academicJournals

Vol. 9(10), pp. 730-735, 11 March, 2015
DOI: 10.5897/AJMR2014.7346
Article Number: DCC968C51326
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Screening of microbial isolates for extracellular fructosyltransferase production

Seema A. Belorkar*, A. K. Gupta and Vibhuti Rai

SOS in Life Sciences, Pt. Ravishankar Shukla University, Raipur-492010 (C.G), India.

Received 22 December, 2014; Accepted 9 February, 2015

Fructooligosaccharides are gaining importance by leaps and bounce every day due to their low caloric value and accompanying health benefits for the consumer. Hence, newer potential sources for the fructosyltransferase (Ftase) enzyme are thoroughly scrutinized. The present work was carried out for preliminary and secondary screening of the microbial isolates for determination of the Ftase producing potential. Three classes of microorganisms' viz. molds, yeasts and bacterial isolates were qualitatively screened. The preliminary screening revealed molds as the most potent group exhibiting greater zone of hydrolysis in the range of 0.30 ± 0.10 to 2.58 ± 0.10 cm. The secondary screening of 20 selected mold isolates was performed in liquid batch culture. Four isolates identified as *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus stallus and Aspergillus versicolor* exhibited higher fructosyltransferase : invertase ratio which is most critical for the synthesis of fructooligosaccharides due to higher transferase activity and low hydrolytic activity. *A. niger* exhibited highest Ftase activity, 36.88 ± 0.23 IU/mg. *A. flavus* produced 21.45 ± 0.33 IU/mg of Ftase. *A. stallus* produced 18.09 ± 0.14 IU/ mg and *A. versicolor* was found to produce 23.78 ± 0.12 IU/mg of Ftase without any cultural medium optimization.

Key words: Prelimnary screening, secondary screening, fructosyltransferase, fructooligosaccharides.

INTRODUCTION

Functional foods like fructooligosaccharides are gaining prime importance in the health market because they provide multiple health benefits like low caloric value, promoting growth of *Bifidobacteria* in colon (prebiotic property), low glycemic index and low carcinogenicity in aid of basic nutrition (Dominguez et al., 2013; Moore et al., 2003 and Slevin et al., 2014). The most popular functional food coming into the lime light is fructo-oligosaccharides (FOS) obtained from sucrose.

FOS are oligosaccharides of fructose containing single glucose unit. They are produced by action of fructosyl-transferase (Ftase) on sucrose. FOS are mainly composed of 1-kestose, nystose and β- fructofuranosyl nystose (2→1) position of sucrose (Sangeetha et al., 2005).

The sucrose is biologically transformed into FOS either by using microorganism itself (Chien et al., 2001; Sanchez et al., 2010) or by enzymes derived from them (Fawkia et al., 2009).

Scientists are constantly attempting to isolate new strains of microorganisms having higher potential for production of the enzyme. Since screening is an elaborate time consuming and tedious process, very few attempts have been made to indigenously isolate the

*Corresponding author. E-mail: seema.belorkar@gmail.com. Tel: +919826196914.

microbe from environment and subject them to screening (Madlova et al., 1999; Reddy et al., 2010; Fernandez et al., 2007). The objective of the present study was to screen the filamentous fungi, yeast and bacteria to enable their usage in industries.

MATERIALS AND METHODS

Preliminary screening of the isolates

Thirty one yeasts, forty two bacteria and fifty four molds isolated from previous work (Belorkar et al., 2013) were selected for prelimnary screening on basis of their colony size as compared to their counterparts in the plates containing selective medium (sucrose- 30.0, $NaNO_{3-}$ 2.5, $MgSO_4.7H_2O$-0.5, KH_2PO_4-1.5 and Agar agar powder -20 at pH 5.50). The basal medium (20 ml in a 100 ml Erlenmeyer flask) used for cultivation of fungi during prelimnary screening contained (g/L): Sucrose- 10.0, $NaNO_{3-}$ 2.5, $MgSO_4.7H_2O$ -0.5 and KH_2PO_4-1.5 at pH 5.50.

Screening for yeast isolates

The modified method of Maugeri and Heernalsteens (2007) was followed in which the medium was replaced by the above defined basal medium at pH 5.50. Loop full of cells were inoculated into 10 ml of basal medium in test tubes and cultivated at 25°C for 24 h. After adequate development of the cultures, they were transferred into 500 ml flasks containing a further 100 ml of the same medium, and cultivated at 25°C for 72 h .The cells were removed by centrifugation at 6000 ×*g* (10 min, 4°C), and the cell-free supernatants were used to screen for transfructosylating activity by plate method containing selective medium. The conditions maintained were for support of fructosyltransferase production.

Screening for molds isolates

The molds were cultivated on basal medium at pH 5.5 with sucrose as the substrate. Screening was carried out under static conditions at 28°C for 72 h. All the experiments were conducted in triplicates. The supernatant was centrifuged at 6000 ×*g* (10 min, 4°C). The cell-free supernatant was used to screen for transfructosylating activity by plate method containing selective medium.

Preliminary screening of Ftase production by fungi

Wells were made aseptically by using cock borer in the Petri plate containing selective media (sucrose- 30.0, $NaNO_{3-}$ 2.5, $MgSO_4.7H_2O$ -0.5, KH_2PO_4-1.5 and Agar powder -20 at pH 5.50). Two hundred microliter of cell-free supernatants of the yeast and mold cultures were individually (crude enzyme) loaded into the well and kept for incubation at 28°C for 12 h. Staining was carried out by spraying triphenyltetrazolium chloride (TTC) reagent to the agar plate and kept for incubation of 20 min in the dark. After washing with the 0.1 M acetate buffer (pH-5), the extracellular production of fructosyltransferase was confirmed by the appearance of the red zone (measured in cm) around the well according to the protocol reported by Reddy et al. (2010).

Screening for bacteria

To select strains of bacteria, the isolates were plated in selective media plates and incubated for 48 h and subjected to TTC staining directly to measure the zone of hydrolysis described by Kim et al. (2000).

Secondary screening

Assay of fructosyltransferase activity

Quantitative assay of fructosyltransferase was based on the procedure used by Yun et al. (1997). The filterate was taken as a crude enzyme with 50% sucrose solution as a substrate at 5.50 pH (0.1 M sodium acetate buffer). The mixture was incubated for 1 h at 60°C. The reducing sugars were estimated by Dinitro-salicylic acid reagent. The enzymatic reaction was terminated by keeping the test tube at 100°C in a water bath for 10 min. One unit of enzyme activity was defined as the amount of enzyme producing 1 µmol of glucose under experimental conditions.

Assay of hydrolytic activity

Sucrose hydrolytic activity was measured according to the method described by Sangeetha et al. (2003). The filtrate was taken as a crude enzyme with 0.50% sucrose solution as a substrate at 5.50 pH (0.1 M sodium acetate buffer). The mixture was incubated for 1 h at 60°C. The reducing sugars were estimated by dinitro-salicylic acid reagent. The enzymatic reaction was terminated by keeping the test tube at 100°C in a water bath for 10 min. One unit of sucrose hydrolytic activity was considered as the amount of enzyme required to produce 1 µmol of glucose under experimental conditions.

Determination of protein concentration

The protein content was determined following Lowry et al. (1951).

Biomass concentration

Biomass concentration was determined in g/100 ml volume of fermentation medium. The biomass obtained by filtration of the fermentation broth was washed thrice with distilled water, air dried and weighed.

Statistical analysis

All the experiments were conducted in triplicate. The results were expressed in Mean ± SE. One-way ANOVA using SPSS 16.0 was performed to analyze the variance in preliminary screening.

Identification of the selected isolates

These four isolates were then identified morphologically by lactophenol cotton blue staining and found to be SSFM1 as *Aspergillus niger* NFCCI2736, SSFM2 as *Aspergillus flavus* NFCCI2734, SAFM30 as *Aspergillus stallus,* RSCDS48 as *Aspergillus versicolor gr.*

RESULTS AND DISCUSSION

Preliminary screening

Thirty one (31) yeast isolates, forty two bacterial isolates

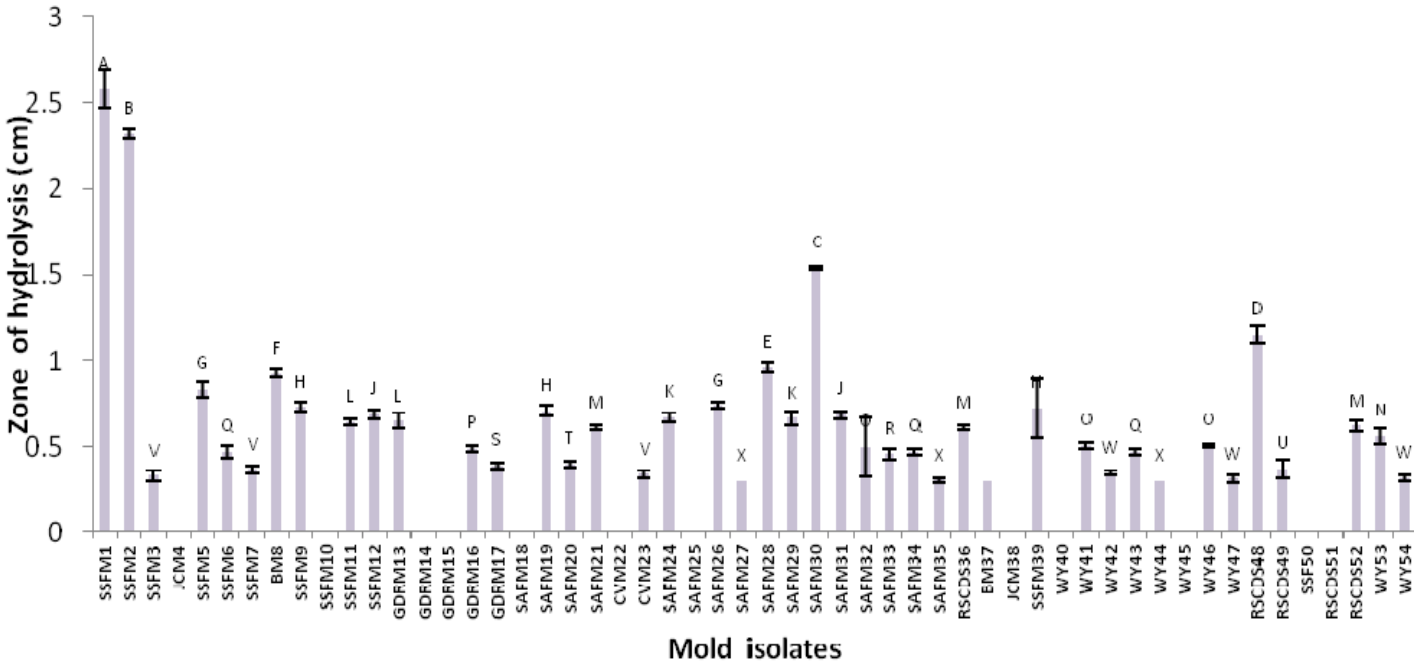

Figure 1A. Zone of hydrolysis (cm) for mold isolates. Same legends represent the zone of hydrolysis of the isolates not significantly different.

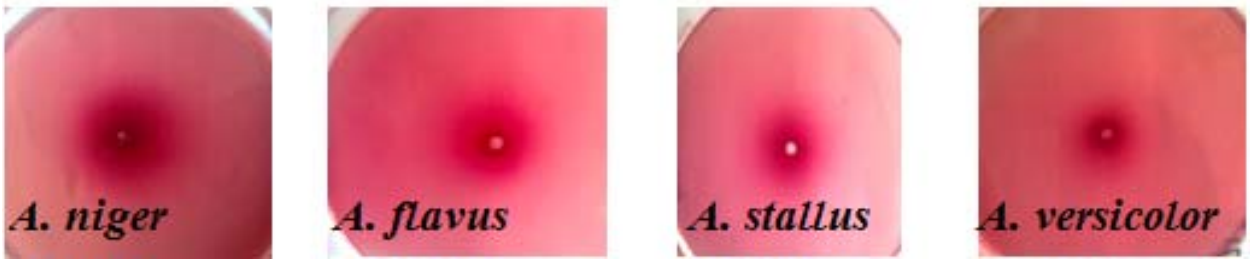

Figure 1B. Zone of hydrolysis exhibited by identified isolates during preliminary screening. Same legends represent the zone of hydrolysis of the isolates not significantly different.

and 54 molds isolated during the previous study were subjected to prelimnary screening. The zone of hydrolysis for molds, yeasts and bacteria are presented in Figures 1A, 2 and 3, respectively. The mold isolates proved to be the most potential Ftase producers. The zone of hydrolysis developed after TTC staining is represented in Figure 1B

The mold isolates exhibited highest zone of hydrolysis ranging from 0.30 ± 0.10 to 2.58 ± 0.10 cm. The largest zone of hydrolysis was recorded for isolate SSFM1 (2.58 ± 0.10 cm) and smallest zone was recorded for SAFM 27, SAFM 35, BM 37 and WY 44. The zone of hydrolysis recorded for molds was found to be similar to the findings of Reddy et al. (2010). Yeasts have been consistently reported to be potent producers of Ftase since the pioneering work of Bacon and Edelman (1950) investigating action of invertase on sucrose. Similarly, isolation of potent bacterial strains like *Psuedomonas* sp. No. 65 from soil has been reported by Kim et al. (1997).

The zone of hydrolysis for yeast isolates was found to be in the range of 0.15 ± 0.01 to 0.58 ± 0.01 cm as given in Figure 2. The maximum zone of hydrolysis was exhibited by RSCDS 24, the value being to 0.58 ± 0.01 cm. The zone of hydrolysis for bacterial isolates was found to be in the range of 0.12 ± 0.01 to 0.54 ± 0.10 cm. The maximum zone of hydrolysis exhibited was SSFB2, the value being 0.54 ± 0.10 cm as given in Figure 3. The bacterial and yeast isolates exhibited less potential for fructosyltransferase production as compared to results of Kim et al, (1997) and Maugeri and Hernalsteens (2007), respectively .

In this study, highest range of zone of hydrolysis was found to be of filamentous fungi among the three classes of microbes, similar to the results reported by Yun (1996).

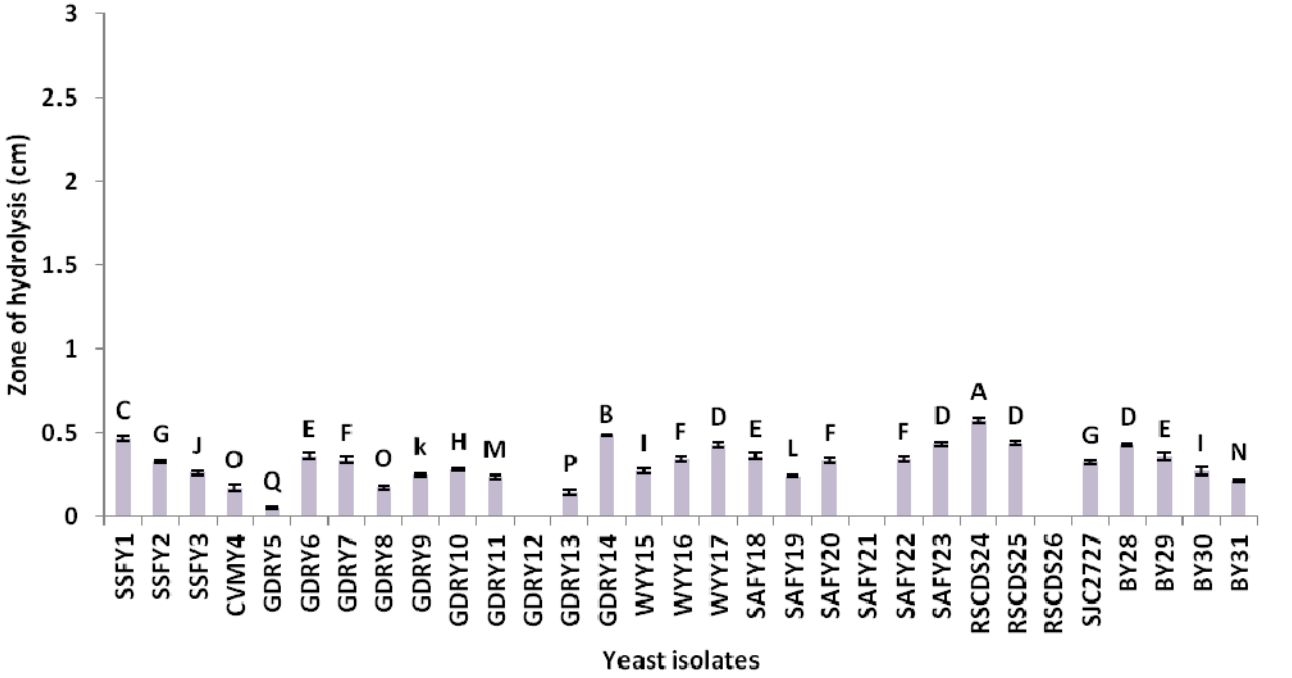

Figure 2. Zone of hydrolysis in cm for yeast isolates. Same legends represent the zone of hydrolysis of the isolates not significantly different.

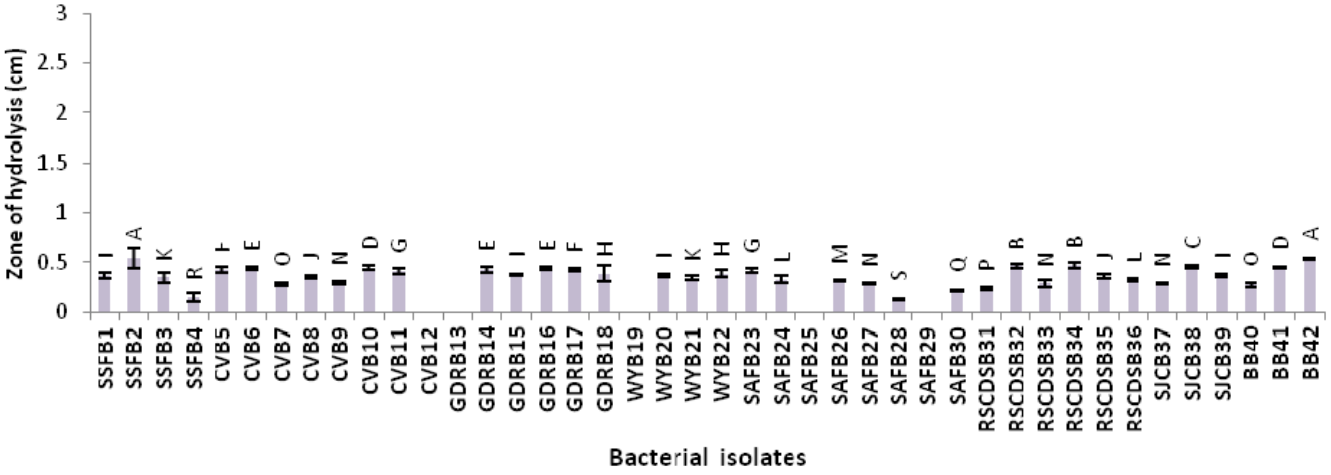

Figure 3. Zone of hydrolysis in cm for bacterial isolates. Same legends represent the zone of hydrolysis of the isolates not significantly different.

The filamentous fungi were therefore, selected for secondary screening in this study as well.

Secondary screening for determination of extracellular Ftase and invertase production

The comparison of zone of hydrolysis of the three microbial groups was subjected to preliminary screening directed in the present study towards selection of molds for secondary screening. Twenty (20) mold isolates were selected for secondary screening in liquid basal medium depending upon their zone of hydrolysis namely SSFM1, SSFM2, SSFM5, BM8, SSFM9, SSFM11, SSFM12, GDRM13, SAFM19, SAFM21, SAFM24, SAFM26, SAFM28, SAFM29, SAFM30, SAFM31, RSCDS36, SSFM39, RSCDS48 and RSCDS52.

The average values of biomass production, Ftase production and invertase (Inv) production by the isolates are presented in Table 1. The most vital parameter in screening experiments for Ftase production is the Ftase :

Table 1. Biomass production, extracellular fructosyltransferase (Ftase) and Invertase (Inv) activity reached in liquid batch fermentation under static condition.

Isolate	Ftase (IU/ml)	Inv (IU/ml)	Protein (mg/ml)	Ftase (IU/mg protein)	Inv (IU/mg protein)	Ftase/Inv ratio	Biomass (g/ 100 ml/ 72 h)
SSFM1*	35.98 ± 0.10	22.02 ± 0.10	0.98 ± 0.006	36.88 ± 0.23	22.56 ± 0.26	1.63	27.60 ± 0.15
SSFM2*	20.39 ± 0.28	13.49 ± 0.10	0.95 ± 0.006	21.45 ± 0.33	14.19 ± 0.07	1.51	17.85± 0.05
SSFM5	7.87 ± 0.10	12.40 ± 0.10	1.25 ± 0.002	6.30 ± 0.07	9.92 ± 0.06	0.63	9.25 ± 0.20
BM8	8.53 ± 0.31	10.22 ± 0.10	1.20 ± 0.002	7.12 ± 0.25	8.53 ± 0.09	0.83	9.70 ± 0.15
SSFM9	6.90 ± 0.18	15.36 ± 0.10	1.32 ± 0.004	5.21 ± 0.12	11.60 ± 0.11	0.44	8.60 ± 0.15
SSFM11	4.42 ± 0.27	21.96 ± 0.18	1.18 ± 0.002	3.71 ± 0.24	18.59 ± 0.15	0.20	7.65 ± 0.55
SSFM12	5.63 ± 0.18	16.33±0.18	1.06 ± 0.004	5.33 ± 0.19	15.46 ± 0.23	0.34	7.40 ± 0.15
GDRM13	5.81 ± 0.18	8.77 ± 0.10	1.35 ± 0.006	4.31 ± 0.14	6.51 ± 0.08	0.66	7.60 ± 0.15
SAFM19	6.78 ± 0.10	19.24 ± 0.18	1.37 ± 0.006	4.93 ± 0.07	13.99 ± 0.19	0.35	7.85 ± 0.15
SAFM21	5.14 ± 0.10	6.23 ± 0.10	0.96 ± 0.006	5.36 ± 0.07	6.49 ± 0.13	0.82	7.30 ± 0.20
SAFM24	5.69 ± 0.10	25.83 ± 0.10	1.12 ± 0.002	5.07 ± 0.09	23.03 ± 0.12	0.22	7.70 ± 0.05
SAFM26	6.96 ± 0.10	13.13 ± 0.10	1.08 ± 0.004	6.42 ± 0.07	12.11 ± 0.06	0.53	8.75 ± 0.25
SAFM28	9.74 ± 0.10	16.82 ± 0.10	1.34 ± 0.004	7.27 ± 0.09	12.55 ± 0.08	0.58	9.75 ± 0.20
SAFM29	5.38 ± 0.10	7.139 ± 0.10	1.02 ± 0.006	5.29 ± 0.12	7.00 ± 0.14	0.75	7.40 ± 0.20
SAFM30*	18.57 ± 0.10	13.67 ± 0.10	1.03 ± 0.006	18.09 ± 0.14	13.31 ± 0.13	1.36	21.30 ± 0.10
SAFM31	1.86 ± 0.18	5.76 ± 0.10	1.25 ± 0.004	1.50 ± 0.13	4.65 ± 0.15	0.32	8.05 ± 0.40
RSCDS36	5.14 ± 0.10	10.67 ± 0.10	1.23 ± 0.004	4.20 ± 0.10	8.75 ± 0.09	0.47	7.30 ± 0.16
SSFM39	6.23 ± 0.10	15.55 ± 0.10	1.09 ± 0.004	5.73 ± 0.11	14.31 ± 0.08	0.40	8.25 ± 0.16
RSCDS48*	29.65 ± 0.10	28.37 ± 0.10	1.25 ± 0.002	23.78 ± 0.12	22.76 ± 0.12	1.04	28.30 ± 0.02
RSCDS52	5.20 ± 0.10	16.58 ± 0.10	1.02 ± 0.002	5.12 ± 0.09	16.27 ± 0.13	0.31	7.30 ± 0.20

All values presented in the table are mean ± SE. *Identified isolates (SSFM1 identified as *Aspergillus niger*; SSFM2 identified as *A. flavus*; SAFM 30 identified as *A. stallus*; RSCDS 48 identified as *A. versicolor*).

Inv ratio (Hidaka et al., 1988; Hidaka 1988). The Ftase : Inv ratio was also determined for each mold and was found to be desirable in SSFM1 (1.63), SSFM2 (1.51), SAFM30 (1.36) and RSCDSM48 (1.04). In the earlier screening experiments, the ratio was reported between 0.3-15.9 (Fernandez et al., 2007; Patel et al., 1994; Reddy et al., 2010). The ratio determines the efficacy of transferase activity for synthesis of FOS. The extracellular Ftase production has been reported to be in the range of 0.053 IU to 660 IU/ml (Maiorano et al., 2008).

The four molds selected from the twenty molds subjected to secondary screening which exhibited high Ftase/Inv ratios was recorded for SSFM1 (1.63), SSFM2 (1.51), SAFM30 (1.36) and RSCDS48 (1.04).

All the 20 mold isolates selected exhibited Ftase production to varying degree ranging from 3.71 ± 0.24 to 36.88 ± 0.23 IU/mg. *A. niger* exhibited highest Ftase activity, 36.88 ± 0.23 IU/mg. *A. flavus* produced 21.45 ± 0.33 IU/mg of Ftase. *A. stallus* produced 18.09 ± 0.14 IU/ mg and *A. versicolor* was found to produce 23.78 ± 0.12 IU/ mg of Ftase.

Conclusion

As FOS has already been given GRAS status, the molds will be promising tool for production of Ftase enzyme for synthesis of FOS. The results of the present screening experiments revealed microbes especially molds to be source exhibiting potential for Ftase production which is gaining importance in food industry for synthesis of prebiotics. The urge of researchers to search for newer Ftase producing microbes can only be quenched with such screening experiments. The present study revealed higher Ftase : Inv ratio of the isolates which can be even enhanced upon optimization of cultural medium. Further optimization experiments will be carried out to boost the Ftase production levels for maximization of FOS synthesis.

Conflict of interests

The authors have not declared any conflict of interests.

REFERENCES

Bacon JSD, Edelman J (1950). The action of invertase preparations. Arch. Biochem. 28: 467-468.

Belorkar SA, Rai V, Gupta AK (2013). Isolation of potential microbial producers of Fructosyl transferase from Baggasse and selected soil sites of Chhattisgarh. Asian. J. Microbiol. Biotech. Environ. Sci.15:785-788.

Chien CS, Lee WC, Lin TJ (2001). Immobilization of *Aspergillus japonicus* by entrapping cells in gluten for production of fructooligo-

saccharides. Enzyme Microb. Technol. 29: 252-257.
Dominguez AL, Rodrigues LR, Lima NM, Teixeira JA (2013). An overview of the recent developments on fructooligosaccharide production and applications. Food Bioprocess Technol .DOI 10.1007/s11947-013-1221-6.
Fawkia M, El B, Azza M, Abdel F, Doaa A, Mostafa FA, Ahmed FA (2009). Production and some properties of fructosyl transferase from *Bacillus subtilis*. J. Appl. Sci. Res. 5: 1132-1141.
Fernandez RC, Ottoni CA, Da Silva ES, Matsubara RMS, Carter JM (2007). Screening of β-fructofuranosidase-producing microorganisms and effect of pH and temperature on enzymatic rate. Appl. Microbiol. Biotechnol. 75:87-93.
Hidaka H (1988). Fructooligosaccharides. In: The Amylase Research Society of Japan (ed) Handbook of amylases and related enzymes. Pergamon. pp. 228-230.
Hidaka H, Hirayama M, Sumi N (1988). A fructooligosaccharide producing enzyme from *A. niger* ATCC 20611. Agric. Biol. Chem. 52:1181-1187.
Kim BW, Kwon HJ, Park HY, Nam SW Park JP, Yun JW (2000). Production of novel transfructosylating enzyme from *Bacillus macerans EG -6*. Biopro. Eng. 23:11-16.
Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951). Protein measurement with the folin phenol reagent. J. Biological. Chem.19:265-275.
Madlova A, Antosova M, Barathova M, Polakovic M, Stefuca V, Bales V (1999). Screening of microorganisms for transfructosylating activity and optimization of biotransformation of sucrose to fructo-oligosaccharides. Chem. Pap. 53:366-369.
Maugeri F, Heernalsteens S (2007). Screening of yeast strains for transfructosylating activity. J. Mol. Cat. B: Enzy. 49:43-49.
Moore NCC, Yang LP, Storm H, Oliva HM, Saavedra JM (2003). Effect of fructooligosaccharide-supplemented infant cereals a double minded randomized trial. Br. J. Nutr. 90:581-587.
Patel V, Saunders G, Bucke C (1994). Production of fructooligosaccharides by *Fusarium oxysporum*. Biotechnol. Lett.16:1139-1144.
Reddy PP, Reddy GSN, Sulochana MB (2010). Screening of β-fructofuranosidase producers with high transfructosylating activity and its 3^2 experimental run studies on reaction rate of enzyme. J. Biol. Sci.10:237-241.
Sanchez OF, Rodriguez AM, Silva E, Caicedo LA (2010). Sucrose biotransformation to fructooligosaccharides by *Aspergillus sp.* N74 free cells. Food Bioprocess Technol. 3:662-673.
Sangeetha PT, Ramesh MN, Prapulla SG (2003). Microbial production of Fructooligosaccharide. Asian. J. Microbiol. Biotech. Environ. Sci. 5:313-318.
Sangeetha PT, Ramesh MN, Prapulla SG (2005). Fructooligosaccharides production using fructosyltransferase obtained from recycling culture of *Aspergillus oryzae* CFR 202. Pro.Biochem. 40:1085-1088.
Slevin MM, Allsopp PJ, Magee PJ, Bonham MP, Naughton VR, Strain JJ, Duffy ME, Wallace JM, MacSorley EM (2014). Supplementation with calcium and short-chain fructooligosaccharides affects markers of bone turnover but not bone mineral density in postmenopausal women. J. Nutr.144: 297-304. Doi: 10.3945/jn.113.188144
Yun JW (1996). Frutooligosaccharides - occurrence, preparation and application. Enzyme Microb. Technol.19: 107-117.
Yun JW, Kim DH, Moon HY, Song CH, Song SK (1997). Simultaneous formation of fructosyltransferase and glucosyltransferase in *Aureobasidium pullulans*. J. Microbiol. Biotechnol. l7: 204-208.