International Journal of Science, Environment and Technology, Vol. 3, No 2, 2014, 708 – 713

ISSN 2278-3687 (O)

BIOTRANSFORMATION OF SUCROSE BY USING THERMOSTABLE AND ALKALINE FRUCTOSYLTRANSFERASE ENZYME ISOLATED FROM MARINE *ASPERGILLUS NIGER*

[1]Amrendra Kumar, [1]Vaishnavi R., [1]Saravanakumar A., [2]Awani Kumar Singh* and [2]Dr. S.K. Tank

[1]CAS Marine Biology, Faculty of Marine Sciences, Annamalai University

[2]Department of Bioscience, Veer Narmada South Gujarat University

(**Corresponding Author*)

Abstract: The biotransformation of sucrose to fructooligosaccharides was investigated using the catalytic action of fructosyltransferase originated from marine *Aspergillus niger*, using whole cell immobilization. The biomass production and enzymatic reaction were carried out in shake flask. A fructosyltransferase enzyme producing fungi locally isolated from sea water from South East cost of India, by using potato dextrose broth medium and identified as *Aspergillus niger*. To evaluate the fructosyltransferase activity two biomass concentrations (6 and 9.5 g L-1) of *Aspergillus niger* mycelia were used. Cultural and physiological characteristics of the isolate were studied. A*spergillus niger* produced an inducible intracellular FTFase enzyme in a high yield (37.04U/ml). Hydrolysis and subsequent analysis showed that the product consisted entirely of D-fructose. The result shows the enzyme reaction reached its maximal activity when the reaction was conducted at 65 ºC, pH 8.0 in 16 hrs.

Keywords: Fructooligosaccharide, *Aspergillus niger*, Whole cell immobilization, FOS production, enzyme assay, sodium alginate.

Introduction

Fructooligosaccharides (FOS) are oligosaccharides of fructose containing a single glucose moiety and have a simple molecular structure rather than the complex molecule from its original sucrose. It is also known as neosugar, inulin, oligofructose or oligofructan. Fructooligosaccharides can be produced by the action of fructosyltransferase from many plants and microorganisms (1, 2). Fructooligosaccharides are composed of 1-kestose (GF2), nystose (GF3), and 1-β- fructofuranosyl nystose (GF4), in which fructosyl units (F) are bound at the β (2 → 1) position of sucrose molecule (GF) (3,4,1,5). When the polymeric grade of fructo-oligosaccharides is low, it has a better therapeutic properties compared than the high polymeric grade. Fructooligosaccharides are widely used in pharmaceutical industry as a functional sweetener; it is known 0.4 and 0.6 time's sweet than sucrose. Its use emerged in

*Received Dec 9, 2013 * Published April 2, 2014 * www.ijset.net*

the 1980s in response to consumer demand for healthier and calorie-reduced foods. These oligosaccharides having low caloric values exhibit several beneficial effects such as anti-carcinogenic properties, decrease level of phospholipids, triglycerides and cholesterol (6). Fructooligosaccharides are industrially produced from sucrose by microbial enzymes with transfructosylating activity. Fructosyltransferase is produced intra- and extracellularly by several microorganisms including bacteria and fungi. The most investigated microorganisms in this subject arc fungi belonging to genera *Aspergillus* (7, 2, 8) *Penicillium* (9) *Aureabasidium* (10) and *Arthrobacter sp.* and *Fusarium* (3). Fructooligosaccharides help the absorption of calcium and magnesium; it is useful for anti diabetic products and is used as prebiotics to stimulate the growth of *bifido bacteria* in the human colon (3).

MATERIALS AND METHODS

Chemicals: All chemicals used were of analytical grade. FOS standards were from Sigma USA and glucose, fructose were from Otto and sucrose of food grade.

Microorganisms and culture conditions: The samples were collected from sea water at Parangipettai coast. The samples were cultured in potato dextrose agar at 28 - 30 °C for 7 – 8 days. The isolated organism was preserved on potato dextrose agar slants for further study (potato infusion agar 200 g; dextrose 20 g; agar 5 g in 1000 ml distilled water) at 4°C. Spore suspension is prepared by scrapping of spores from the PDA plates by sterilized inoculated loop, diluted to the concentration nearly $2X10^7$ spores / ml in sterilized saline and kept at -20±1°C by subculture once in a month.

Production of fructosyltransferase enzyme by whole cell immobilization process: The enzyme production media was prepared in 250 mL flask and added amount of culture media was 100 mL. The composition of the culture medium was potato dextrose broth; 5gm dissolved in 100 ml distilled water autoclaving 125°C for 20 min. The 10 mL of 24 h old inoculums was transferred into 100 mL culture media with 1 mL mycellia suspension were incubated at 30°C with shaking at 240 rpm for 24-32 hrs, after incubation the culture media is centrifuged at 8000 rpm for 15 min at 4°C. After centrifugation the whole pellet was taken for immobilization. The pellet was mixed with 20% w/v sodium alginate and the mixture was extruded drop wise through needle (1D 1.0 mm) into 0.1 M $CaCl_2$ solution by peristaltic pump. Droplets were instantly transformed into spherical beads (2-3 mm in diameter) by exchange of Na^+ in droplets and Ca^+ in solution. The immobilized cells (beads) were kept in

2% of calcium chloride solution for enzymatic reaction and the microorganisms at a concentration of 20 g were mixed with the sodium alginate at a concentration of 1% w/v.

Optimization of parameters for FOS production

The various parameters were used in this study such as temperature (50 ^{0} C, 55 ^{0} C, 60 ^{0} C, 65 ^{0} C) pH (7.0, 7.5, 8.0, 8.5 and9.0), agitation (100, 150, 200 and 250 RPM), incubation time (12 hrs, 16 hrs, 20 hrs, 24 hrs, 28 hrs and 32 hrs), substrate level (50%, 60% and 70 % sucrose solution).

Effect of surfactant and different concentration metallic salt and inhibitors were also optimized. Surfactants (phenylmethylsulphonylfluoride (PMSF), EDTA, cystine, SDS, Tween-80 and Triton X- 100), metallic salt and inhibitors ($FeCl_2$, $HgCl_2$, $MgCl_2$, $AgNO_3$, $CuCl_2$, $PbCl_2$, $CaCl_2$, $NiCl_2$ and $MnCl_2$) were used in respect of distilled water as a control.

Enzyme assay: For determining of enzyme activity 2.5 mL of enzyme is mixed with sucrose solution 60% w/v as substrate, 0.2 M citrate buffer (pH 5.5) and incubated at 60°C for 1 hrs using shaker incubator. The reaction was terminated by keeping reaction mixture in water bath of 100°C for 15 min. Glucose, fructose and trisaccharides like GF_2, GF_3 and GF_4, etc. were then determined by HPLC. One unit of activity of transfructosylation is defined as amount of enzyme activity which catalyses the formation of 1μ mol glucose per min and one unit of hydrolytic activity was defined as the amount of enzyme which catalyses the formation of 1μ mol fructose per min under these conditions, quantitative conversion analysis of sucrose to FOS by fructosyltranferase enzyme was done by HPLC.

Result and Discussion

Microorganism culture and Identification: Marine microbes having unexplored potentiality, this experiment will explore one of the criteria for the evaluation of microorganisms and other parameters, which were available for the production of enzymes, were the ample growth of mycelia at the cultivation.

Whole cell immobilization: After spraying the mixture of sodium alginate and pelletized culture in calcium chloride solution, gel of calcium – alginate thus formed in the presence of Ca^{++} ions entraps the cells in the matrix and the resultant beads contain 90-95% water and only up to 10% solids, within 3 hrs of spraying the beads like hard and stored on 4 ^{0}C.

Optimization of different parameters

Conditions and transfructosylating activities of cells in a batch reaction at temperature of 65°C, pH 8.0, 200 RPM were optimized in 60% sucrose concentration. A typical course of

saccharide concentrations in such an experiment is shown in (Fig. 2. A and B), Approximately a 60 % conversion of sucrose was achieved in 16 hrs of reaction time with a high selectivity to fructooligosaccharide production which follows from the low concentration values of fructose produced through the hydrolytic activity. In this study first time we are reporting the production of fructooligosaccharide from marine microbes source for fructosyltransferase enzyme, this observation differ from the observations published by other authors to optimization of parameter for fructooligosaccharide production. (11) Found the optimum pH of 5.5 using the free cells of *Aureobasidium pullulans*, when the enzyme activity sharply decreased below pH 5 and above pH 6.5. (12) Worked with the immobilized mycelium of *Aspergillus japonicus* that had an optimum activity at the pH 5.5 and above pH 6 the activity rapidly decreased. (13) reported that the effect of temperature in range from 55 to 60°C for catalytic activity of fructosyltransferase enzyme from *Aspergillus niger,* a typical graph of HPLC showing conversion of sucrose to FOS, there were no significant role of agitation had reported for effect of FOS production (Figure 1 and 2).

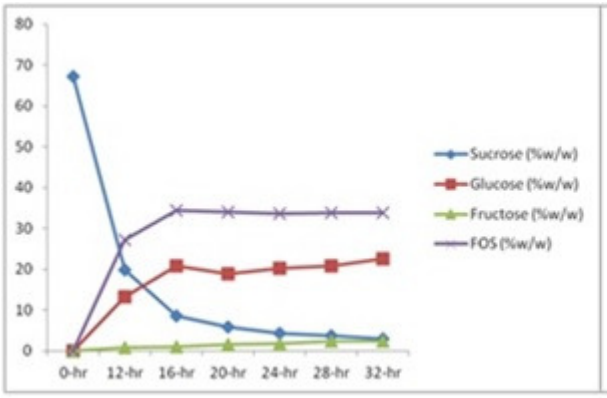


Fig 1.

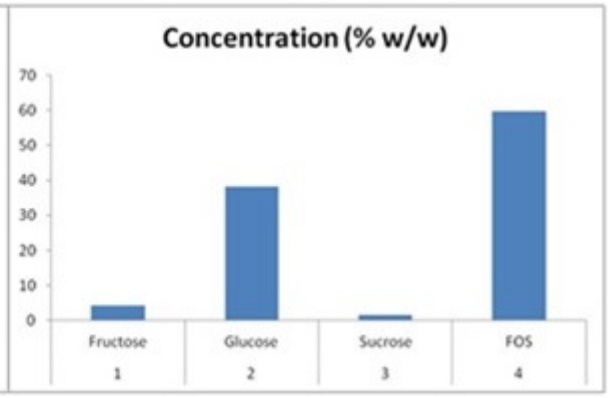


Fig. 2

Fig.1 and 2. Final conversion of sucrose to FOS on different incubation time after evaluating all parameters

Effect of inhibitors, surfactants and metallic salt on enzyme activity

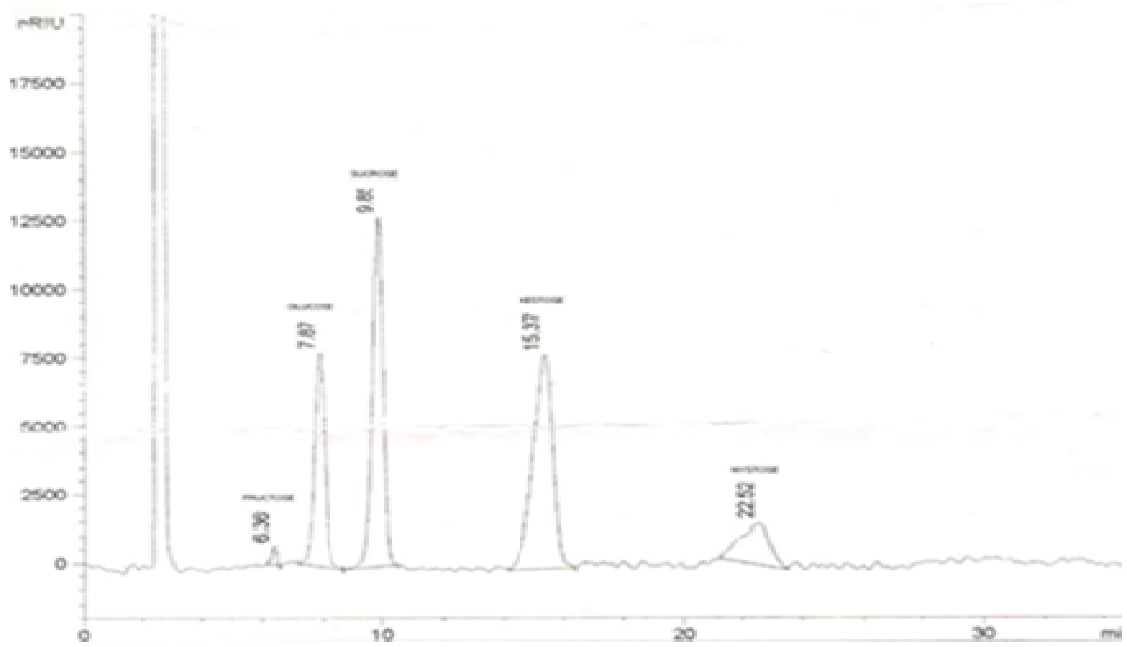
The some surfactants used were: phenylmethylsulphonylfluoride (PMSF), EDTA, cystine, SDS, Tween-80 and Triton X- 100. In the presence of SDS and Triton X-100 were stimulated the protease activity. phenylmethylsulphonylfluoride (PMSF), EDTA, cystine and Tween-80 were slightly reduced**.** Effect of different metals in the concentration of 1, 3, 5, and 10 mM ($FeCl_2$, $HgCl_2$, $MgCl_2$, $AgNO_3$, $CuCl_2$, $PbCl_2$, $CaCl_2$, $NiCl_2$ and $MnCl_2$,) on fructosyltransferase activity showed that metals such as Mn could stimulate the

fructosyltransferase activity. In contrast, it was strongly inhibited by Hg and Pb, Hg^{2+} ions were inhibitory to the fructosyltransferase activity and Mn^{2+} (104, 109, 112 and 132% residual activity at 1, 3, 5 and 10 mM) were enhancing the same where as all the other ions tested were having a neutral effect, neither enhancing nor inhibiting it.

Table. 1.

Sample/ hrs for HPLC analysis	Area of Fructose	Area of Glucose	Area of Sucrose	Area of Kestose (GF2)	Area of Nystose (GF3)	Area of (GF4)	Wt. of Sample mg	% ww Fructose	% ww Glucose	% ww Sucrose	% ww Kestose	% ww Nystose	% ww F+G+S	% ww Water Content	K + N	%w/w Total Solid	% ww Total FOS (DWB)
0 Hrs	12875.6	230956	243965	407408	165912	19307.8	1010	0.000	0.000	74.163	0.000	0.000	74.163	37.93	0.00	62.07	0.00
12 Hrs	13829.9	228535	225210	392858	165968	ND	1044	0.000	4.715	48.762	12.242	1.802	53.477	40.38	1.04	59.62	23.26
16 Hrs	16411.9	264147	246552	451083	201276	ND	1017	0.000	7.497	36.138	16.543	3.116	43.635	38.45	19.66	61.55	33.94
20 Hrs	16352.7	238363	196835	395583	182275	ND	1039	0.000	8.393	24.993	18.757	4.358	33.386	43.46	23.12	56.54	40.88
24 Hrs	19574.5	273680	71518.2	156315	183763	ND	1064	0.428	10.684	20.860	22.598	6.923	31.972	41.21	29.52	58.79	50.21
28 Hrs	3093.0	78211	695226.0	208668	18431	0.0	1020	0.410	12.960	14.204	22.664	8.859	27.574	44.54	31.52	55.46	56.84
32 Hrs	3870.0	93204	636247.0	242622	26523	0.0	1020	0.665	13.203	13.566	22.190	9.513	27.434	4036	31.70	59.64	53.16

HPLC conversion table of FOS production



Typical graph of HPLC showing conversion of sucrose in to different compound as per RT

Conclusion: In the present study, screening of thermostable or alkaline stable fructooligosaccharide producing enzyme fructosyltransferase were investigated and optimized against different parameter by the action of fructosyltransferase of marine origin microbes. The *Aspergillus niger* isolated from sea water allowed achieving high cell density cultures and showing independence stability of temperature 60^0 C and pH 8.0 at 16 hrs. The FTase specific transfructosylating activity showed a dependence on the biomass concentration and the reaction time, even though, the volumetric productivity also showed

dependence on incubation time of period, substrate concentration (60%) with specific inhibitors, surfactants and metallic salt concentrations.

REFERENCES

[1] Yun JW (1996) Enzyme Microb Technol: 19, 107.

[2] Hidaka H Hirayama M. Sumi N (1988) A fructooligosaccharideproducing enzyme from *Aspergillus niger* ATCC 20611. Agric Biol Chem 52**:**1181–1187.

[3] Sangeetha PT Ramesh MN Prapulla SG (2005a) Fructooligosaccharide production using fructosyl transferase obtained from recycling culture of *Aspergillus oryzae* CFR 202. *Process Biochem* 40:1085–1088.

[4] Kaplan H Hutkins RW (2000) Fermentation of fructooligosaccharides by lactic acid bacteria and bifidobacteria. Appl Environ Microbiol 66**:** 2682–2684.

[5] Kühbauch W (1972) Veränderungen der Gehalte an Glucose, Fructose, Saccharose und Fructosan sowie des Polymerisationsgrades von Fructosanmolekülen in Blättern und Stengeln einiger Knaulgrassorten während des Wachstums *Landwirtsch Forsch* 2: 173–181.

[6] Biedrzycka E Bielecka M (2004) Prebiotic Effectiveness of Fructans of Different Degrees of Polymerization. *Trends Food Sci Technol* 15:170–175.

[7] Hirayama M. Sumi N. Hidaka H. (1989) Purification and properties of a fructooligosaccharide-producing fructofuranosidase from *Aspergillus niger* ATCC 20611. Agric Biol Chem 53: 667–673.

[8] Chen J. Sharma S Quiocho FA Davidson AL (2001) Trapping the transition state of an ATP-binding cassette transporter: evidence for a concerned mechanism of maltose transport. Proc Natl Acad Sci USA. 98: 1525–1530.

[9] Poonawalla FM Patel KL Iyengar MRS (1965) Production by penicillium chrysogenum and fungi in submerged fermentation, appl microbial 13: 652-659.

[10] Sangeetha PT Ramesh MN Prapulla SG (2005b) Maximization of fructooligosaccharide production by two stage continuous process and its scale up. *J. Food Eng* 68: 57–64.

[11] Jung KH Lim JY Yoo SJ Lee JH Yoo MY(1987) Biotechnol Lett 9: 703.

[12] Chang H Yoo I Kim B (1994) High density cell culture by membrane-based cell recycle. Biotechnol Adv 12: 467–487.

[13] Sánchez Oscara Guío Felipea García Dianaa Silva Edelbertob Caicedo Luisa (2007) Fructooligosaccharides production from sucrose by *Aspergillus sp.* N74 in a hybrid bioreactor Proceedings of European Congress of Chemical Engineering (ECCE-6) Copenhagen 16-20.