Journal of Microbiology and Biotechnology
Vol. 7. No. 3, 204-208, 1997

NOTE

Simultaneous Formation of Fructosyltransferase and Glucosyltransferase in *Aureobasidium pullulans*

YUN, JONG-WON*, DONG-HYUN KIM, HYE-YEON MOON, CHII-HYUN SONG, AND SEUNG-KOO SONG[1]

Department of Biotechnology, Taegu University, Kyungbuk 712-714
[1]Department of Chemical Engineering, Pusan National University, Pusan 609-735, Korea

Aureobasidium pullulans possesses the capacity for simultaneous formation of fructosyltransferase and glucosyltransferase in various sugar media including sucrose, maltose, glucose and fructose. Among them, sucrose (300 g/l) was the most suitable carbon source for fructosyltransferase production, while fructose (100 g/l) gave the maximal production of glucosyltransferase. There existed a critical concentration for the optimal formation of enzymes in sucrose, glucose and fructose media. By contrast, no effect of maltose concentrations up to 300 g/l was observed. The specific activity of the glucosyltransferase on maltose medium was highest during the early period of fermentation, after which a sharp decrease occurred, whereas fructosyltransferase activity on sucrose medium maintained a nearly constant rate for a given culture period. Concomitant production of fructosyltransferase and glucosyltransferase was investigated with different combinations of lower concentrations of sucrose and maltose. Maltose supplementation in sucrose media and sucrose addition to maltose media enhanced the activity ratios of fructosyltransferase to glucosyltransferase as compared to that of non-supplemented media. Several polymers and surfactants were added in an attempt to enhance enzyme production, and supplementation of polyoxyethylene-sorbitan monolaurate (Tween 20) promoted fructosyltransferase production by 20%.

In recent years, a great number of new sweeteners such as fructooligosaccharides (FOS), isomaltooligosaccharides (IMO) and galatooligosaccharides have been commercially produced because they have many health-promoting properties such as being low calorie, non-cariogenicity, the prevention of dental caries, and act as a growth promotor for bifidobacteria in the human gut (7, 12, 16). Amongst them, fructooligosaccharides which have 1-3 fructosyl units bound to the β, 2-1 position of sucrose and isomaltooligosaccharides, in which one or more α-glucosidic linkages, with or without α-1,4-glucosidic linkages, have attracted special attention because of two important factors (5, 8, 10, 17, 18). Firstly, production processes are not complicated and costs are relatively low; secondly, their taste is similar to conventional sweeteners such as table sugar, high fructose corn syrup and starch-derived sugars.

FOS are being commercially produced using fructosyltransferases (FTase, EC 2.4.1.9) derived from numerous microorganisms such as *Aspergillus*, *Aureobasidium*, *Fusarium* species, etc. (6, 9, 19, 20, 23). On the other hand, IMO has been produced from starch by the cooperative enzymatic actions of several starch-debranching enzymes (11, 15). More recently, the authors and some other researchers have made an effort towards the direct production of IMO from maltose by using a single enzyme system of glucosyltransferase rather than a complex enzyme system (1, 2, 21, 22).

Aureobasidium pullulans used in this work is known as one of the major producers of FTase which is involved in FOS production. The authors previously reported that this strain can also produce intracellular glycosyltransferase (GTase, EC 2.4.1.24) which is responsible for forming IMO from maltose with high activity (21, 22).

In the present study, to facilitate a more precise and detailed study, simultaneous formation of FTase and GTase in *A. pullulans* was studied by changing carbon sources and by the addition of several polymers and surfactants into the basal fermentation medium.

Chemicals

The sucrose and maltose used throughout the experiments were food-grade commercial products. All other chemicals were of analytical grade.

*Corresponding author
Phone: 82-53-850-6556. Fax: 82-53-850-6509.
E-mail: jwyun@biho.taegu.ac.kr.
Key words: *Aureobasidium pullulans*, fructosyltransferase, glucosyltransferase, oligosaccharides

Enzyme Production

FTase was produced from an aerobic flask culture of *A. pullulans* KFCC (Korean Foundation of Culture Collection) 10524 under the conditions described below. The medium contained per liter of demineralized water: yeast extract 10 g, K_2HPO_4 5 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, $NaNO_3$ 10 g with defined amounts of sucrose; and pH was adjusted to 6.0 before sterilization. Unless otherwise specified, 200 g/l sucrose and 100 g/l maltose were employed for the production of FTase and GTase. The sugars were autoclaved separately and then added aseptically to the ingredients to give the final concentration required. Fermentations were performed at 30°C at an agitation speed of 200 rpm. After centrifugation at 8000 g for 30 min, the resulting supernatant was analyzed to measure the extracellular activity of FTase while the compacted cells were redissolved in deionized water and then used to determine the intracellular activity of FTase as previously mentioned. For all cases of GTase production, no extracellular GTase was excreted. Thus, only the intracellular activity of GTase was evaluated after preparation of the cell suspension.

Enzyme Assay

The activities of FTase and GTase were determined by measuring the amount of glucose released under the following conditions respectively: reaction mixture consisted of 7.5 ml of 700 g/l sucrose (FTase) or maltose (GTase), 2.3 ml of 0.1 M sodium citrate buffer (pH 5.5) and 0.2 ml of enzyme solution; pH, 5.5; temperature, 55°C; reaction time, 1 h. Glucose was assayed by the glucose-oxidase/peroxidase method using a Sigma Diagnostics Kit (No. 510, Sigma Diagnostics, MO, U.S.A.). For both FTase and GTase, one enzyme unit was defined as the amount of enzyme activity required to produce one μmol of glucose per minute under the conditions described above.

Analytical Methods

Residual sugars were directly analyzed by HPLC (Shimadzu Co., Kyoto, Japan) using an Aminex HPX-42C column (0.78×30 cm, Bio-rad, U.S.A.) and a refractive index detector (Shimadzu Co., Kyoto, Japan). The column temperature was maintained at 85°C. Water was used as a mobile phase at 0.6 ml/min.

Effect of Carbon Sources on FTase Production

To investigate the best carbon source and its concentration for FTase production, several sugars, namely sucrose, maltose, glucose and fructose were examined. As shown in Fig. 1, the specific enzyme yields increased with respect to the initial concentrations up to 300 g/l, thereafter the FTase yields sharply decreased. Thus it is clear that sucrose is the best carbon source and its optimum concentration is 300 g/l based on the specific enzyme production rate. Where glucose and fructose were used as a carbon source, specific enzyme production re-

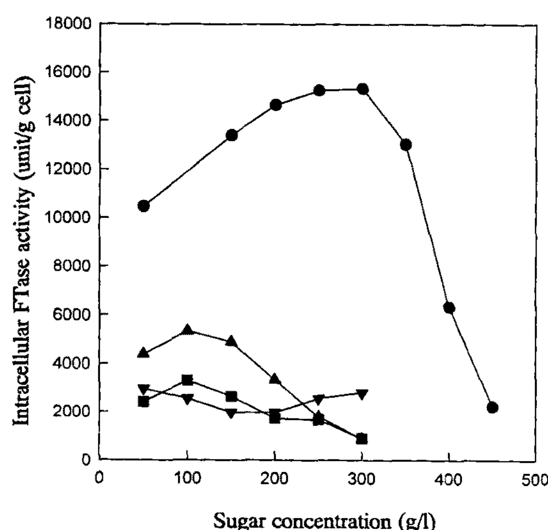

Fig. 1. Intracellular FTase activities with different sugar concentrations: sucrose (●), maltose (▼), glucose (■), fructose (▲).
Enzyme activity was measured after cultivation of 100 h under basal medium supplemented with various sucrose concentrations.

mained at a very low level as compared to that of sucrose, and were also significantly depressed at high concentrations. By contrast, maltose concentrations within the range of 50~300 g/l did not affect enzyme production. Chen *et al.* (3) reported contradictory results, that specific FTase production increased consecutively up to 500 g/l. This difference might be resulted from a dissimilar degree of inhibition of cell growth at high concentrations of sucrose media.

Production of FTase with Sucrose

The growth pattern of *A. pullulans* and FTase production were observed for 120 h in basal medium with 200 g/l sucrose as a sole carbon source. Cell growth and enzyme production reached maxima at around 8 g/l and 96 units/ml, respectively after 108 h of cultivation and then leveled off (Fig. 2). However, specific FTase production (unit/g biomass) were nearly constant towards the end of fermentation. Chen *et al.* (3) reported different results in that specific β-fructofuranosidase production by *Aspergillus japonicus* in sucrose media decreased with time at low sucrose concentrations (50~100 g/l) but increased at elevated higher sucrose concentrations (150~200 g/l).

Effect of Carbon Sources on GTase Production

The most striking result from the substrate screening for GTase production was that higher specific enzyme yield was observed on media containing fructose rather than maltose within the ranges from 50 to 150 g/l, but thereafter enzyme production was markedly reduced in the fructose media. However, maltose supported superior

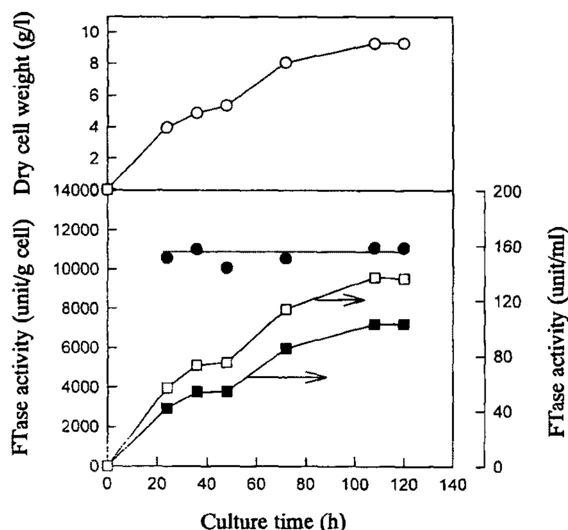

Fig. 2. Typical growth curve and FTase activity on 200 g/l sucrose medium: specific FTase activity (●), intracellular FTase activity (■), extracelluar FTase activity (□).
Dry cell weight was determined by heating the compacted cells at 120°C for 2 h.

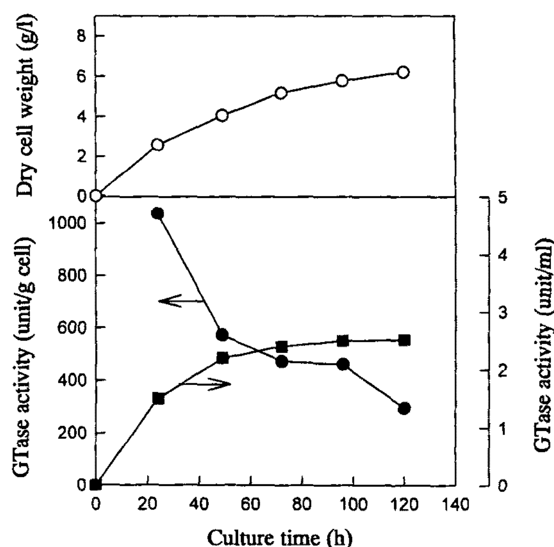

Fig. 4. Typical growth curve and GTase activity on 100 g/l maltose medium: specific GTase activity (●), intracellular GTase activity (■).

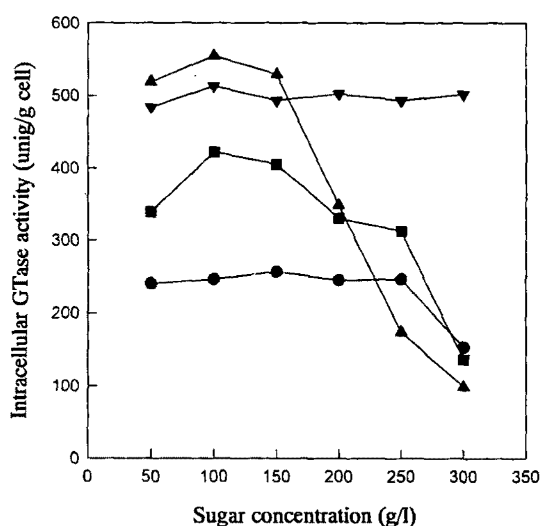

Fig. 3. Intracelluar GTase activities with different sugar concentrations: sucrose (●), maltose (▼), glucose (■), fructose (▲).
Enzyme activity was measured after cultivation of 100 h under basal medium supplemented with various maltose concentrations.

GTase yields at even higher concentrations, up to 300 g/l. Consequently, it appears that maltose is the best inducer for GTase production in *A. pullulans* and its initial concentration is not an important factor for consideration.

Production of GTase with Maltose

The time course of GTase production with an initial maltose concentration of 100 g/l is shown in Fig. 4. Cell growth and enzyme production reach maxima at around 6.2 g/l and 3.7 units/ml, respectively after 96 h cultivation and then leveled off. Enzyme production based on the fermentation broth increased with time while specific enzyme production was decreased during the entire fermentation period. Hayashi *et al.* (5) also reported that maltose was the best carbon source for intracellular GTase production from *Aureobasidium* sp. ATCC 20524 and its optimal concentration was 20 g/l, which is considerably lower level than that indicated by our results. It is known that the fermentation kinetics of GTase production vary with strains. Chen *et al.* (2) reported that *Aspergillus niger* CCRC 31494 produced extracellular GTase following a mixed growth-associated manner. On the other hand, Hayashi *et al.* (5) reported that production of intracellular GTase in *Aureobasidium* sp. ATCC 20524 behaved in a growth-associated manner. From the results shown in Fig. 4, it seems that the kinetics of GTase production in *A. pullulans* are also of the growth-associated type. Unlike FTase production, significant loss of specific GTase activities was observed as the culture proceeded. It appears that this phenomenon is closely related with enzyme instability at the lower pH values (4~4.5) found during fermentation. This fact contrasts well with the case of FTase which is quite stable in an acidic culture condition.

Simultaneous Formation of FTase and GTase

Considering the aforementioned results, it is likely that high concentrations of sucrose and maltose were not appropriate for the simultaneous formation of FTase and GTase. Therefore, simultaneous formation of these two

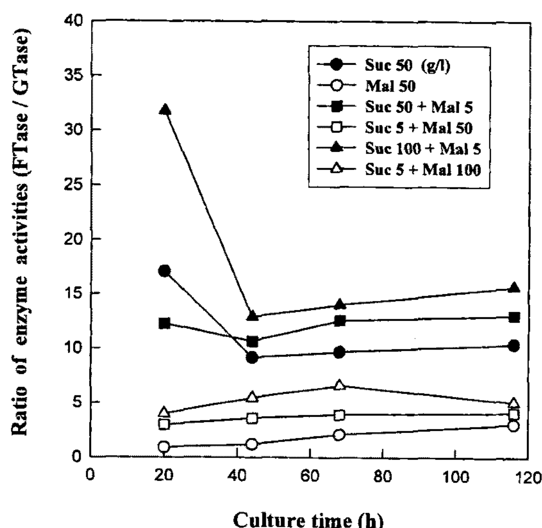

Fig. 5. Ratio of FTase to GTase activity with the combination of low concentration of sucrose and maltose.

enzymes was carried out with different combinations of lower concentrations of sucrose and maltose in the ranges 50~100 g/l. As shown in Fig. 5, in all the media combinations tested, the enzyme yields were increased in proportion to sugar concentrations. Furthermore, focused on 50 g/l media, both maltose supplementation in sucrose media and sucrose addition in maltose media enhanced the activity ratios of FTase to GTase compared with those of either only maltose or only sucrose media. It is interesting to note that the activity ratio in the 50 g/l sucrose media alone is much higher than the media containing 5 g/l maltose within 40 h, but the ratios were reversed as culture proceeds. Moreover, the activity ratios were maintained nearly constant except for the early fermentation period. This result supported the hypothesis that both FTase and GTase are growth-associated types, and that maltose and sucrose have a stimulatory effect on FTase and GTase production, respectively.

Effect of Surfactants and Polymers on Simultaneous Formation of Enzymes

The effect of surfactants and polymeric additives on enzyme production have been investigated by several researchers (13, 14). It has been reported that the enhancement effect of these additives resulted from efficient spore dispersion, rheological properties of the medium, availability of nutrients and oxygen, and the physiological functions of the cells (4). To investigate this effect, several well-known additives were supplemented into the basal medium to a final concentration 1 g/l, and the results were analyzed by measuring total enzyme activity rather than specific activity (because it was difficult to quantitify the biomass accurately due to severe morphological changes after additive supplementation). As illustrated in Table 2, most of the polymers tested did not stimulate enzyme production of either FTase or GTase beyond basal levels. However, Tween 20 (polyoxyethylenesorbitan monolaurate, Sigma Chemical, St. Louis, MO, U.S.A.) promoted total FTase production by 20% by comparison with the fermentation broth. This enhancement of enzyme production could be explained by morphological changes (from compact to loose pellet form) and reduced residual substrates after additive supplementation (data not shown). However, Tween 20 did not affect GTase production, which is probably due to the different enzyme location within the cells or the different physiological function of the cell for enzyme production.

Table 1. Simultaneous formation of intracellular FTase and GTase with various carbon sources in *A. pullulans*.

Sugars (100 g/l)	Dry cell weight[a] (g/l)	Specific enzyme production (unit/g cell)	
		FTase	GTase
Glucose	6.6	1309.4	437.7
Fructose	5.1	1603.9	515.2
Maltose	6.6	1103.6	471.4
Sucrose	8.1	8599.7	363.6

[a]Dry cell weight was measured after 2-h drying at 120°C.

Table 2. Effect of surfactants and polymers on simultaneous production of FTase and GTase in *A. pullulans*.

Additives	Enzyme production (unit/ml broth)[a]	
	FTase	GTase
Polymers[b]		
CMC	135.3 (81.3)	3.45 (89.8)
PEG 3350[c]	140.3 (84.6)	3.40 (88.5)
PEG 8000[c]	158.8 (95.7)	3.80 (99.0)
PVA	138.2 (83.3)	3.63 (94.5)
Surfactants[d]		
Triton X-100	136.8 (82.5)	3.46 (90.1)
Tween 20	199.4 (120.2)	3.84 (100)
Tween 80	175.9 (106.0)	3.59 (93.4)
Polypep	189.9 (106.0)	3.82 (99.5)
Control	165.9 (100)	3.84 (100)

[a]Numbers in the parentheses mean relative activities to that of control. [b]The polymer concentration added into standard medium was 1 g/l. CMC, carboxymethylcellulose; PEG, polyethyleneglycol; PVA, polyvinylalcohol. [c]Numbers mean molecular weight of each product. [d]The surfactant concentration added into basal medium was 1 g/l. Tween 20, polyoxyrthylenesorbitan monolaurate; Polypep, a proprietary protein digest from Sigma product.

Acknowledgement

This research was supported by the Taegu University research grants, 1997.

REFERENCES

1. Chen, W. C. 1995. Production of β-fructofuranosidase by *Aspergillus japonicus* in batch and fed-batch cultures. *Biotechnol. Lett.* **17**: 1291-1294.
2. Chen, W. C., S. L. Lee, and P. Y. Tung. 1996. Effect of initial maltose concentration, agitation and aeration rates on the production of glucosyltransferase by *Aspergillus niger*. *Biotechnol. Lett.* **18**: 489-494.
3. Chen, W. C. 1996. β-Fructofuranosidase production by *Aspergillus japonicus* in shaking batch cultures as affected by initial sucrose concentration. *Biotechnol. Lett.* **18**: 69-72.
4. Chen, W. C. 1996. Production of β-fructofuranosidase by *Aspergillus japonicus*. *Enzyme Microb. Technol.* **18**: 153-160.
5. Hayashi, S., M. Nonokuchi, K. Imada, and H. Ueno. 1990. Production of fructosyl-transferring enzyme by *Aureobasidium* sp. ATCC 20524. *J. Ind. Microbiol.* **5**: 395-400.
6. Hayashi, S., K. Ito, M. Nonoguchi, Y. Takasaki, and K. Imada. 1991. Immobilization of a fructosyl-transferring enzyme from *Aureobasidium pullulans* sp. on *Shirasu* porous glass. *J. Ferment. Bioeng.* **72**: 68-70.
7. Hidaka, H., T. Eida, T. Takizawa, T. Tokunaga, and Y. Tashiro. 1986. Effect of fructooligosaccharides on intestinal flora and human health. *Bifidobacteria Microflora* **5**: 37-50.
8. Hidaka, H., T. Eida, and Y. Saitoh. 1987. Industrial production of fructooligosaccharides and its application for human and animals. *Nippon Nogeikagaku Kaishi* (in Japanese). **61**: 915-923.
9. Jung, K. H., J. Y. Lim, S. J. Yoo, J. H. Lee, and M. Y. Yoo. 1987. Production of fructosyltransferase from *Aureobasidium pullulans*. *Biotechnol. Lett.* **9**: 703-708.
10. Kuriki, T., M. Tsuda, and T. Imanaka. 1992. Highly branched oligosaccharides production by the transglucosylation reaction of neopullulanase. *J. Ferment. Bioeng.* **73**: 198-202.
11. Kuruki, T., M. Yanase, H. Takata, Y. Takesada, T. Imanaka, and S. Okada. 1993. A new way of producing isomalto-oligosaccharide syrup by using the transglycosylation reaction of neopullulanase. *Appl. Environ. Microbiol.* **59**: 953-959.
12. Kohmoto, T., F. Fukui, H. Takaku, and T. Mitsuoka. 1991. Does-response test of isomaltooligosaccharides for increasing fecal *Bifidobacteria*. *Agri. Biol. Chem.* **55**: 2157-2159.
13. Metz, B. and N. W. F. Kossen. 1977. Biotechnology review: The growth of the molds in the form of pellets. *Biotechnol. Bioeng.* **19**: 781-199.
14. Sharma, A. and S. R. Padwai-Desai. 1985. On the relationship between pellet size and aflatoxin yield in *Aspergillus parasitious*. *Biotechnol. Bioeng.* **27**: 1577-1580.
15. Takaku, H. 1988. *Handbook of amylase and related enzymes*, p. 215-217, The Amylase Research Society of Japan ed. Pergamon Press, Oxford.
16. Wada, K., J. Watanabe, J. Mizutani, M. Tomoda, H. Suzuki, and Y. Saitoh. 1992. Effect of soybean oligosaccharides in a beverage on human fecal flora and metabolites. *Nippon Nogeikagaku Kaishi* **66**: 127-135.
17. Yun, J. W., K. H. Jung, J. W. Oh, and J. H. Lee. 1990. Semibatch production of fructo-oligosaccharides from sucrose by immobilized cells of *Aureobasidium pullulans*. *Appl. Biochem. Biotechnol.* **24/25**: 299-308.
18. Yun, J. W., K. H. Jung, Y. J. Jeon, and J. H. Lee. 1992. Continuous production of fructo-oligosaccharides from sucrose by immobilized cells of *Aureobasidium pullulans*. *J. Microbiol. Biotechnol.* **2**: 98-101.
19. Yun, J. W. and S. K. Song. 1993. Production of high-content fructo-oligosaccharides by the mixed-enzyme system of fructosyltransferase and glucose oxidase. *Biotechnol. Lett.* **15**: 573-576.
20. Yun, J. W., M. G. Lee, and S. K. Song. 1994. Batch production of high-content fructo-oligosaccharides by the mixed-enzyme system of β-fructofuranosidase and glucose oxidase. *J. Ferment. Bioeng.* **77**: 159-163.
21. Yun, J. W., M. G. Lee, and S. K. Song. 1994. Production of panose from maltose by intact cells of *Aureobasidium pullulans*. *Biotechnol. Lett.* **16**: 359-362.
22. Yun, J. W., M. G. Lee, and S. K. Song. 1994. Continuous production of isomalto-oligosaccharides from maltose syrup by immobilized cells of permeabilized *Aureobasidium pullulans*. *Biotechnol. Lett.* **16**: 1145-1150.
23. Yun, J. W. 1996. Fructooligosaccharides - occurrence, preparation and application. *Enzyme Microb. Technol.* **19**: 107-117.

(Received January 31, 1997)