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# Enhancement of Extracellular fructosyltransferase Production by *Aspergillus stallus* Through Batch Fermentation

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**Fructooligosaccharides are now well known for their prebiotic properties. Moreover, they lower cholesterol levels, phospholipids and triglycerides in the blood, as well as the diastolic blood pressure. Their synthesis is now commonly accomplished by microbial fructosyltransferase (Ftase) enzyme which has gained parallel importance in food market. The present study intends to maximize the Ftase production of *A. stallus* by amending the nutritional, physical and cultivation conditions of the mold. The investigation of effect of physical parameters like incubation period, pH and temperature revealed the optimum conditions to be day 4 at pH 6.00 and 30$^0$C incubation temperature . Sucrose was the best utilized carbon source for Ftase production at 5 % concentration and supported 36.05±0.10 IU/ml of enzyme .Beef extract was optimally utilized at 2% as nitrogen source enhancing Ftase production to 36.05 ± 0.10 IU/ml. The study of additives revealed 123.33% increase in Ftase production due to $MgSO_4$. The introduction of shaking conditions increased the Ftase production to 68.73 ± 0.10 IU/ml.The total enhancement of Ftase production was 201.44%.**

**Keywords:** Fructooligosaccharides,, fructosyltransferase, batch fermentation and cultural amendments.

Fructooligosaccharides are now well established as excellent prebiotics in health market after receiving Gras status from FDA[1] .Naturally, their occurance in trace amounts is prominently found in onion, garlic, rye, wheat, tomatoes and animal products as honey[2]. However, another potential source is synthesis of FOS by employing microbial FTases which has been successfully attempted in past by many researchers[3-5].

Microbial Ftases synthesize FOS utilising sucrose as a sole substrate. The Ftase is classified as β-fructofuranosidases (EC 3.2.1.26).The transferase activity on sucrose is demonstrated only under high sucrose concentration[6-7].

The enzyme reaction mechanism in *A pullulans* [8] was elaborated as follows

Sucrose (G-F) + Sucrose (G-F) → Glu-fru-fru ($GF_2$) + Glu (released) step- 1

1-Kestose ($GF_2$) + Sucrose (G-F)n→Glu-fru-fru-fru ($GF_3$) + Glu (released) step-2

Nystose ($GF_3$) + Sucrose (G-F)n→Glu-fru-fru-fru-fru ($GF_4$) + Glu (released) step-2

($1^F$-fructofuranosylnystose)

The prebiotic properties of FOS are attracting immense attraction[9]. Moreover, the health benefits registered include lower cholesterol levels, phospholipids and triglycerides in the blood, as well as the diastolic blood pressure[3,10-11].

The experimental strategy for maximum synthesis of FOS has been varying from worker to worker. Rajoka and Yasmeen used gamma irradiation for strain improvement whereas some researchers cloned the Ftase genes in yeast for enhancement of the Ftase production[12]. The more

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practical approach followed by some researchers was enhancement of Ftase by optimization of the physico-cultural parameters of the enzyme production. Optimization was done either by experimental designing[13-15] or by single factor replacement method[16-17]. The present study aims at investigation of degree of increment in Ftase production by alterations in physico-chemical parameters and its potential for industrial applications.

## MATERIALS AND METHODS

**Isolation and maintainance of the mold**

The mold was isolated in the screening studies was observed to have Ftase production potential (18). It was morphologically identified to be *A. stallus* at Agharkar research institute, Pune. The culture is maintained on czapeckdox medium at $40^0$C. Subculturing is done after every 1 month.

**Preparation of the inoculum**

The innoculum was standardized to have $2.6 \times 10^6$ cfu conidia/ml for all the inoculations.

**Batch fermentation studies for production of Ftase**

The basal medium used for Ftase production contained 20 ml of basal medium was transferred to separate 100 ml cotton plugged conical flasks[19]. The flasks were then sterilized in an autoclave for 15 min at 121°C and cooled at room temperature. 0.8 ml inoculum was transferred to each flask. All the experiments were run parallel in triplicates.The filtrate after harvesting biomass was used as source of extracellular enzyme. The biomass was separated, washed and weighed.

**Ftase enzyme assay**

0.1 ml of crude enzyme was added to 1.0 ml of 50% sucrose solution and incubated for 1 h at 60°C. 2 ml of DNSA reagent was added to the reaction mixture (20). The enzymatic reaction was terminated by keeping the test tube at 100°C in a water bath for 10 min.

**Total protein content**

The total protein was assayed following Lowry *et al.* (21).

**Determination of Biomass**

The cell mass was determined by filtration of the fermentation broth, washed with distilled water and weighed.

**Statistical analysis**

All the experiments were repeated at least three times and the results were reproducible. The mean and standard errors were determined. The data was subjected to t- test and one way ANOVA using SPSS (16.0) software.

**Optimization of cultural and nutritional parameters for Ftase production**

Effect of incubation period, temperature and initial pH of the medium on ftase production

The effect of incubation period was investigated from day 1-day 10. The enzyme assay, total proteins and cell mass determination after every 24h interval till 10 days was performed.

The effect of incubation temperature was investigated at 10°C, 20°C, 30°C, 40°C, 50°C and 60°C on production of enzyme. The fermentation was terminated on day 4. The initial pH of the medium was adjusted to 3.0, 4.0, 5.0, 5.5, 6.0, 7.0 and 8.0 in the experimental setup.

**Qualitative and quantitative screening of carbon sources for optimum Ftase production**

The effect of replacement of sucrose by glucose, lactose, fructose, galactose, inulin and starch in the basal medium was investigated. Quantitative effect of the selected carbon source was also studied within a range of 1% to 10%.

**Qualitative and quantitative screening of nitrogen sources for optimum Ftase production**

Effect of different organic and inorganic nitrogen sources such as peptone, yeast extract, yeast extract + peptone, meat extract, urea, beef extract, ammonium nitrate, ammonium sulfate and potassium nitrate were evaluated for the optimum enzyme production. Quantitative effect of the selected nitrogen source was also studied within a range of 0.25% to 2.0% on Ftase production.

**Effect of additives, metal ions, vitamins and amino acids**

Various additives and metal ions (0.1%) were added to the basal medium to investigate its effect on Ftase production viz. calcium chloride, copper sulphate, zinc sulphate, ferric sulphate, tween-80, poly ethylene glycol, carboxy methyl cellulose, sodium dodecyl sulphate and ethylene di-amine tetra acetic acid was investigated. The effect of 0.01% vitamin solution supplemented in the basal media was investigated for biotin, pyridoxin-HCl, riboflavin, p-aminobenzoic acid and nicotinic acid. Similarly the effect of 0.01%

amino acid solution of alanine, aspartic acid, cysteine, cystine, glutamine, histidine, leucine, lysine, tyrosine and thiamine supplemeted in basal medium was investigated.

**Effect of agitation**

The effect of introduction of shaking condition (240rpm) on production of fructosyltransferase was investigated.

## RESULTS AND DISCUSSION

The optimization of enzyme production has been carried out by many workers either by single factor replacement or by experimental designing to increase the enzyme yield. The effect of incubation period, different temperatures, pH, carbon sources, nitrogen sources, different additives and shaking conditions were assessed on the production of Ftase by *A. stallus.*

**Effect of incubation period, temperature and initial pH of the medium on ftase production**

The study on effect of incubation period revealed day 4 was the best time for optimum Ftase production 22.80 ±0.10 IU/ml and specific activity of 18.59 ± 0.05IU/mg of protein.The details of enzyme produced on each day is given in Table -1.The biomass of *A. stallus* was recorded to be 1.15 ± 0.05 g/ 100 ml on the 1st day of incubation. The biomass increased up to the 4th day, the value being 33.20 ± 0.10 g/ 100 ml and remained nearly stable up to 6th day. A gradual decline was observed up to 10th day with the biomass recorded as 26.90 ± 0.15 g/ 100 ml (Table-1).

Figure-1a shows the results that temperature had a significant effect on extracellular fructosyltransferase production. At 10°C, no growth was observed and no extracellular fructosyltransferase production was recorded. The activity started increasing thereafter with a maximum value at 30°C (20.30 ± 0.03 IU/ mg protein) and then showed a decline at 40°C (15.16 ± 0.13 IU/ mg protein). However, further rise in temperature was inhibitory to the growth of *A. stallus.* No activity was recorded at 50°C and 60°C.

Different pH, ranging from 3.0 to 8.0, was selected to study the effect of pH on enzyme production (Figure -1b). The statistical analysis indicates that pH had a significant effect on extracellular fructosyltransferase production. The extracellular fructosyltransferase production increased with increase in pH up to 6.0 and gradually decreased with further increase in the pH up to 8.0. The optimum pH which supported maximum extracellular fructosyltransferase

**Table 1.** Effect of incubation period on extracellular fructosyltransferase production by *Aspergillus stallus*

| Period | Biomass(g) | Ftase (IU/ ml) | Protein(mg/ ml) | Ftase (IU/ mg protein) |
|---|---|---|---|---|
| Day 1 | 1.15 ± 0.05 | 0.18 ± 0.02 | 0.11 ± 0.002 | 6.81 ± 0.14 |
| Day 2 | 15.75 ± 0.15 | 12.88 ± 0.18 | 0.85 ± 0.002 | 15.14 ± 0.22 |
| Day 3 | 21.30 ± 0.05 | 18.57 ± 0.14 | 1.02 ±0.002 | 18.09 ± 0.14 |
| Day 4 | 33.20 ± 0.10 | 22.80 ± 0.10 | 1.22 ± 0.001 | 18.59 ± 0.05 |
| Day 5 | 33.15 ± 0.10 | 16.81 ± 0.10 | 0.96 ± 0.002 | 17.35 ± 0.09 |
| Day 6 | 33.15 ± 0.05 | 13.24 ± 0.31 | 0.74 ± 0.002 | 17.74 ± 0.36 |
| Day 7 | 26.25 ± 0.20 | 10.95 ± 0.10 | 0.70 ± 0.004 | 15.55 ± 0.23 |
| Day 8 | 26.20 ± 0.20 | 6.29 ± 0.10 | 0.68 ± 0.002 | 9.12 ± 0.18 |
| Day 9 | 26.15 ± 0.05 | 5.68 ± 0.10 | 0.62 ± 0.002 | 9.10 ± 0.13 |
| Day 10 | 26.90 ± 0.15 | 2.78 ± 0.10 | 0.60 ± 0.002 | 4.59 ± 0.15 |

**Table 2.** Effect of vitamins on extracellular fructosyltransferase production (IU/ mg protein) by *Aspergillus stallus*

| Vitamins(0.01%) | Ftase(IU/ mg protein) | Vitamins(0.01%) | Ftase(IU/ mg protein) |
|---|---|---|---|
| Control | 20.08 ± 0.17 [a] | Riboflavin | 19.12 ± 0.12[c] |
| Biotin | 18.11 ± 0.11[d] | p-amino benzoic acid | 18.12 ± 0.11[d] |
| Pyridoxin-HCl | 19.23 ± 0.10[b] | Nicotinic acid | 19.23 ± 0.12[b] |

Mean within a column with different lowercase letter are statistically significant (P<0.05)

activity of 20.32 ± 0.11 IU/ mg protein was 6.0. The minimum extracellular fructosyltransferase production was registered at pH 3.0 of 17.18 ±0.12 IU/ mg protein. At pH 8.0, the activity was 15.35 ± 0.16 IU/ mg protein.

**Qualitative and quantitative screening of carbon sources for optimum Ftase production**

Five different carbon sources *i.e.* fructose, glucose, maltose, inulin and starch were substituted in place of sucrose in the basal medium and fructosyltransferase activity was assayed in culture filtrate. The effect of different carbon sources on extracellular production of fructosyltransferase by *A. stallus* was investigated and recorded in Figure 2(primary axis). The maximum activity was recorded in medium containing sucrose as sole source of carbon. The extracellular fructosyltransferase production was 20.32 ± 0.10 IU/ mg protein. The minimum fructosyltransferase production was recorded to be 16.10 ± 0.20 IU / mg protein with glucose as sole source of carbon in the medium.

The result on the effect of optimum concentration of sucrose in the culture medium for extracellular fructosyltransferase production is given in Figure 2(Secondary axis). The minimum fructosyltransferase production was recorded at 1% sucrose concentration to be 20.32 ± 0.10 IU/ mg protein. The optimum concentration of sucrose was found 5% at which the extracellular fructosyltransferase production increased to 23.57 ± 0.10 IU/ mg protein .

**Qualitative and quantitative screening of nitrogen sources for optimum Ftase production**

The effect of different organic and inorganic nitrogen sources were investigated on extracellular fructosyltransferase production by the four selected fungi. The sources experimented were 0.25% of yeast extract, beef extract, peptone, yeast extract (YE) + peptone, potassium nitrate, ammonium sulphate, ammonium phosphate and ammonium chloride were used to replace sodium nitrate in the basal medium (Figure-3). The preferred nitrogen source for Ftase production was found to be the beef extract, the value being 26.01 ± 0.12 IU/ mg protein. The optimum activity was supported at 2.0% of beef extract in the culture medium (29.40 ± 0.08 IU/ mg protein). However, the production did not increase with further increase in the concentration of beef extract.

**Effect of additives, metal ions, vitamins and amino acids**

Effect of additives supplemented in trace amounts on the Ftase production was investigated.

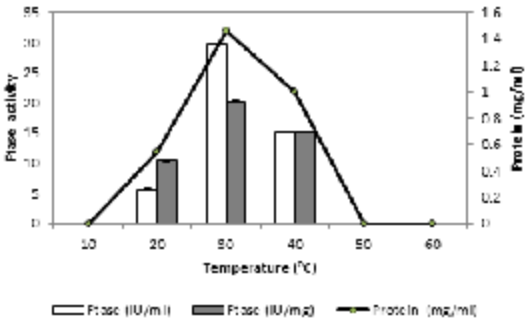



**Fig. 1a.** Effect of temperature on extracellular fructosyltransferase production by *Aspergillus stallus.* Values with different alphabet labels are statistically significant at p<0.05

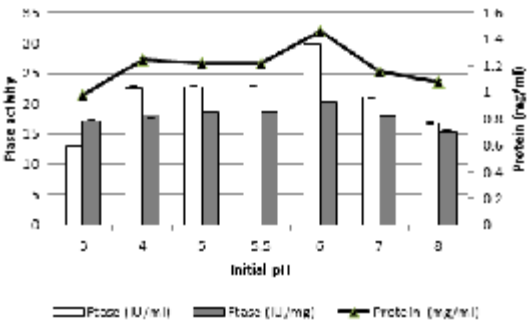



**Fig. 1b.** Effect of initial pH on extracellular fructosyltransferase production by *Aspergillus stallus.* Values with different alphabet labels are statistically significant at p<0.05

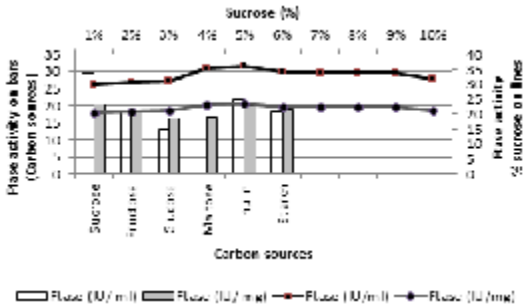
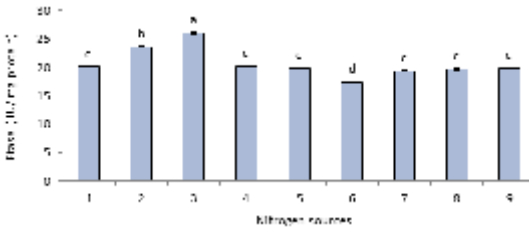
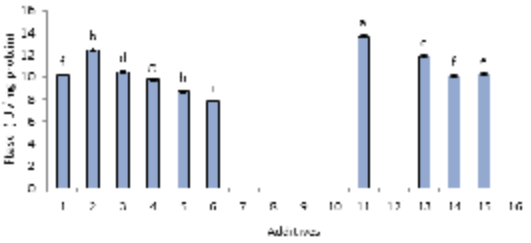



**Fig. 2.** Effect of different carbon sources on extracellular Fructosyltransferase production by *Aspergillus stallus.* Values with different alphabet labels are statistically significant at p<0.05



(1- Yeast extract, 2- Sodium nitrate, 3- Beef extract, 4- Peptone, 5- YE + Peptone, 6- Potassium nitrate, 7- Ammonium sulphate, 8- Ammonium phosphate, 9- Ammonium chloride)

**Fig. 3.** Effect of different nitrogen sources on extracellular fructosyltransferase production by *Aspergillus stallus.* Values with different alphabet labels are statistically significant at p<0.05. (legend as per figure-3)



(1-Control, 2-Magnesium sulphate, 3-Maganese sulphate, 4-Calcium chloride, 5-Copper sulphate, 6-Zinc sulphate, 7-Ferric sulphate, 8-Tween 80, 9-Tween 20, 10-Polyethylene glycol, 11-CMC, 12-SDS, 13-Magnesium chloride, 14-Potassium chloride, 15-Sodium chloride, 16-EDTA)

**Fig. 4.** Effect of different additives on extracellular fructosyltransferase production by *Aspergillus stallus.* Values with different alphabet labels are statistically significant at p<0.05. (legends as per figure-4)

The effect of additives like magnesium sulphate, manganese sulphate, calcium chloride, copper sulphate, zinc sulphate, ferric sulphate, tween-80, tween-20, polyethylene glycol, carboxy methyl cellulose, sodium dodecyl sulphate, magnesium chloride and potassium chloride was investigated (Figure-4). Also the effect of vitamins like, biotin, pyridoxin-HCl, riboflavin, p-aminobenzoic acid and nicotinic acid was determined on Ftase production. The effect of amino acids like, alanine, aspartic acid, cysteine, cystine, glutamine, histidine, lysine, tyrosine, thiamine, leucine and lysine supplemented in the basal medium was investigated. A statistically significant positive response was seen by addition of magnesium sulphate, manganese sulphate, sodium chloride, carboxy methyl cellulose, magnesium chloride and potassium chloride which exhibited increase in the production of fructosyltransferase. The highest

**Table 3.** The effect of amino acids on extracellular Fructosyltransferase production (IU/ mg protein) by *A. stallus.*

| Amino acid (0.01%) | Ftase(IU/ mg protein) | Amino acid (0.01%) | Ftase(IU/ mg protein) |
|---|---|---|---|
| Control | $20.08 \pm 0.17^{a}$ | Histidine | $12.21 \pm 0.10^{h}$ |
| Alanine | $15.23 \pm 0.11^{f}$ | Lysine | $17.23 \pm 0.02^{d}$ |
| Aspartic acid | $19.33 \pm 0.23^{b}$ | Tyrosine | $16.22 \pm 0.13^{e}$ |
| Cysteine | $13.22 \pm 0.14^{g}$ | Thiamine | $15.26 \pm 0.11^{f}$ |
| Cystine | $12.23 \pm 0.14^{h}$ | Leucine | $18.88 \pm 0.10^{c}$ |
| Glutamine | $11.22 \pm 0.12^{i}$ | Lysine | $15.23 \pm 0.12^{f}$ |

Mean within a column with different lowercase letter are statistically significant (P<0.05)

**Table 4.** Effect of shaking on production of extracellular fructosyltransferase by *A. stallus*

| Organism | Condition | Ftase(IU/ ml) | Protein(mg/ ml) | Ftase(IU/ mg protein) |
|---|---|---|---|---|
| *Aspergillus stallus* | Static | 53.11 ± 0.10 | 1.81 ± 0.002 | 29.40 ± 0.08 |
| | Shaking | 68.73 ± 0.10 | 1.91 ± 0.002 | 35.49 ± 0.11** |

Superscripts on values exhibits statistical significance between static and shaking conditions in the organism: *(P<0.05); ** (P<0.01) and *** (P<0.001)

relative activity was found to be 135.26% under the influence of carboxy methyl cellulose.

**Influence of Vitamins**

The vitamins could not influence the enzyme activity in A. *stallus*

**Influence of amino acids**

The effect of amino acids (0.01%) like alanine, aspartic acid, cysteine, cystine, glutamine, histidine, lysine, tyrosine, thiamine, leucine and lysine supplemented in the basal medium was investigated for Ftase production by the fungal isolates. None of the tested amino acids supported high enzyme production as compared from the control.

**Influence of shaking on production of extracellular fructosyltransferase**

The effect of shaking on fructosyltransferase enzyme production is presented in Table-4. *A. stallus* exhibited a rise of fructosyltransferase production from static (29.40 ± 0.08 IU/ mg protein) to shaking (35.49 ± 0.11 IU/ mg protein) condition.

## DISSCUSSION

The increase in biomass production was found to be associated with Ftase production in the study on *Aureobasidium pullulans* by Yun *et al*[22]. The present study also report similar observations of increase in Ftase production parallel to biomass up to day 4 of incubation. The growth pattern was found normal along with time period of incubation. The incubation period studied for 10 days revealed maximum extracellular Ftase on day 4, pH 6.0 and temperature $30^0$C. However, Patil and Butle reported optimum pH to be 5.5 for *Syncephalastrum recemosum Cohn*[23]. The pH of the medium is critical since it determines the ionic sate of the nutrients and hence their solubility in the medium and absorbtion by the catalysing organism[16] .

Sucrose was found to be the best utilized source by almost all researchers as in case of the present study. Sucrose in the medium induces the production of Ftase by the microbe. The concentration of sucrose greatly influenced the Ftase production[6-7]. 5% sucrose was found to be indusive for Ftase above which the Ftase production suffered a decline upto 10 %.

Organic sources of nitrogen like beef extract supplemented the growing organisms with growth factors naturally and supported maximum enzyme yield. In contrast, Urea was reported to be the best supporting nitrogen source for ftase with 110% increase in Ftase production has been reported by Ottoni *et al.,*[24]. Shaking was reported to be ineffective by Dhake and Patil[19] where as in present study it increased the yield by 29.41%. The optimization increased the overall production of Ftase up to 201.44 % which will have promising results during scale up process.

## CONCLUSION

It is therefore concluded that *A. stallus* used in this study for expression of extracellular Ftase exhibited a much higher positive response to the optimising parameters as compared to the previous reports, with a final hike of 201.44 % in Ftase production. The results are promising for scaling up process in a bioreactor with much higher Ftase production desirable for industrial usage.

## REFERENCES

1. Godshall, M. A. Future directions for the sugar industry. http://www.spriinc.org/buton10bftpp.html. 2007 Cited Dec 07
2. Sangeetha, P. T., Ramesh, M. N., Prapulla, S. G. Recent trends in the microbial production, analysis and application of fructooligosaccharides. *Trends Food Sci Technol*., 2005;**16**: 442–457.
3. Katapodis, P., Kalogeris, E., Kekos, D., Macris, B.J. Biosynthesis of fructo-oligosaccharides by Sporotrichum thermophile during submerged batch cultivation in high sucrose media. *Appl. Microbiol. Biotechnol.,* 2004; **63**:378–382.
4. Lim, J. S., Lee, J. H., Kim, J. M., Park, S.W., Kim, S.W. Effects of morphology and rheology on neo-fructosyltransferase production by Penicillium citrinum. *Biotec. Bioprocess . Eng.,* 2006; **11**:100–104.
5. Barbosa, G. N. O. Production of fructosyltransferase by *Rhodotorula sp*. Master of Science, University of Campinas, Sao Paulo, Brazil.,2007.
6. Chen, W. C., Liu, C. H. Production of b-fructofuranosidase by *Aspergillus japonicus. Enzyme. Microb. Technol*., 1996; **18**:153–160.
7. Yun, J. W. Fructooligosaccharides-occurrence, preparation, and application. *Enzyme. Microb. Technol.,* 1996; **19**: 107–117.

8. Jung, K.H., Yun, W. J., Kang, K.R., Lim, J.Y., Lee, J.H. Mathematical model for enzymatic production of fructooligosaccharides from sucrose. *Enzyme Microb. Technol.,* 1989*;* **11**: 491–494
9. Courtois, J. Oligosaccharides from land plants and algae: production and applications in therapeutics and biotechnology.*Curr. Opinion in Microbiol*., 2009; 261-273.
10. Nguyen, Q. D., Rezessy-Szabó, J. M., Bhat, M. K., Hoschke, A. Purification and some properties of β-fructofuranosidase from *Aspergillusniger* IMI303386. *Process Biochemistry, 2005;* 2461-2466. (DOI: 10.1016/j.procbio.2004.09.012)
11. Maiorano, A. E., Piccoli, R. M., Silva, E. S., Rodrigues, M. F. A. Microbial production of fructosyltransferases for synthesis of prebiotics. *Biotechnology* Letters., 2008; **30**: 1867-1877.
12. Rajoka, M. I., Yasmeen, A. Improved productivity of bfructofuranosidase by a derepressed mutant of Aspergillus niger from conventional and non-conventional substrates. *World J . Microbiol . Biotechnol*., 2005; **21**:471–478
13. Sangeetha, P. T., Ramesh, M.N., Prapulla, S.G. Maximization of fructooligosaccharide production by two stage continuous process and its scale up. *J Food Eng* ., 2005; **68**:57–64.
14. Vandakova, M., Platkova, M., Antosova, M., Bales, V., Polakovic, M. Optimization of cultivation conditions for production of fructosyltransferase by *Aureobasidium pullulans. Chem . Pap .,* 2004*;* **58**(1):15–22.
15. Chen, W. C. Production of b-fructofuranosidase by Aspergillus japonicus in batch and fed-batch cultures. *Biotechnol. Lett, ,* 1995; **17**:1221–1924.
16. Kim, B. W., Kwon, H. J., Park, H. Y., Nam, S.W., Park, J. P. Yun, J. W. Production of a novel transfructosylating enzyme from *Bacillus macerans* EG–6. *Bioprocess Engineering*., 2001; **23**: 11-16.
17. Wang, L. M., Zhou, H. M. Isolation and identification of a novel *Aspergillus japonicus JN19* producing b-fructofuranosidase and characterization of the enzyme. *J . Food Biochem* ., 2006; **30**:641–658.
18. Belorkar, S. A., Gupta, A. K. Rai, V. Screening of microbial isolates for extracellular fructosyltransferase production. *Afri. J. of Microbio. Rese.*, 2015; **9**: 730-735.
19. Dhake, A. B., Patil, M. B. Effect of substrate feeding on production of fructosyltransferase by *Penicillium purpurogenum. Braz. J . Microbiol*., 2007; **38**: 194-199.
20. Miller, G. L. Use of di-nitro salicylic acid reagent for determination of reducing sugar. *Analytical Chemistry., 1959;* 31, 426-428.
21. Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R. J. Protein measurement with the folin phenol reagent. *J. Biol. Che.,* 1959; **193**: 265-275.
22. Yun, J. W., Kim, D. H., Kim, B.W., Song, S. K. Comparison of sugar compositions between inulo- and fructo-oligosaccharides produced by different enzyme forms. *Biotechnol. Lett.,2008;* 19:553–556.
23. Patil, M. B., Butle, A. Fructosyltransferase production by indigenously isolated *syncephalastrum racemosum cohn .Journal of Global Biosciences* ., 2014; **3**; 597-603.
24. Ottoni, C. A., Fernandez, C., Piccoli, R. M., Moreira, R., Maresma, B. G., Silva, E. S. D., Rodrigues, M. F. A., Mairano, A. E. Media optimization for â-Fructofuranosidase production by *Aspergillus oryzae. Braz. J. Chem. Eng*., 2012; **29**: 49-59 . http://dx.doi.org/10.1590/S0104-66322012000100006 .

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