OPTIMISATION OF FRUCTOOLIGOSACCHARIDES (FOS) PRODUCTION USING BOTH INTRACELLULAR AND EXTRACELLULAR FRUCTOSYLTRANSFERASE (FT-ASE) PRODUCED BY *ASPERGILLUS* sp. ISOLATED FROM AN INDONESIAN SUGAR FACTORY

By

TRIANTARTI, ARIS TOHARISMAN, YAHYA KURNIAWAN, HENDRO SANTOSO and SIMPING YULIATUN

Indonesian Sugar Research Institute
triantarti@gmail.com

KEYWORDS: Fructosyltransferase, Fructooligosaccharide, Extracellular, Intracellular.

Abstract

FRUCTOOLIGOSACCHARIDES (FOS) were produced from sucrose with a number of desirable characteristics such as low calories, non-carcinogenic effect, safe for diabetics, and having bifidus-stimulating functionality. *Aspergillus* sp. isolated from the Wonolongan Sugar Mill area in Indonesia was a potential isolate for FOS production. The aim of our research was to determine the optimum condition for conversion of sucrose to FOS using both intracellular and extracellular FT-ase of *Aspergillus sp.* Wet cells were used as a source of intracellular FT-ase while extracellular FT-ase consisted of crude FT-ase (broth medium) and semi pure FT-ase. Semi pure FT-ase was FT-ase in broth medium after isolation using ethanol 60% (v/v) and diluted back to the initial volume of broth medium using buffer pH 7. The effect of varying pH from 3–7, varying concentration of extracellular FT-ase, varying concentrations of wet cells and varying incubation times were tested in this experiment. All reactions for FOS production were conducted at 50°C containing sucrose 40% (w/v) as a substrate in the enzymatic reaction. Results showed that the optimum pH for FOS production was 7, both for intracellular and extracellular FT-ase. The optimum condition for FOS production using intracellular FT-ase was 2 g wet cells /100 mL substrate followed by 4 h incubation, and FOS yield reached 39.1% of the initial sucrose concentration. The optimum condition for FOS production using crude extracellular FT-ase was 1.5 times dilution using buffer pH 7 followed by 8 h incubation time and FOS yield reached 29.1%. The optimum condition for FOS production using semi-pure extra cellular FT-ase was stock semi-pure FT-ase without any dilution followed by 8 h incubation and FOS yield reached 25.2%. This experiment showed that both intracellular and extracellular FT-ase of *Aspergillus sp.* could be used for FOS production.

Introduction

Fructooligosaccharides (FOS) is a prebiotic which is composed mainly of 1-kestose (GF_2), nystose (GF_3) and fructofuranosyl nystose (GF_4). Industrial scale for FOS production is done mainly using fungal enzymes. Indonesian Sugar Research Institute (ISRI) has isolated *Aspergillus* sp. from a sugar mill area in Indonesia for FOS production namely *Aspergillus sp. FOS-1(ISRI).* Fermentation has been optimised for producing FT-ase in flask fermentation. Fermentation broth as a source of crude FT-ase was used for conversion of sucrose to FOS (Toharisman *et al.*, 2008). The objective of this research project was to determine the optimum conditions for conversion of sucrose to FOS using both intracellular and extracellular FT-ase of *Aspergillus sp. FOS-1(ISRI).*

Materials and methods

Cultivation conditions

Fermentation using *Aspergillus sp. FOS-1(ISRI*) was conducted in a 15 litre fermentor filled with 10 litres of fermentation medium. The composition of the fermentation medium was the same as the previous experiment (Toharisman *et al.,* 2008). Fermentation was conducted at 30°C for 24 hours. pH was controlled at 7.0 with aeration 1.0 vvm and agitation 100 rpm. At the end of fermentation, the broth was separated from the fungal cells by filtration. Wet cells were used as a source of intracellular FT-ase while extracellular FT-ase consisted of crude FT-ase (broth medium) and semi-pure FT-ase. Semi-pure FT-ase was FT-ase in broth medium after isolation using ethanol 60% (v/v) and diluted back to the initial volume of broth medium using buffer pH 7. FOS concentration was determined using HPLC as in the previous experiment (Toharisman *et al.,* 2008)

Effect of pH on the conversion of sucrose to FOS

Effect of pH on the conversion of sucrose to FOS was conducted only for intracellular enzyme, while the optimum condition for conversion using extracelluler FT-ase was determined in the previous experiment (Toharisman *et al.,* 2008). Two grams of wet cells were added to the buffer containing 40% sucrose with pH between 3 and 7. Cells in buffer with various pH were incubated at 50°C for 1 hour.

Conversion of sucrose to FOS using intracellular and extracellular FT-ase

All reactions for FOS production were conducted in buffer pH 7 containing sucrose 40% (w/v) as a substrate for 8 hours at 50°C. Crude enzyme and semi-pure enzyme were diluted with buffer pH 7. Ratios of crude enzyme to buffer pH 7 (v/v) were various at 1:2, 2:1, and one treatment without dilution. Cells of *Aspergillus sp.* were added in buffer pH 7 containing 40% sucrose, at various cell concentrations i.e. 2, 4 and 8% (w/v).

Results and discussion

Table 1 shows the effect of various pHs on conversion of sucrose to FOS using wet cells of *Aspergillus sp. FOS-1(ISRI)* as a source of intracellular enzyme. Optimum pH for conversion was pH 7. There was no FOS synthesised on reaction between pH 3–5; on the other hand, invertase enzyme or hydrolytic enzyme was active in that pH range. Fernandez *et al.* (2004) reported a similar result that the hydrolytic activity of *Aspergillus* sp 27H isolated from soil was highest at low pH (pH 4). The optimum pH of intracellular enzyme was the same as the optimum pH of extracellular enzyme (Toharisman *et al.,* 2008).

Table 1—Effect of various pH on the carbohydrate composition after enzymatic reaction using wet cells of *Aspergillus sp. FOS-1(ISRI)* with sucrose as a substrate.

pH	Carbohydrate composition of product after enzymatic reaction (%)		
	Glucose + fructose	Sucrose	Kestose
3	100	0	0
4	100	0	0
5	92.3	7.7	0
6	49.2	24.4	26.4
7	31	20.4	48.5

Figure 1 shows the effect of various concentrations of wet cells on FOS production after incubation for 8 hours. The results showed that the highest FOS production was 2 g wet cells/100 mL substrate followed by 4 h incubation time, and FOS yield reached 39.1% of the initial sucrose concentration. The higher concentration of wet cells on the conversion reaction resulted in

the faster degradation of FOS during the long incubation time. It indicated that wet cells of *Aspergillus sp. FOS-1(ISRI)* contained not only FT-ase, which produced FOS from sucrose, but also had a hydrolysing enzyme capable of hydrolysing FOS. However, in this experiment when the enzymatic reaction was conducted at optimum condition, high concentration of FOS was still produced. Others have reported that pellets of *Aspergillus oryzae* CFR 202 could be reused for six recycles. *Aspergillus* sp. *FOS-1(ISRI)* was tested only for three recycles with consistent results.

Figure 2 shows the effect of various concentrations of crude enzyme on FOS production. The optimum condition for FOS production using crude extracellular FT-ase was 1.5 times dilution using buffer pH 7 followed by 8 h incubation time, and FOS yield reached 29.1%. The longer incubation time resulted in a higher concentration of FOS produced, except for crude enzyme without any dilution. This profile was different to the conversion using wet cells. It seemed that the activity of the hydrolysing enzyme on the enzymatic reaction using crude enzyme was not as high as its activity on the conversion process using wet cells.

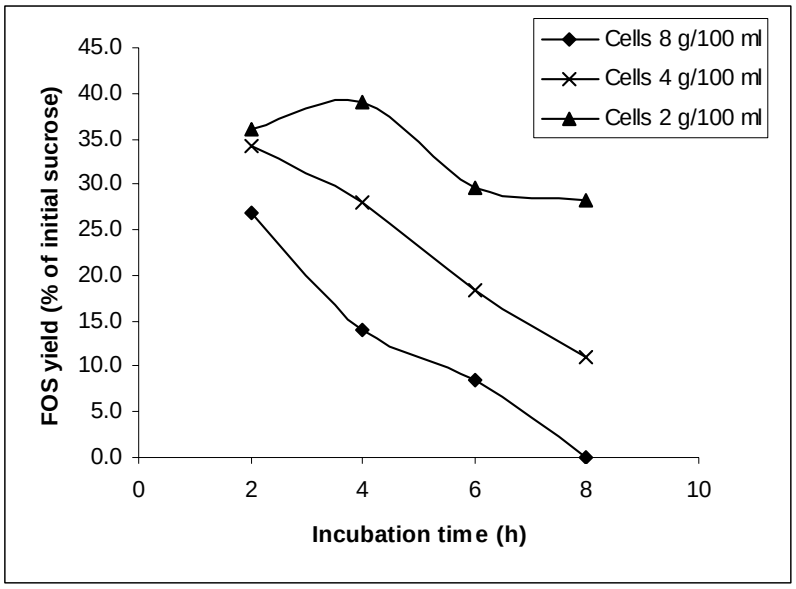

Fig. 1—Effect of various concentrations of wet cells on FOS production.

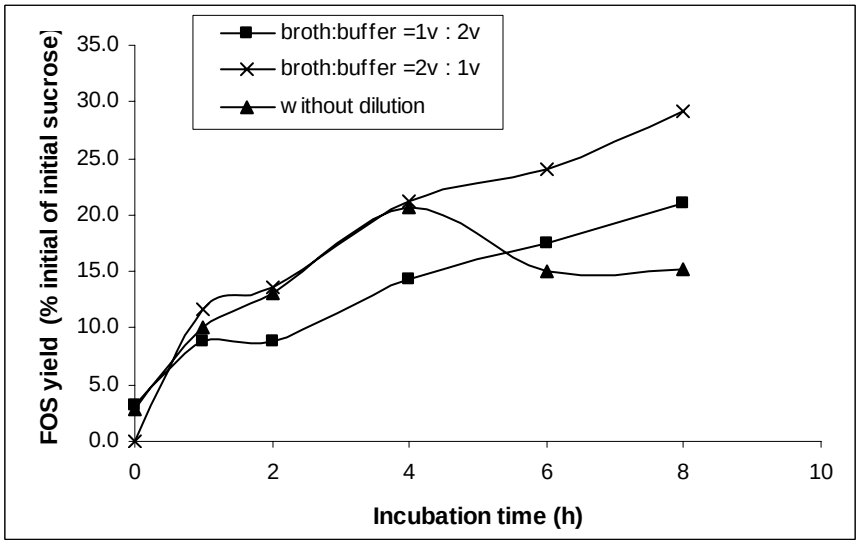

Fig. 2—Effect of various concentrations of crude enzyme on FOS production.

Semi-pure FT-ase was prepared by isolation using ethanol 60% (v/v) and diluted back to the initial volume of broth medium using buffer pH 7. Smith and Luenser (1982) also reported the possibility of isolating FT-ase from black yeast *Aureobasidium pullulans* by water-soluble organic solvent. Figure 3 shows the effect of various concentrations of semi-pure FT-ase on FOS production.

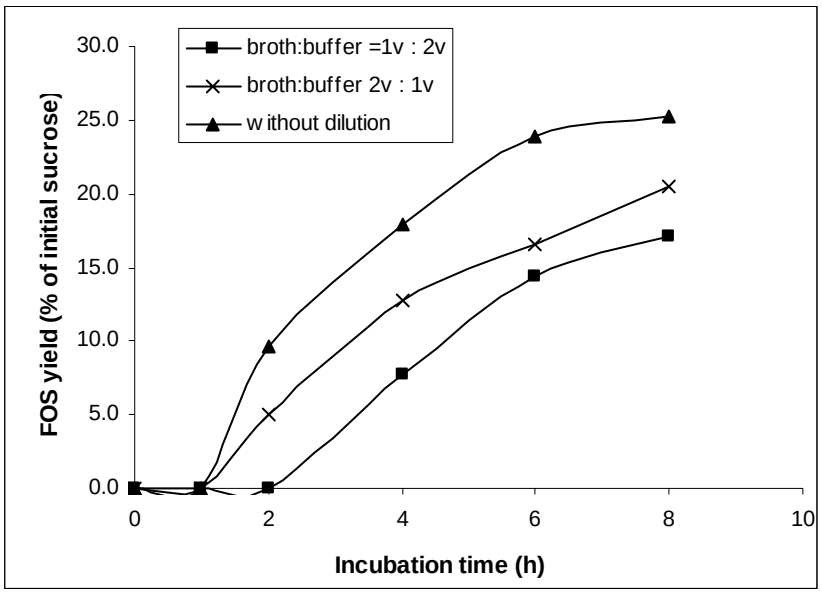

Fig. 3—Effect of various concentrations of semi-pure FT-ase on FOS production.

The optimum condition for FOS production using semi-pure extracellular FT-ase was stock semi-pure FT-ase without any dilution followed by 8 h incubation and FOS yield reached 25.2%. FOS syrup produced using semi-pure FT-ase was free from residual compounds previously present in the fermentation broth medium.

Conclusions

FOS can be produced using both intracellular and extracellular FT-ase enzyme from *Aspergillus sp. FOS-1(ISRI)*. So far the highest yields of FOS resulting from enzymatic reaction using intracellular FT-ase enzyme, crude extracellular FT-ase and semi-pure extracellular FT-ase were 39.1, 29.1 and 25.2% (w/w of initial sucrose concentration) respectively.

REFERENCES

Fernandez, R.C., Marezme, B.B., Juarez, A. and Martinez, J. (2004). Production of fructooligosaccharides by fructofuranosidase from *Aspergillus sp* 27H. Journal of Chemical Technology and Biotechnology, 79: 268–272.

Sangeeta, P.T., Ramesh, H.N. and Prapulla, S.G. (2005). Fructooligosaccharide production using fructosyl transferase obtained from recycling culture of Aspergillus oryzae CFR 202. Process Biochemistry 40(3-4): 1085–1088.

Smith, J.A. and Luenser, S.J. (1982). Process for the production of fructose transferase enzyme. US Patent 4309505.

Toharisman, A., Triantarti and Santoso, H. (2008). Optimisation of fructooligosaccharides production from plantation white sugar. Proc. of the Int. Conf. IS-2008. Meeting the Challenges of Sugar Crops and Integrated Industries in Developing Countries. IAPSIT 11 – 14 September 2008, Al-Arish, Egypt.

OPTIMISATION DE LA PRODUCTION DE FRUCTOOLIGOSACCHARIDES (FOS) A L'AIDE DE FRUCTOSYLTRANSFERASE (FT-ASE), INTRA ET EXTRA CELLULAIRE PRODUITE PAR *Aspergillus* spp. ISOLEE DANS UNE SUCRERIE EN INDONESIE

Par

TRIANTARTI, ARIS TOHARISMAN, YAHYA KURNIAWAN,
HENDRO SANTOSO et SIMPING YULIATUN

Indonesian Sugar Research Institute
triantarti@gmail.com

MOTS-CLÉS: Fructosyltransferase, Fructooligosaccharide, Extracellulaire, Intracellulaire.

DES FRUCTOOLIGOSACCHARIDES (FOS) ont été produites à partir de saccharose avec un certain nombre de caractéristiques intéressantes telles que peu de calories, effet non cancérogène, sans danger pour diabétiques et ayant une fonctionnalités bifidus stimulante. Un isolat d'*Aspergillus* trouvé dans l'usine sucrière de Wonolongan en Indonésie s'est avéré avoir un potentiel pour la production de FOS. Le but de notre recherche était de déterminer la condition optimale pour la conversion de saccharose en FOS à l'aide de fructosyltransferase intracellulaire et extracellulaire provenant de cet isolat d'*Aspergillus*. Des cellules en milieu liquide ont été utilisées comme source de FT-ase intracellulaire tandis la FT-ase extracellulaire provenait d'un milieu de culture brut et aussi de la FT-ase semi purifiée. La FT-ase semi purifiée provenait d'un bouillon de culture après précipitation à l'aide d'éthanol à 60% (v/v) et dilués au volume initial du bouillon de support à l'aide de solution tampon de pH 7. Les effets de varier le pH de 3 à 7, des concentrations variées de FT-ase extracellulaire de différents niveaux provenant de différentes concentrations des cellules en milieu liquide et une gamme de periode d'incubation ont été évalués dans cette étude. Toutes les réactions pour la production de FOS ont été menées à 50°C dans un substrat de saccharose à 40% (p/v) pour la réaction enzymatique. Les résultats ont démontré que le pH optimal pour la production de FOS était de 7 pour la FT-ase intracellulaire comme extracellulaire. La condition optimale pour la production de FOS à l'aide de FT-ase intracellulaire était de 2 g de cellules pour 100 ml de substrat en milieu liquide pour une perdiode d'incubation de 4 h ce qui résultat en un rendement de FOS à 39.1% de la concentration initiale en saccharose. La condition optimale pour la production de FOS à l'aide de la FT-ase extracellulaire à l'état brut, était une dilution de 1.5 à l'aide du tampon de pH 7 suivi par une periode d'incubation de 8 h ce qui mena à un rendement de FOS atteignant 29.1%. La condition optimale pour la production de FOS à l'aide de la FT-ase extra cellulaire semi-purifiée était l'utilisation de la solution stock sans dilution pour une periode d'incubation de 8 h ce qui donna un rendement de FOS de l'ordre de 25.2 %. Cette expérimentation a démontré que la FT-ase intracellulaire et extracellulaire provenant d'une espèce *Aspergillus* pouvait être utilisée pour la production de FOS.

OPTIMIZACIÓN DE LA PRODUCCIÓN DE FRUCTOOLIGOSACÁRIDOS (FOS), USANDO TANTO FRUCTOSILTRANFERASA (FT-ASA) INTRA Y EXTRA CELULAR, PRODUCIDA POR *ASPERGILLUS sp.* AISLADOS EN UNA FÁBRICA DE AZÚCAR INDONESIA

Por

TRIANTARTI, ARIS TOHARISMAN, YAHYA KURNIAWAN,
HENDRO SANTOSO y SIMPING YULIANTUN.
Indonesian Sugar Research Institute
triantarti@gmail.com

PALABRAS CLAVE: Fructosiltranferasa, Fructooligosacárido, Extracelular, Intracellular.

Resumen

LOS FRUCTOOLIGOSACÁRIDOS (FOS) fueron producidos a partir de sacarosa con un conjunto mde características deseables tales como, bajas calorías, sin efectos cancerígenos, seguros para diabéticos y con funcionalidad bifido estimulante. El *Aspergillus sp* para la producción de FOS fue aislado en áreas del Central Wonolongan en Indonesia. El propósito de nuestra investigación era determinar las condiciones óptimas de conversión de la sacarosa en FOS, usando tanto la FT-asa intra y extracelular del *Aspergillus sp.* Se utilizó pasta de levadura como fuente de FT-asa intracelular, mientras la FT-asa extracelular consistió en FT-asa cruda (medio de cultivo) y FT-asa semipura. La Ft-asa semipura fue la FT-asa del medio de cultivo después de ser aislada usando etanol al 60% w/v y diluida de nuevo al volumen del medio de cultivo con solución buffer pH 7. En este experimento se probó el efecto de variar el pH de 3–7, variando la concentración de FT-asa extracelular, variado la concentración de la pasta de levadura y el tiempo de incubación. Todas las reacciones para la producción de FOS se realizaron a 50°C, conteniendo sacarosa al 40% (w/v) como sustrato en la reacción enzimática. Los resultados mostraron que el pH óptimo para la producción de FOS era 7, tanto para ft-asa intra como extra celular. La condición óptima para la producción de FOS empleando FT-asa intracelular fue de 2 g de pasta de levadura/ ml de sustrato, con 4 horas de incubación y el rendimiento de FOS alcanzó 1% de la concentración inicial de sacarosa. La condición óptima para la producción de FOS empleando FT-asa cruda extracelular fue con dilución 1.5 veces empleando buffer a pH 7, seguida de 8 horas de incubación y el rendimiento de FOS alcanzó 29.1%. La condición óptima para la producción de FOS usando FT-asa extracelular semipura fue conservar la Ft-asa semipura sin dilución, seguida de 8 horas de incubación y el rendimiento de FOS alcanzó 25.25. Este experimento mostró que tanto la FT-asa intra como extracelular del *Aspergillus sp* .podían ser empleadas en al producción de FOS.