Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 23, No. (1) : 2021 : 84-88

ISSN-0972-3005

MICROBIAL ENZYMATIC PRODUCTION OF FRUCTOOLIGOSACCHARIDES FROM SUCROSE IN AGRICULTURAL HARVEST

JAYALAKSHMI J., A. MOHAMED SADIQ[A] AND V. SIVAKUMAR[B]

[a]Adhiparasaksthi College of Arts and Science, Kalavai 632 506, India
[b]Department of Biochemistry, Adhiparasaksthi College of Arts and Science, Kalavai 632 506, India

(Received 2 December, 2020; Accepted 30 December, 2020)

Key words : *Fructooligosaccharides, Banana peel, Fructosyltransferase,* Penicillium citrinum.

Abstract – Fructooligosaccharides (FOS) derived from sucrose by microbial enzyme Fructosyltransferase (FTase) especially from fungal sources has received particular attention due to its beneficial effects such as prebiotics, low calorific, non-cariogenic, bifidogenic and its mass production. This work is aimed to maximize the fructooligosaccharide production from cheap agricultural harvests instead of using expensive commercial sucrose. Cheaper alternatives carbon (sucrose) sources selected for this study were Banana, Banana peel, Papaya and Cane molasses. Fungal strain *Penicillium citrinum* is having higher Fructosyltransferase activity was collected from sugarcane mill. This fungus was fermented in four fermentation medium containing four selected carbon sources. The activity of FTase was estimated by DNS method at regular intervals. It was found that Banana peel and Cane molasess shows significantly ($p<0.002$) higher activity when compared to control. For the industrial production, Banana peel and cane molasses would be a promising cheaper alternative carbon sources for FOS production.

INTRODUCTION

Fructooligosaccharides (FOS) have received particular interest because of their excellent biological and functional properties for use as prebiotic compounds. They are used as components of functional foods and have received GRAS (Generally recognized as safe) status from the FDA (Food and Drug administrations-US) (Patil *et al.*, 2011).

In response to an increasing demand from the consumer for healthier and calorie-controlled foods, a number of so-called alternative sweeteners such as palatinose and various oligosaccharides including Isomaltooligosaccharides, soybean oligosaccharides, and fructooligosaccharides have emerged since the 1980's. They are important primarily because of their functional properties rather than sweetness. Of all the new products introduced so far, microbial fructooligosaccharides (FOSs) from sucrose have attracted special attention and are attributed to the expansion of the sugar market by several factors. First, mass production is not complicated. Second, the sweet taste is very similar to that of sucrose, a traditional sweetener (Yun, 1996).

Fructooligosaccharides (FOS) are naturally occurring mixture of non-digestible sugars that constitute one of the most established groups of prebiotic oligosaccharides in the world (Ahmad, 2010). Along with prebiotic property it also used as an alternative sugar substitute in food industry.

FOSs is oligosaccharides of fructose containing a single glucose moiety and has a simple molecule structure rather than the complex molecule from its original sucrose. FOS is composed by 1-Kestose (GF2), nystose (GF3) and 1-β-D-fructofuranosylnystose (GF4) in which fructosyl units (F) are bound at the β (2-1) position of sucrose molecule (GF) (Hidaka H *et al.*, 1986).

FOSs has a number of interesting properties. First, FOSs has low sweetness intensity since they are only about 1/3rd as sweet as sucrose. This property is quite useful in the various kinds of foods where the use of sucrose is restricted by its high sweetness. Second, FOSs are calorie free that is they are scarcely hydrolysed by the digestive enzyme

and not utilised as an energy source in the body, thus they are safe for diabetics. Thirdly, they are non-cariogenic that is why they are not used by streptococcus mutans to form acids and insoluble β-glucan that are the main culprits in dental caries. Finally FOSs encourages the growth of bifidobacteria and discourages the growth of potentially putrefactive microorganism that has a tendency to cause diarrhoea. FOSs has an important physiological property (i.e.) they decrease the level of Serum Cholesterol, Phospholipids and Triglycerides.

FOS can be found in plants and vegetables including Onion, Garlic, Asparagus, Jerusalem artichoke, Sugar beet etc. but generally in low concentration. The industrial scale recovery from these plants is not economical since their low concentration, for this reason, FOS are produced commercially via biosynthetic as well as hydrolytic methods using microbial enzymes. The raw material of this reaction is sucrose and the product mixture contains unconverted sucrose besides GF2,GF3 and GF4 and glucose as a by-product. The latter component is a strong competitive inhibitor of the synthesis. Elimination of the formed by-product component can result an increase in the product yield.

The partial hydrolysis of inulin is also used practically for FOS production. Inulin recovered from Jerusalem artichoke (*Helianthus tuberosus*) and chicory (*Cichoriumintybus*) species is used commonly as substrate of endoinulinases(E.C 3.2.1.7) by the industry to produce GF2-GF4 FOS (Csanadi and Sisak, 2008).

Two classes of enzymes are particularly useful for FOSs production at industrial scale: fructosyltransferases (EC 2.4.1.9) and β-fructofuranosidases, also called invertases (EC 3.2.1.26). β -fructofuranosidase from fungi *Aureobasidium pullalans, Aspergillus niger* and *Aspergillus oryzae* were used to produce short chain FOSs. Another enzyme capable of synthesizing FOSs is the fructosyltransferase which transfructosylates the sucrose into FOS (Lotthida Inthanavong, 2011). Fructosyltransferases possess a higher transferring activity than β-fructofuranosidases (Antosova and Polakovic, 2001). Although these proteins differ in their subunit structure, molecular weight, degree of glycosylation, chemical susceptibility and substrate specificity, they all display both hydrolytic and transfer activities which limit the FOSs production to the use of high sucrose concentrations.

MATERIALS AND METHODS

Materials: 3, 5 Dinitrosalicylic acid, Sucrose, Dextrose and all other chemicals used were of analytical grade. FOS standard used were of from Sigma chemicals.

Isolation and preliminary screening: Different soil samples were collected from Sugar cane fields in Kalavai village, India and also from bagasse dumping area of Tiruvalangadu sugar mill, India. To isolate Fructosyltransferase producing fungal strains, soil samples were serially diluted and spread on PDA medium at 30 °C. Petri plates were constantly observed for the appearance of colonies with greater diameter and cultures were purified by repeated transfer of cultures and maintained in PDA.

Secondary screening of Fructosyltransferase producing microbes: The isolated organisms from the preliminary screening were selected and fermented in preculture media containing sucrose as carbon source at pH 6.5 as described by (Toharisman, *et al.,* 2009). Fungal were inoculated in 50 mL pre culture medium. Composition of pre medium was sucrose (200g/L), yeast extract (12g/L), Carboxy methyl cellulose (2g/l), magnesium sulphate (2g/L) and pH 6.5. The flask was kept in a shaker at 200 rpm and grown for 18 hour at 30 °C. After 18 hours of inoculation, 5 mL of both the media were centrifuged at 600 rpm for 20 minutes at 4 °C. After centrifugation the supernatant was used as a source of extracellular enzyme and the remaining pellet is homogenized with pestle and mortar using 0.2M Sodium acetate buffer, pH 6.5 and centrifuged at 10000 rpm for 10 min and the supernatant was used as intracellular enzyme. Then the enzyme solution (5 mL and 10 mL) was added with the reaction mixture (50 mL) contained 10 g/L sucrose in 50 mm sodium acetate buffer (pH 6.5) and incubated at 60 °C for 20 minutes. Then this mixture was used for DNS assay to find the enzyme activity. It was found that *Penicillium citrinum* (identified morphologically in Microbiology lab of Adhiparasakthi college of arts and science, Kalavai, India) from soil of sugarcane mill shows higher enzyme activity and it was selected for this study.

Fermentation culture or Biomass production: From the pre-culture, 5 mL was taken and inoculated into 100 mL fermentation medium in 200 mL flask. The composition of fermentation medium comprised of sucrose rich substrates like cane molasses, banana,

banana peel and papaya in four separate flasks of 200g/L each, di-potassium hydrogen phosphate (4g/L), carboxy methyl cellulose (2g/l), potassium di-hydrogen phosphate (9g/L). The flasks were kept in a shaker and grow at 30 °C and samples were taken for analysis at regular intervals.

Carbon sources in fermentation medium

- Finely chopped and autoclaved banana (200 g) was taken in fermentation medium 1.
- The pH of the fresh chopped banana peel was adjusted to 7 with acetate buffer and sterilised in autoclave for 15mins at 121°C. After cooling, 200 g of sterilized banana peel was added with fermentation medium 2.
- Ripened papaya fruit was taken from the local garden for research purpose. From that sterilised and chopped 200 g was added with fermentation medium 3.
- Molasses were collected from sugar mill for research purpose. 200 mL of sterilized molasses was added with fermentation medium 4.

Enzyme assay: After incubation the fermentation medium was sampled with regular intervals of 24 and 48 hours to measure the enzyme activity. For enzyme activity determination of all the four different substrates medium were centrifuged at 600 rpm for 20 minutes at 4 °C. After centrifugation, both extracellular and intracellular enzymes were used for the enzyme assay by DNS method (Miller, 1959). One unit of total FTF activity was defined as the amount of biocatalyst that liberates 1µmol of the reducing sugars (Glc and Fru) from sucrose per min at the standard assay conditions.

Analysis of FOS by TLC: The thin layer chromatography (TLC) was used for qualitative analysis of FOS products from the fermentation medium. TLC was done by using 0.2 mm thick silica gel TLC plate (Merck). Before development, Silica gel layer were pre-treated with 0.02M Sodium acetate. The samples were spotted 10 µL onto TLC plate, then the plates were developed in solvent mixture consisting of butanol:ethanol:water [5:3:2 v/v]. After development the plates were dried for 15 minutes in a warm air, then sprayed with colouring reagent using sprayer and dried the plates in a room temperature (10 minutes) and then for colour production the plates were kept heating at 120 °C for 20 minutes (Kalarina *et al.*, 2003).

RESULTS AND DISCUSSION

The main objective of this research was to produce FOS with minimal cost. For this four different cheap carbon sources were used for FOS production in optimized condition. They were Banana, Banana peel, Cane molasses and Papaya.

DNS assay

FOS production was carried out in four sets of fermentation medium (1, 2, 3, 4) each differs only in their carbon source. Along with this four sets of non-inoculated fermentation medium (5, 6, 7, 8) were incubated and its composition was similar to the first four respectively except *Penicillium citrinum* inoculation. This act as blank to nullify the effect of reducing sugar present in carbon sources as this interferes in DNS assay.

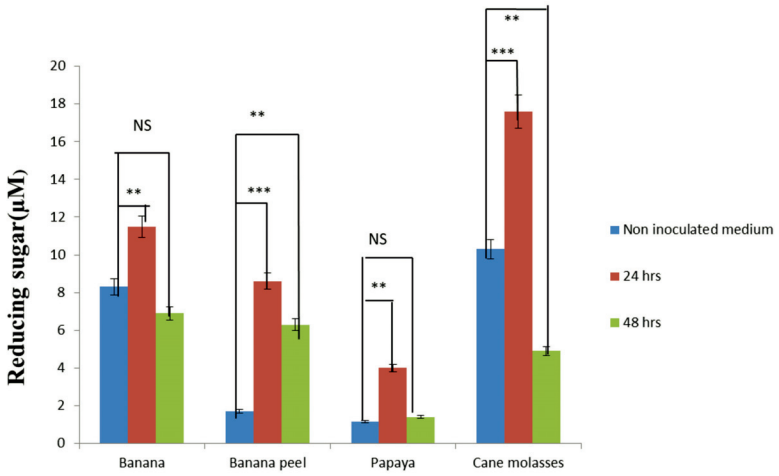

Fig. 1. Amount of reducing sugar in 24hr and 48hr sample: Amount of reducing sugar in the all carbon sources were compared with its corresponding non inoculated blank shows significant ($p<0.002$) increase in reducing sugar at 24 hrs culture than 48 hrs. NS-Non significant, ** $p<0.05$, ***$p<0.002$

Figure 1 shows the reducing sugar in the all carbon sources compared with its corresponding non inoculated blank shows significant ($p<0.002$) increase in reducing sugar at 24 hrs culture than 48 hrs. Mycelial growth continued up to 48 hr and observed decrease in enzyme activity.

Of the four different carbon sources employed, maximum FTase (6.9±0.15 U/mL/min) was obtained for Banana peel and (7.3±0.29 U/mL/min) for cane molasses at 24 hr (Figure 2).

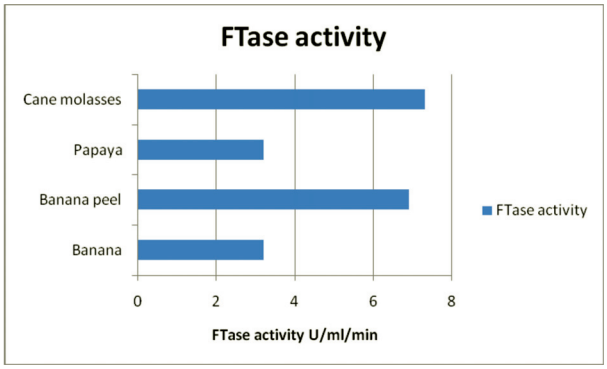

Fig. 2. Fructosyltransferase activity of four carbon sources at the end of 24hours.

From Table 1 it was clear that Fructosyltransferase activity was greater in banana peel and sugarcane molasses. Fructosyltransferase activity was also present in banana and papaya but only in negligible amount.

Four different carbon sources as listed in Table 1 employed for this study shows that Banana peel and cane molasses were the best inducer of FTase production by *Penicillium citrinum*. Patil *et al.,* (2011) obtained similar results that banana wastes were found superior to commercial production of β-fructofuranosidases. Baig *et al*. (2003) had similar results for the production of cellulose by banana waste and suggested that banana can be used effectively as the component in the medium for the production of enzymes.

Sugarcane molasses also shows higher Fructosyltransferase activity. Dorta *et al* (2006) suggested that *Aspergillus japonicas* FCL 119T and *Aspergillus niger* ATCC 20611 used cane molasses as a good substitute for sucrose for the production of Fructosyltransferase as well as FOS.

TLC Analysis of FOS: TLC plates were run with fructose as standard and non-inoculated medium as blank. As fructose is a monosaccharide it moves fast and high R_f but FOS is a oligosaccharide having more than two fructose it moves slower than fructose and having R_f less than fructose. So based on the degree of polymerization (DP) of fructose well detected spots of FOS were obtained near standard fructose. Table 2 shows the R_f value of samples and standards.

Table 2. R_f value of standards and samples

Carbon sources	R_f value
Fructose	0.512
Banana	0.476
Banana Peel	0.470
Papaya	0.474
Sugarcane Molasses	0.478
Standard FOS	0.479

CONCLUSION

The experimental results provided in this study have revealed the cheaper alternative substrates for the production of fructooligosaccharides instead of using commercial expensive sucrose as substrate. As the four carbon sources used as substrate for fructooligosaccharide production are rich in sucrose. Banana, Banana peel, Papaya and Sugarcane molasses were the four carbon sources used for fructooligosaccharide production by *Penicillium citrinum* using fructosyltransferase. From the result it was found that Cane molasses and

Table 1. Activity of fructosyltransferase in different carbon sources at 24hrs.

Carbon sources	Reducing sugar (μmol)		Fructosyltransferase Activity (U/mL/min)
	Inoculated medium (Mean)	Non-inoculated medium (Mean)	
Banana	11.5	8.3	3.2±0.5
Banana peel	8.6	1.7	6.9± 0.15
Papaya	4.4	1.15	3.2±0.56
Sugarcane molasses	18.2	10.9	7.3±0.29

Banana peel were shown more fructosyltransferase activity than the other two.

Though the Banana contains fructooligosaccharides in it and was not detectable by TLC method as it may be in trace amount. After banana was added in fermentation medium the fungi Penicillium present in it used the sucrose content of banana and shows fructosyltransferase activity and fructooligosaccharide production. This produced fructooligosaccharides was detected by TLC. This shows that banana is suitable as substrate for fructooligosaccharide production.

Banana peel and cane molases were found to be the best carbon source for fructooligosaccharide production. In conclusion, a higher yield of FOS production by *Penicillium citrinum* was possible by banana peel and molases. The supplementation of banana peel and molases with nitrogen nutrients showed better production of fructooligosaccharide by fructosyltransferase of *Penicillium citrinum*.

Cane molases and Banana peel offers a good option, if researches on the possibilities of augmenting its nutrition status are carried out. It is recommended that the study on fructooligosaccharide production using cane molases and banana peel should be conducted on large scale. Extensive toxicological and acceptability tests should be performed before the product is approved for large scale consumption.

Conflict of Interest: The authors have indicated they have no potential conflicts of interest to disclose.

REFERENCES

Ahmad, N. 2010. Production of Fructo-oligosaccharides from the Enzymatic Conversion of Coconut Sugar Catalyzed by Fructosyl transferase in a Batch Reactor (Doctoral dissertation, UMP).

Antosova, M. and Polakovic, M. 2002. Fructosyl transferases: the enzymes catalyzing production of fructooligosaccharides. *Chemical Papers-Slovak Academy of Sciences.* 55(6) : 350-358.

Baig, M.M.V., Mane, V. P., More, D. R., Shinde, L. P. and Baig, M. I. A. 2003. Utilization of banana agricultural waste: production of cellulases by soil fungi. *Journal of Environmental Biology.* 24 (2) : 173-176.

Csanádi, Z.S. and Sisak, C.S. 2008. Production of short chain fructooligosaccharides. *Hungarian Journal of Industry and Chemistry.* 36 (1-2).

Dorta, C., Cruz, R., de Oliva-Neto, P. and Moura, D.J. C. 2006. Sugarcane molasses and yeast powder used in the fructooligosaccharides production by *Aspergillus japonicus*-FCL 119T and *Aspergillus niger* ATCC 20611. *Journal of Industrial Microbiology and Biotechnology.* 33 (12) : 1003.

Hidaka, H., Eida, T., Takizawa, T., Tokunaga, T. and Tashiro, Y. 1986. Effects of fructooligosaccharides on intestinal flora and human health. *Bifidobacteria and Microflora*. 5 (1) : 37-50.

Inthanavong, L. 2011. Production and Characterization of the Fructosyl transferase (levansucrase) from *Geobacillus stearothermophilus* and Its Application in the Synthesis of Novel Fructooligosaccharides (Doctoral dissertation, McGill University Library)

Kalarina, R., Jana P., Andrej, O., Karol, F. and Tatana, G. 2003. *Jour. of. Plant Chroma.*16 : 52-57.

Miller, G.L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*. 31 (3) : 426-428.

Patil, P.R., Reddy, G.S.N. and Sulochana, M.B. 2011. Production, optimization and characterization of â-fructofuranosidase by chrysoniliasitophila PSSF84-a novel source. *Indian Journal of Biotechnology.* 10 : 56-64.

Toharisman, A. and Santoso, H. Triantarti, 2009. Optimization of fructooligosaccharides production from plantation white sugar. Available at http:// www.sugarresearch. org.Available at http:// www.sugarresearch.org.

Yun, J.W. 1996. Fructooligosaccharides—occurrence, preparation and application. *Enzyme and Microbial Technology.* 19 (2) : 107-117.