RESEARCH ARTICLE

Production of beta-fructofuranosidase using lignocellulosic residue-fungal biomass co-immobilized in alginate gel

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Chavhan Arvind

Cite this article as:
Gadpayle Raju U (2016) Production of beta-fructo-furanosidase using lingo-cellulosic residue-fungal biomass co-immobilized in alginate gel, *Int. J. of Life Sciences*, A6:25-28.

Acknowledgement
The kind gift of fungal strain *Aspergillus flavus* H-NOB by Heena Nechwani, M.Sc. and technical support extended by Prof. Sanjay P. Timande, HOD, Dept of Microbiology, D.B. Science College, Gondia is duly acknowledged.

ABSTRACT

Lignocellulosic biomass, such as agro-industrial wastes produced during and after crop harvesting, represents a highly rich source of carbon and energy which has been under-utilized. Such lignocelluosic material can be a good nutrition source during fermentation for microorganisms for the production of valuable industrial chemicals, nutraceuticals, pharmaceuticals, enzymes, etc. Immobilization of microorganisms in polymeric gels like alginate provides an alternative in fermentation technology with certain advantages. Immobilization of microorganisms on agro-industrial residues permits direct contact of cells with substrate as well as facilitates easier isolation and separation of products from fermentation medium, an advantage over solid state fermentation (SSF) and submerged fermentation (SmF). In the present work, β-fructofuranosidase (EC 3.2.1.26) (FFase) production by a wild type strain *Aspergillus flavus* H-NOB using lignocellulosic agro-industrial materials viz., sugarcane bagasse and paddy straw residues co-immobilized with fungal biomass in alginate gel was investigated. FFase production by *A. flavus* H-NOB under immobilized condition ranged from 21.65 to 23.75 U/ml for sugarcane bagasse residues and 16.15 to 17.85 U/ml for paddy straw residues, respectively. Optimal pH and temperature for FFase production was 5.5 and 30°C for both the lignocellulosic wastes under immobilized condition. The FFase production kinetics showed a liner progression curve of FFase activity levels for upto 60 h. The study results demonstrate that lignocellulosic waste residues can be successfully utilized as nutritional source for *A. flavus* H-NOB for the production of FFase under alginate-immobilized condition.

Keywords: Agro-industrial Waste, Lignocellulose, *Aspergillus*, beta-Fructofuranosidase, Immobilization, Alginate

INTRODUCTION

Filamentous fungi are given much attention due to their ability to produce a large variety of valuable biomolecules as well as industrially important products (Fenice *et. al.,* 1998). Furthermore, their ability to grow on a large number of organic waste materials ranging from toxic effluents or solids wastes to agro-industrial materials make them suitable for bioconversion of a waste into value added product. Agro-industrial wastes represent a large deposit of carbon and energy as well as source of other nutrients (Pandey, 2000). It is composed of a very heterogenous and chemically complex

materials generated from agriculture process, crop processing, forestry, poultry, animal husbandry, fruit processing, etc. *Aspergillus* spp. has been reported for the production of various industrially important products such as enzymes using various organic materials including agro-industrial waste (Alegre *et. al.,* 2009; Mussatto *et. al.,* 2009). The *Aspergillus* spp. have also been variously reported for the production of β-fructofuranosidase (EC 3.2.1.26, FFase) (Balasubramaniem *et. al.,* 2001; Gosh *et. al.,* 2001; Ashokkumar *et. al.,* 2002). FFase enzyme hydrolyses sucrose into reducing sugars-glucose and fructose as well as produce fructooligosaccharides (FOS) through transfructosylating activity. It is extensively used in food industries and confectionaries as sweetner enhancing ingredient. Furthermore, the FOS have great pharmaceutical potential due to their low caloric, anti-diabeticand prebiotic activity (Sangeetha *et. al.,* 2005). Immobilization of cells has been reported to increase cell productivity due to microenvironmental- and functional- stability (Santos *et. al.,* 2005; Mussatto *et. al.,* 2009). Furthermore, immobilization facilitates operation advantage over solid state fermentation (SSF) and submerged fermentation (SmF) in easier separation of products from cell biomass in batch as well as in continuous systems (Fenice *et. al.,* 1998; Santos *et. al.,* 2005). In the present study, immobilization of lignocellulosic residues along with fungal biomass in alginate gel was attempted and subsequently production of FFase under immobilized condition was studied.

MATERIALS AND METHODS

Fungal strain

The fungal strain used in the present study was a wild type *Aspergillus* strain, identified and designated as *Aspergillus flavus* H-NOB having extracellular β-D-fructofuranosidase activity SMF and SSF (Nechwani et. al., 2012).The growth media or media ingredients used in the present study were purchased from HiMedia laboratories, Mumbai, India.

Preparation of lignocellulosic residues

Sugarcane bagasse and paddy straw were used as lignocellulosic agro-industrial wastes for the production of FFase under immobilized condition. The materials were collected from local sugarcane juice vender and from paddy field after crop harvesting. The collected materials were cleaned, washed and boiled in water for 10 minutes followed by drying in oven at 60°C O/N, mechanically reduced by grinding, sifted with wire gauge to obtain a size range of 1-2 mm residues and autoclaved at 121°C for 15-20 min before use. The sterile lignocellulosic residues thus obtained were used for further experiments.

Inoculum preparation

A. flavus H-NOB strain used in the experiment was maintained on potato dextrose agar (PDA-HiMedia) plates and spores were collected by scraping in sterile 20% glycerol-water after the strain was grown on PDA at 28°C for 5-6 days. About 1.5 x 10^7spores were inoculated into Erlenmeyer flasks (250 ml) containing sterile agro-industrial residues (0.5 g each, separately) suspended in 50 ml of sterile semi-synthetic medium having following composition (% w/v): sucrose 20, yeast extract 2.5, $NaNO_3$ 0.2, K_2HPO_4 0.5, $MgSO_4.7H_2O$ 0.05, KCl 0.05.The flasks were incubated at 28-30°C for 24 h under shaking (180 rpm). This medium was referred as inoculum for immobilization experiments.

Alginate immobilization

For alginate immobilization, inoculum centrifuged at 2000 rpm 5 min; residues re-suspended in 25 ml of fresh sterile semi-synthetic medium and mixed slowly with 3% (w/v) Na-Alginate prepared in 25 ml of fresh sterile semisynthetic medium with constant stirring. The resultant mixture was added dropwise into 2% w/v$CaCl_2$solution in water with the help of 10 ml syringe and beads allowed to harden for 1 h. The average beads size (diameter) ranged 3-4 mm. The beads were then rinsed with sterile medium and later used for enzyme production studies (Fig 1).

FFase activity

β-fructofuranosidase activity in medium was determined by measuring the reducing sugars released by the hydrolysis of sucrose by enzyme present in medium. In brief, 0.1 ml of suitably diluted extract was mixed with 0.2 ml of 1M sucrose, as substrate, in 0.05M sodium acetate buffer (pH 5.0) and incubated for 15 min at 37°C.The reducing sugar was quantified using 3',5'-dinitrosalisilic acid (DNS) (Miller *et. al.,* 1959) spectro-photometrically at 540nm. The protein was quantified spectro-photometrically at 660nm as per Lowry *et. al.* (1951) method, using bovine serum albumin (BSA) as standard. One unit of enzymatic activity was determined as the amount of enzyme necessary to produce 1 μmol of reducing sugar per minute under the assay condition.

RESULT AND DISCUSSION

Effect of Temperature and pH on FFase production

Beta-fructofuranosidase production by *A. flavus* H-NOB immobilized on agro-industrial lignocellulosic material residues in alginate gel peaked to 1.43 U/ml in 24 h on bagasse residues and 0.58 U/ml on paddy straw at pH 5.5& 30°C (Fig. 2, A& B).The FFase production lowered for bagasse and paddy straw residues with increase with temperature while at 45°C it dropped to almost 10%& 45% of peak value, respectively. Compared to paddy straw residues the FFase production was about 1.4 times higher with sugarcane bagasse residues.

Kinetics of FFase production

Production kinetics of beta-fructofuranosidase at optimal pH and Temperature was carried out as determined from previous experiment. The alginate beads (50 each of bagasse and paddy straw, separately) in 25 ml sterile medium/100 ml Erlenmeyer flask were incubated at pH 5.5 and 30°C. FFase activity was found relatively low in the medium with paddy straw residues (0.098 U/ml/h at 24 h) while that was with sugarcane bagasse residues was high (0.149 U/ml/h at 24 h) with a maximum reaching 2.93±0.96 U/ml and 4.46±1.064 U/ml by 24 h, (Fig. 3). The maximum amount of FFase in incubated medium reached to 17.0 U/ml with paddy straw residues and 22.7 U/ml with sugarcane bagasse at the end of incubation period. Kinetically, under immobilized condition, FFase production progressed linearly upto 60 h as evident from graph. Further, such production kinetics can be exploited for continuous production systems.

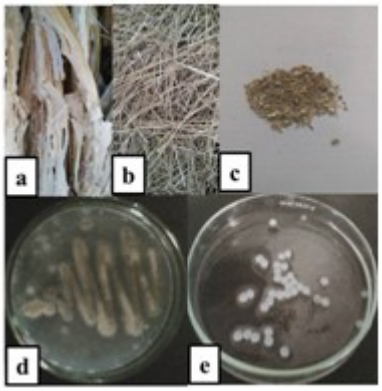

Fig. 1: A collage of photographs showing sugarcane bagasse (a), paddy straw (b), residues (1-3 mm size)(c), wild type strain *Aspergillus flavus* H-NOB on PDA (d) and residue-*A. flavus* biomass co-immobilized alginate beads.

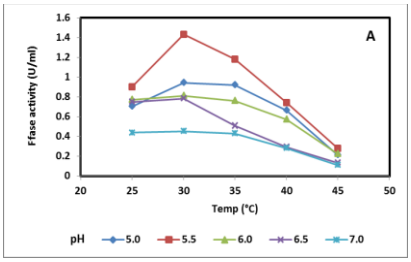

Fig. 2.A

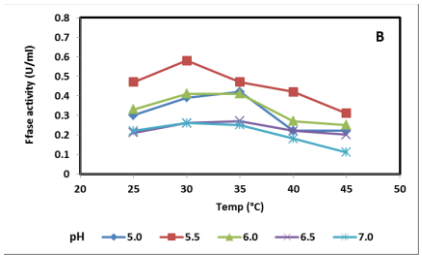

Fig. 2.B

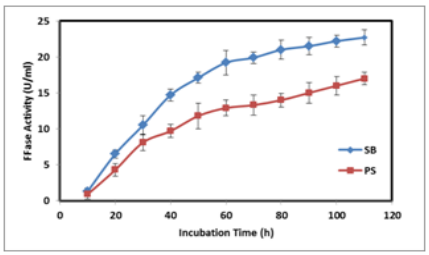

Fig. 3:

Fig. 2. Effect of Temperature and pH on production of FFase by *A. flavus* H-NOB under alginate-immobilized condition on agro-industrial residues- (A) sugarcane bagasse and (B) paddy straw. The were incubated in semisynthetic media for 48 hr at different pH and Temperature. The FFase activity was determined from incubated medium at the end of incubation as described in Materials and Methods.

Fig. 3. Production kinetics of FFase on lignocellulosic residues by *A. flavus* under immobilized condition. Erlenmeyer flask containing 50 beads of each type of residues incubated separately in sterile 25 ml semisynthetic medium at 30°C and pH 5.5 for desired period. The FFase activity was determined as described in Materials and Methods.

CONCLUSIONS

The results obtained in the present study indicates that the wild strain *Aspergillus flavus* H-NOB can be successfully utilized for the production of FFase using lignocellulosic agro-industrial waste under alginate gel-immobilized condition on sugarcane bagasse and paddy straw residues. The optimal FFase production was found at pH of 5.5 &30°C and had linear progression curve till 60 h that can be utilized for designing a continuous production system. The study results successfully demonstrate that immobilization of lignocellulosic residues along with fungal biomass can be an alternative fermentation systems for the production of valuable products such as enzymes.

REFERENCES

Alegre ACP, Polizeli MLTM, Terenzi HF, Jorge JA, Guimarães LHS (2009) Production of thermostable invertase by *Aspergillus caespitosus* under submerged or solid state fermentation using agro industrial residues as carbon source. Braz. J. Microbiol. 40:612- 622.

Ashokkumar B, Gunasekaran P (2002) B-fructofuranosidase production by 2-deoxyglucose resistant mutant of *Aspergillus niger* in submerged and solid state fermentation. Ind. J. Expt. Biol. 40:1032-1037.

Balasubramaniem AK, Nagarajan KV, Paramasamy G (2001) Optimization of media for β-fructouranosidase production by *Aspergillus niger* in submerged and solid state fermentation. Process Biochem 36(12): 1241-1247.

Fenice M, Giambattista RD, Raetz E, Leuba JL, Federici F (1998) Repeated batch and continuous production of chitinoytic enzymes by *Penicillium janthinellum* immobilized on chemically-modified macroporus cellulose. J. Biotechnol. 62: 119-131.

Gosh K, Dhar A, Samanta TB (2001) Purification and characterization of an invertase produced by *Aspergillus ochraceus* TS. Indian J. Biochem. Biophys. 38:180-185.

Lowry OH, Rosebrough NJ, Farr AL, Randal RJ (1951). Protein measurement with the folin phenol reagent. J. Biol. Chem. 193:265- 275.

Miller GL (1959) Use of dinitrisalicylic acid reagent for determination of reducing sugar. Anal. Chem. 31:426-429.

Mussatto SI, Aguilar CN, Rodgrigues LR, Teixeira JA (2009) Fructooligosaccharides and b-fructofuranosidase production by *Aspergillus japonicas* immobilized on lignocellulosic materials. J. Mol. Catalysis B: Enzymatic. 59: 76-81.

Nechwani HA, Gadpayle RU, Timande SP (2012) Studies on fungal beta-fructofuranosidase production in solid state fermenatation. In the proceedings of national conference on 'Technological Options for Sustainable Solid Waste Management', pp.59-62.

Pandey A, Soccol CR, Nigam P, Soccol VT (2000). Biotechnological potential of agro-industrial residues. I - Sugarcane bagasse. Biores. Technol. 74:69-80.

Sangeettha PT, Ramesh MN, Prapulla SG (2005). Recent trends in the microbial production, analysis and application of fructooligosaccharies. Trends Food Sci. Tech. 16(10):442457.

Santos JC, Silva SS, Mussatto SI, Carvalho W, Cunha MAA (2005). Immobilized cells cultivated in semi-continuous mode in a fluidized bed reactor for xylitol production from sugarcane bagasse. World J. Microbial Biotech. 21: 531-535.