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Characterization of Fructosyltransferase Produced by *Syncephalastrum racemosum Cohn*

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ABSTRACT
An indigenously isolated strain *Syncephalastrum racemosum Cohn* was found to produce fructosyltransferase (Ftase) enzyme. The Ftase was purified by conventional purification techniques to homogeneity. SDS PAGE revealed the molecular weight of the purified Ftase to be 37 KD. Optimum pH for activity was 5.5 while an optimum temperature was recorded to be 55ºC. Mg^{++} and Mn^{++} were able to restore the activity of the metal chelator treated purified Ftase. The Ftase was found to be glycoprotein in nature. PMSF, PCMB, DTNB, β-mercaptoethanol and iodoacetate did not inhibit the activity completely, but the surfactants were able to inhibit the activity completely.

Keywords: Fructosyltransferase, enzyme, fungi, microbial enzyme, oligosaccharides.

1. Introduction
Fructooligosaccharides are functional food ingredients. Other classes of oligo - saccharides are galactooligosaccharides, isomaltooligosaccharides, inulinooligosaccharides and soybean oligosaccharides. Fructooligosaccharides are important because of their favorable functionalities such as being low caloric, non cariogenic, prevent dental caries, and act as growth promoter for bifidobacteria in human gut [1-3]. Fructooligosaccharides (FOS) have 1-3 fructosyl units bound to β, 2-1 position of sucrose. Fructosyltransferase [EC 2.4.1.9] is widely used enzyme for production of FOS. It catalyses the formation of FOS from sucrose. Ftase is reported to be produced by many microorganisms, including *Bacillus macerans* [4], *Streptococcus salivarius* [5], *Aspergillus niger* [6], *Aureobasidium pullulans* [7] and *Fusarium oxysporum* [8]. *Syncephalastrum racemosum Cohn*, isolated in our laboratory from the rotten woods was found to produce both extracellular and intracellular Ftases. The present paper deals with the purification and characterization of extracellular Ftase produced by *S. racemosum Cohn.*

2. Materials and methods
Microorganism: *S. racemosum Cohn* was maintained on PDA slants and subcultured every 2 months to keep it viable.

2.1 Culture conditions
The cultivation medium has the composition as mentioned by Patil and Butle (2014). The medium contained per litre: $NaNo_3$ 3 g, KH_2PO_4 1 g, $MgSO_4.7H_2O$ 0.5 g, $FeSO_4.7H_2O$ 0.01 g and Sucrose 30 g. pH was adjusted to 5.5 before sterilization at 121 lb PSI for 15 min. Sucrose was autoclaved separately and added to the growth medium before inoculation [9].

2.2 Enzyme production
Sterilized medium in a conical flask with 1/5th of its capacity was inoculated with spore suspension containing 10^8 spores/mL and incubated at 37 ºC for 10 days under aerobic and static condition. The mycelial mass was removed by filtration and filtrate was clarified by centrifugation at 9886 *g* for 20 min (Remi C24). The supernatant designated as crude Ftase was used for further purification of enzyme.

2.3 Purification of Ftase
Ammonium sulphate precipitation of the crude enzyme was carried out as suggested by Patil and Shastri (1982) [10]. The precipitated proteins were solubilized in 0.2 M sodium acetate buffer, pH 5.5 (SAB), and dialyzed against the same for 18 h at 4 ºC. Next day the dialyzed

fraction was again centrifuged (2000 rpm) and used for further purification.

2.4 Ion Exchange chromatography
DEAE cellulose was used for this step and the column length was 30 × 1.5 CM. Proteins were loaded at the proportion of 0.5 mg /CM of the column. Elution of the bound proteins was performed by applying NaCl gradient from 0.05 to 0.3 M in SAB. Maximum proteins were eluted in the gradient of 0.2 to 0.25 M NaCl.

2.5 Gel filtration Chromatography
The proteins eluted out from ion exchange chromatography were passed through the Sephadex G-75 column as described by Patil and Shastri (1982) [11].

2.6 SDS Gel electrophoresis
The proteins eluted from the gel filtration chromatography were run in the electrophoretic field using SDS PAGE and comparing the R_f with that of the standard molecular weight markers including phosphorylase *b* (94 kDa), BSA (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20 kDa) and α-lactalbumin (14.4 kDa) [11].

2.7 Protein estimation
Protein estimation was carried out by the method of Lowry *et al*., (1951) using BSA as standard protein [12].

2.8 Carbohydrate estimation
Determination of carbohydrate content was performed by the method of Dubois *et al*. (1956) using D- glucose as standard [13].

2.9 Ftase assay
Ftase assay was performed by the method of Dhake and Patil (2006). One unit of enzyme activity was defined as that amount of enzyme which liberates 1mM glucose under experimental conditions [14].

2.10 Optimum pH and pH stability
pH dependence of purified Ftase was determined by incubating the purified fraction with buffers ranging from pH 3.0 to 7.0 as per the method of Dhake and Patil (2006) with suitable controls. The buffers used were 0.1 M sodium citrate for pH 3.0, 0.1M sodium acetate for pH 4 to 6 and 0.1M Citrate-Phosphate buffer for pH 7.0 [14].

2.11 Optimum temperature and temperature stability
Purified Ftase was exposed to different temperatures (20 to 70 ^oC) for activity and stability as per the method of Dhake and Patil (2006) [14].

2.12 Effect of metal ion chelators and metal ions on Ftase activity
This was monitored again as suggested by Dhake and Patil (2006) [14].

2.13 Effect of inhibitors and activators
Ftase was treated with various compounds as suggested by Patil and Shastri (1985) before the determination of activity [11].

Table 1: Purification scheme of Ftase produced by *S. racemosum Cohn.*

Fraction	Volume (ml)	Activity U/ml	Total Units	Proteins Mg/ml	Specific activity	Fold purification	Recovery %
Crude	100	50	5000	3.4	14.7	1	100
0-90% ASP*	20	150	3000	5.2	28.84	2	60
DEAE cellulose fraction	15	150	2250	1.75	85.71	5.8	45
Sephadex G75 fraction	10	185	1850	0.62	298	20	37

ASP*: Ammonium Sulphate Precipitate

2.14 Glycoprotein nature of the protein
Major carbohydrate content of purified fraction was determined by TLC (Upadhyay *et al*. 1997) using various carbohydrate as standards. R_f values were determined by the formula: R_f = The distance travelled by the solute/ The distance travelled the solvent [15].

2.15 Estimation of proteins in terms of amino acids
Proteins in terms of amino acids were measured by the method described by Spice (1959) using purified tyrosine as standard [16].

2.16 Estimation of Tryptophan in Ftase
Tryptophan content in purified Ftase was measured by the method described by Spande and Witkop (1967) using pure tryptophan as standard amino acid [17].

2.17 Statistical analysis
All results were statistically analyzed by the method described by Walpole (1985). P value was set at < 0.05 for significance [18].

3. Results
Results presented in table 1 show the purification scheme of Ftase by following ion exchange and gel filtration chromatography. The enzyme was purified to homogeneity with a good yield with 20 fold purification (Fig.1, 2, and 3).

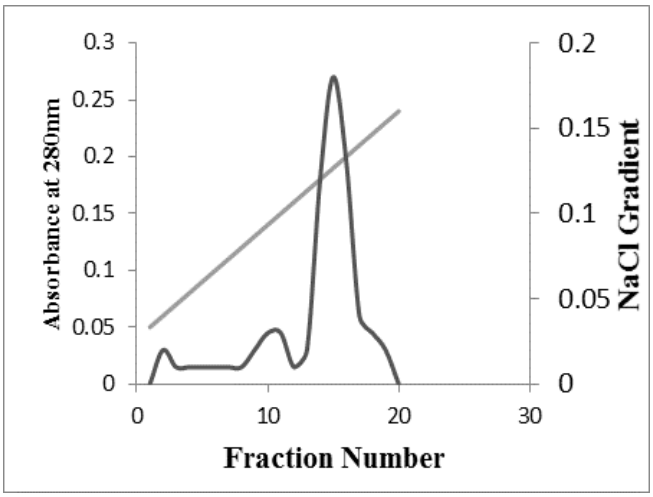


Fig 1: Elution profile of Ftase on DEAE Cellulose column.

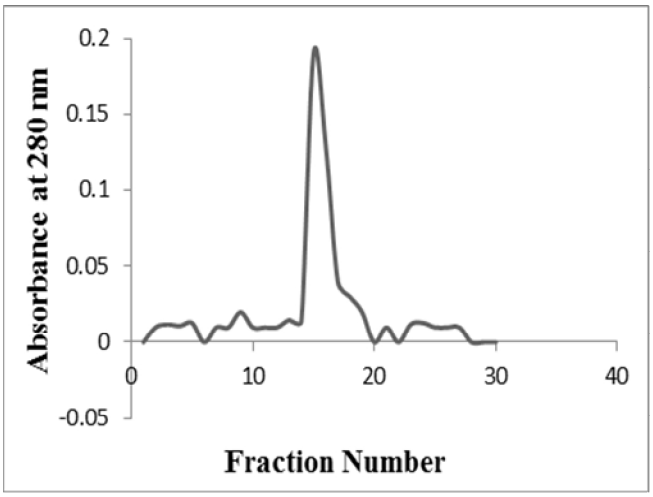
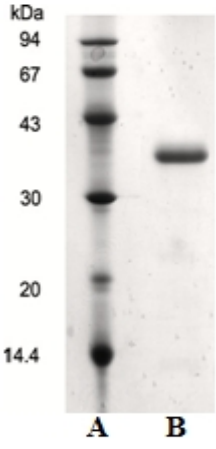


Fig 2: Elution pattern of Ftase on Sephadex G 75 column.

SDS PAGE demonstrated the purified fraction to be homogeneous with the molecular weight of 37 KD as shown in fig 3.Molecular weight of 37 kD as shown in Fig.3.



(A) Left lane: molecular mass markers from GE Healthcare including phosphorylase *b* (94 kDa), BSA (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20 kDa) and α-lactalbumin (14.4 kDa). **(B)**Right lane: representing purified Ftase

Fig 3: SDS Polyacrylamide gel electrophoresis of Ftase.

Purified Ftase was found to have optimum pH of 5.5 and stability from the pH 4 to 6.5 only (Fig. 4). Optimum temperature was recorded at 55 ^{o}C and the enzyme lost its activity drastically at 65 ^{o}C (Fig. 7)..

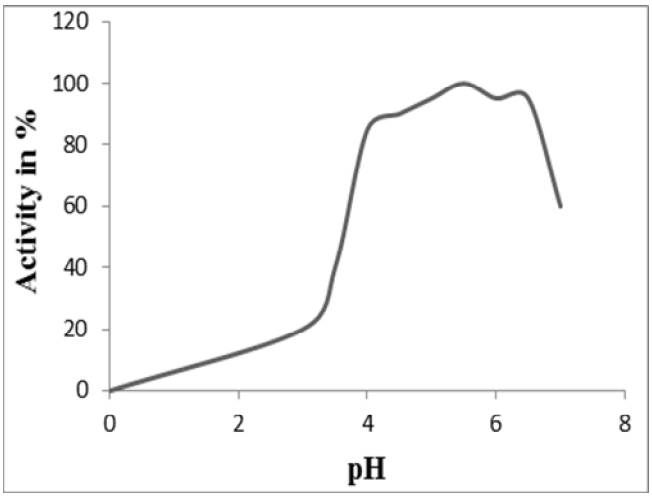


Fig 4: Optimum pH and pH stability of Ftase activity.

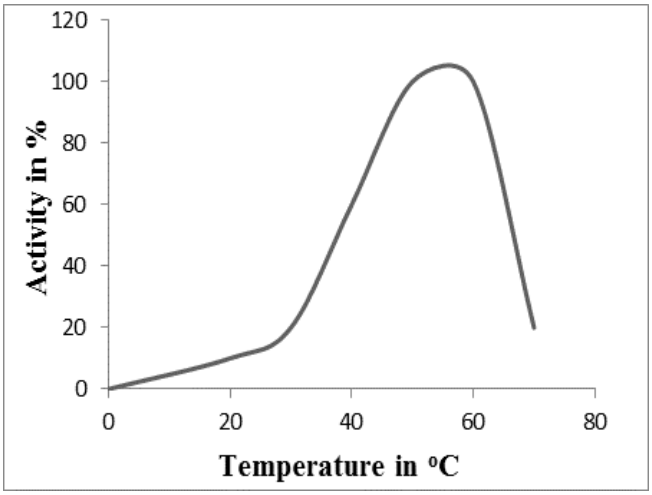


Fig 5: Optimum temperature and temperature stability of Ftase activity.

Incubation of purified Ftase with EDTA and 1, 10 phenanthroline lost the enzyme activity but the activity was restored back by Mg^{++} and Mn^{++} ions. Hg^{++}, Pb^{++} and Sb^{++} were found to inhibit the activity completely (Fig. 6).

Carbohydrate analysis has indicated the purified protein to be glycoprotein in nature; however, TLC of the purified fraction did not reveal clearly the presence of any specific carbohydrate moiety.

Measurement of protein in terms of amino acids has shown 94 µg amino acids in 100 µg proteins. The tryptophan content was found to be 11 µg in 100 µg proteins (table 2).Treatment of purified fraction with SDS, triton X100, tween 20 and tween 80 decreased the activity. PMSF, p-chloromercuribenzoate, dithionitrobenzoic acid, iodoacetate and β-mercaptoethanol did not decrease the activity completely as that of surfactants however, these compounds were found to decrease the activity

to less than 50% (Fig. 5).

Table 2: Properties of purified Ftase produced by *Syncephalastrum racemosum Cohn*

Property	Observation
Molecular weight	37 KD
Optimum pH	5.5
pH stability	4.0 to 6.5
Optimum temperature	55 ºC
Temperature stability	35 to 65 ºC
Nature of protein	Glycoprotein
Carbohydrate moiety	Not identified
Amino acids/100 µg protein	94
Tryptophan content/100 µg protein	11

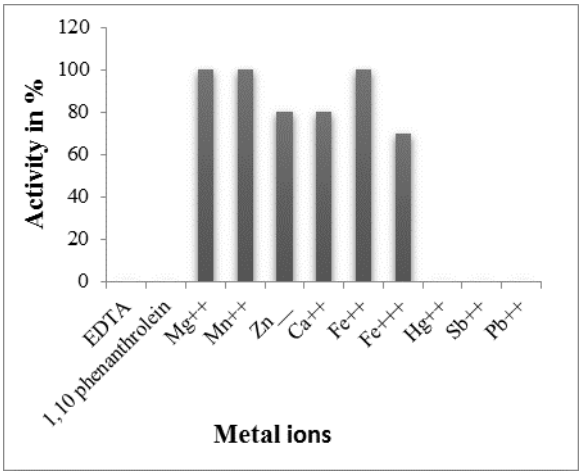


Fig 6: Effect of metal ion chelators and metal ions on Ftase activity.

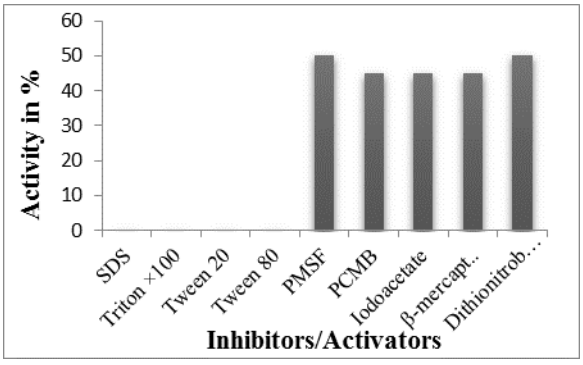


Fig 7: Effect of inhibitors and activators on activity of Ftase.

4. Discussion

All experiments were carried out in triplicate and were analyzed by single linear regression analysis. Ftase produced by *S. racemosum Cohn* was purified by conventional methods to purity. The enzyme was quite thermostable with its optimum activity at 55 ºC and optimum pH of 5.5. Similar properties with respect to pH and thermostability were found with the Ftase produced by *P. purpurogenum* [14]. The purified Ftase was homogeneous on SDS PAGE indicating the monomeric nature of the protein with the molecular weight of 37 KD showing that the purified Ftase of *S. racemosum Cohn* was also comparable with that of Ftase produced by other microorganisms [1-3]. The amino acid content of the purified fraction was less in total protein content indicating presence of some other moiety in protein fraction which was endorsed by the glycoprotein nature of the purified Ftase. Tryptophan content was 11 µg/100 µg proteins showing the presence of good aromatic amino acid content. Treatment with metal ion chelators lost the enzyme activity, while Mg^{++} and Mn^{++} ions could restore the activity of Ftase. A similar observation was found by Dhake & Patil for the Ftase produced by *P. purpurogenum* [14].

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6. References

1. Hidaka H, Hirayama M, Sumi N. A Fructo oligosaccharide producing enzyme from *A. niger* ATCC 20611. Agric. Biol. Chem. 1988; 52:1181-1187.
2. Kohmoto T, Fukui F, Takaku H, Mitsuoka T. Dose response test of Isomaltooligosacchrides for increasing fecal bifidobacteria. Agric Biol Chem 1991; 55:2157-2157.
3. Wada K, Watanabe J, Mizutani J, Tomoda M, Suzuki H, Saitoh Y, Nippon NK. Effect of soybean oligosachharides in a beverage on human fecal flora and metabolites. J Agric Chem Soc Japan 1992; 66:125-137.
4. Park JP, Oh TK, Yun JW. Purification and characterization novel fructosylating enzyme from *Bacillus macerans.* Process Biochemistry 2001; 37:471-476.
5. Song DD, Jocoues NA. Purification and enzymic properties of Fructosyltransferase of *Streptococcus salivarius* ATCC 25975. Biochem J 1999; 341:285-291.
6. Hocine LL, Wang Z, Jiang B, S. Xu. Purification and partial characterization of fructosyltransferase and invertase from *A. niger*. J Biotechnol 2000; 81:73-84.
7. Yun JW, Kim DH, Moon HY, Song CH, Song SKJ. Sim ultaneous formation of Fructosyltransferase and Glucosyltransferase in *Aureobasidium pullulans.* Microbiol Biotechnol 1997; 7:204-208.
8. Patel V, Saunders G, Bucke C. Production of fructooligosachharides by *F. oxysporum*. Biotechnol Lett 1994; 11:1139-1144.
9. Patil MB, Butle A. Fructosyl transferase production by Indigenously isolated *Syncephalastrum racemosum Cohn.* J Global Biosci 2014; 3(2):597-603.
10. Patil M, Shastri NV. Effect of fungicides and phenolics on extracellular production of proteases by *A. alternate.* J Ferment Technol, Japan 1982; 60:37-40.
11. Patil M, Shastri NV. Purification and properties of proteases produced by *A. alternate*. Regulation of production by fructose. J Biosci 1985; 9:1-11.
12. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein estimation by Folin phenol reagent. J Biol Chem 1951; 193:265-275.
13. Dubois M, Gillies KA, Hamilton RK, Ribbers BA, Smith F. Colorimetric method of determination of sugars and related substances. Anal Biochem 1956; 28:350-356.

14. Dhake AB, Patil MB. Production and properties of cell wall bound fructosyltransferase by *Penicillium purpurogenum*. Nagpur Univ Sci Journal 2006; 6:97-111.
15. Upadyay A, Upadhyay K, Nath N. Biophysical Chem, Mumbai- 400004: HPH, 1997.
16. Joseph RS. Calorimertic procedures for amino acids. In Methods in Enzymol, Vol 3, Acad Press, N.Y, 1957; 467-471.
17. Spande TF, Witkop B. Determination of the tryptophan content of proteins with N-bromosuccinimide. Methods in Enzymol 1967; 11:498-532.
18. Walpole R. The statistical methods, Macmilan publishing company, 1982.