speed affecting on the cell growth was investigated varying from 75 to 300 rpm. However, these agitation speeds did not affect the cell growth among various sucrose concentrations (Figure 3). The FOS production maximized at 65 g/L was obtained with 150-rpm agitation speed. As compared to the FOS production yield, this was maximized at a 385-rpm agitation speed (Dominguez *et al.*, 2012).

As a result, the FOS production was optimized at 50 g/L sucrose, 20 g/L yeast extract and 150-rpm agitation speed, which the FOS concentration of 65 g/L was obtained from 4 h of culture time. From the HPLC analysis, 1-kestose and sucrose were found separately in the sample taken at this highest production of FOS (Figure 4). The profiles of sugars and FOS (i.e. 1-kestose) were summarized in Figure 5, from the FOS production by the yeast strain ML1 in a 3L fermenter under optimal conditions mentioned above.

Conclusion

The present work showed the yeast strain ML1 was potential for use in the batch production of FOS, which is applicable in industry. An optimization of different cultivation to enhance the FOS production is ongoing investigation.

Acknowledgement

This work was supported by the Department of Biotechnology, Faculty of Engineering and Industrial Technology, Silpakorn University.

References

Crittenden, R.G. and Playne, M.J. 1996. Production, properties and applications of food grade oligosaccharides. Trends in Food Science and Technology 7: 353–361.

Dominguez, A., Nobre, C., Rodrigues, L.R., Peres, A.M., Torres, D., Rocha, I., Lima, N. and Teixeira, J. 2012. New improved method for fructooligosaccharides production by Aureobasidium pullulans. Carbohydrate Polymers 89: 1174– 1179.

Fujita, K., Kuwahara, N., Tanimoto, T., Koizumi, K., Iizuka, M. and Minamiura, N. 1994. Chemical structures of hetero-oligosaccharides produced by *Arthrobacter* sp. K-1 beta-fructofuranosidase. Bioscience Biotechnology and Biochemistry 58: 239–243.

Gibson, G.R. and Roberfroid, M.B. 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. Journal of Nutrition 125: 1401–1412.

Gupta, A.K. and Bhatia, I.S. 1980. Glucofructosan biosynthesis in *Fusarium oxysporum*. Phytochemistry 19: 2557-2563.

Hidaka, H., Hirayama, M. and Sumi, N. 1988. A fructooligosaccharide producing enzyme from *Aspergillus niger* ATCC 20611. Agricultural and Biological Chemistry 52: 1181–1187.

Hirayama, M., Sumi, N. and Hidaka, H. 1989. Purification and properties of a fructooligosaccharide-producing β-fructofuranosidase from *Aspergillus niger* ATCC 20611. Agricultural and Biological Chemistry 53: 667–673.

Lim, J.S., Parl, M.C., Lee, J.H., Park, S., W. and Kim, S.W. 2005. Optimization of culture medium and conditions for Neo-fructooligosaccharides production by Penicillium citrinum. European Food Research and Technology 221: 639-644.

Mussatto, S., Aguilar, C., Rodrigues, L.R. and Teixeira, J.A. 2009a. Fructooligosaccharides and β-fructofuranosidase production by *Aspergillus japonicus* immobilized on lignocellulosic materials. Journal of Molecular Catalysis B: Enzymatic 59: 76–81.

Mussatto, S., Aguilar, C., Rodrigues, L.R. and Teixeira, J.A. 2009b. Colonization of *Aspergillus japonicus* on synthetic materials and application to the production of fructooligosaccharides. Carbohydrate Research 344(6): 795–800.

Rao, V.A. 2001. The prebiotic properties of FOS at low intake levels. Nutrition Research 21: 843–848.

Sangeetha, P.T., Ramesh, M.N. and Prapulla, S.G. 2005a. Recent trends in the microbial production, analysis and application of Fructooligosaccharides. Trends in Food Science and Technology 16: 442–457.

Sangeetha, P.T., Ramesh, M.N. and Prapulla, S.G. 2005b. Fructooligosaccharide production using fructosyl transferase obtained from recycling culture of Aspergillus oryzae CFR 202. Process Biochemistry 40: 1085–1088.

Silva, J.B., Cavalcante Fai, A.E., Santos, R.D., Bassob, L.C. and Pastorea, G.M. 2011. Parameters evaluation of fructooligosaccharides production by sucrose biotransformation using an osmophilic Aureobasidium pullulans strain. Procedia – Food Science 1: 1547-1552.

Spiegel, J.E., Rose, R., Karabell, P., Frankos, V.H. and Schmitt, D.F. 1994. Safety and benefits of fructooligosaccharides as food ingredients. Food Technology 48: 85–89.

Vandáková, M., Platková, Z., Antošová, M., Báleš, V. and Polakovič, M. 2004. Optimization of cultivation conditions for production of fructosyltransferase by Aureobasidium pullulans. Chemical Paper 58(1): 15–22.

Vega-Paulinoa, R.J. and Zúniga-Hansen, M.E. 2012. Potential application of commercial enzyme preparations for industrial production of short-chain fructooligosaccharides. Journal of Molecular Catalysis B: Enzymatic 76: 44–51.

Yun, J.W. 1996. Fructooligosaccharides - occurrence, preparation and application. Enzyme Microbial Technology 19: 107–117.