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Original Research Paper

Enhanced production of β-D-fructofuranosidase by *Saccharomyces cerevisiae* using agro-industrial wastes as substrates

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ABSTRACT

Agro-industrial wastes as carbon and nitrogen sources are evaluated to develop a cost effective media formulation for β-D-fructofuranosidase (FFase) production by *Saccharomyces cerevisiae* GVT263 to replace more costly sucrose, peptone, yeast extract and malt extract. Banana leaf powder (BL) and groundnut oil cake (GOC), which were significant and promising carbon and nitrogen sources for FFase production along with $MnSO_4$, inoculum size (IS) and incubation period (IP) are selected and optimized. Maximum FFase production with 9-fold increase (from 400 U mL^{-1} in basal medium to 3587 U mL^{-1}) was obtained using BL 4%, GOC 4%, $MnSO_4$ 0.06%, IS 0.5% in 48 h. This is the first report on FFase production using BL and GOC as sole source of carbon and nitrogen.

1. Introduction

β-D-Fructofuranosidase, (FFase) (EC 3.2.1.26) which catalyzes the hydrolysis of terminal non-reducing β-D-fructofuranoside residue in sucrose, raffinose and related β-D-fructofuranosides, is produced by bacteria, fungi, higher plants, and some animals (Belcarz et al., 2002). However, *Saccharomyces cerevisiae* is the most preferred organism for FFase production due to its high sucrose-fermenting ability. FFase is widely used in food and beverage industry and one of the important applications of FFase is for production of non-crystallizable sugar (invert sugar) syrup from sucrose, which is used as a humectant in the manufacture of soft centered candies and fondants. FFase is also used whenever sucrose containing substrates are subjected to fermentation, viz. production of alcoholic beverages, lactic acid, glycerol and also in the manufacture of artificial honey and plasticizing agents. In addition to hydrolytic activity, FFase also exhibits transfructosylating activity at high sucrose concentrations, that may be used to obtain fructooligosaccharides (FOS), used as prebiotic formulations (Emine et al., 2010).

Medium composition is one of the most important parameters when enzymes are produced for industrial purposes because around 30–40% of production cost is estimated to be the cost of growth medium, particularly contributed by carbon and nitrogen sources (Laxman et al., 2005). Thus, to reduce the production costs, efforts are being made to develop organic carbon and nitrogen supplements from agro-wastes equivalent to commercially available sucrose, yeast extract, malt extract and peptone. Agro-industrial wastes are mainly composed of complex carbohydrates and crude proteins that might serve as nutrients for microbial growth and production of enzymes. Agricultural wastes are either sold as cattle feed, composted, burnt or disposed into landfill, which leads to adverse environmental impacts (Couto and Sanroman, 2005). The extent of total losses in these commodities is approximately estimated as 20–30% of the total production, amounting to a huge loss. The failure or inability to salvage and reuse such materials economically results in the unnecessary waste and depletion of natural resources (Essien et al., 2005). Thus, the development of potential value-added processes using these wastes is highly attractive and becomes an environment friendly method of waste management.

The objective of this work is to examine the prospects of using different agro-industrial wastes as carbon and nitrogen sources for production of FFase by *S. cerevisiae* GVT263 and optimization of significant variables. Initial screening of different substrates was done to understand the significance of their effect on product (FFase) formation, then a few better ingredients were selected for further level of statistical screening by Plackett–Burman design and the selected variables were optimized by response surface methodology (RSM) for maximizing the enzyme production.

2. Materials and methods

2.1. Microorganism, culture conditions

S. cerevisiae GVT263, a high FFase-producing mutant developed in this lab, was used in the present study (Venkateshwar et al., 2009).

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The strain was grown in yeast extract malt extract (YEME) broth (basal medium) containing sucrose-1%, peptone-0.5%, yeast extract-0.3%, malt extract-0.3% at 30 °C, pH 6.0 and 200 rpm.

2.2. Substrates

Different substrates used in the present study are banana peel (BP), banana leaf (BL), mango peel (MP), pineapple peel (PP), sugarcane pressmud (SPM), cabbage leaf (CL), sweet lime peel (SLP), orange peel (OP), sugarcane leaf (SCL), cauliflower stalks (CFS), jack fruit peel (JFP), potato peel (PoP), spent grain powder (SG), corn leaf (CoL), corn cob (CC), sunflower oil cake (SOC), cotton seed oil cake (CSOC), groundnut oil cake (GOC), coconut oil cake (COC), soyabean oil cake (SBOC), red lentil powder (RL), green gram husk (GGH) and dry yeast cells (YC). All these substrates, except yeast cells were collected freshly from local markets and farms, cleaned, sliced, dried at 60–70 °C, blended to fine powder, packed in air tight zipped polythene covers and stored in moisture free container at room temperature. The dry yeast cells used were of commercial, granulated food grade yeast. All these raw materials were directly used as substrates in the fermentation media without any pre-treatment.

2.3. Analysis of substrates

The carbon and nitrogen contents of the substrates were analysed by CHNS analyser, the total sugars were estimated by anthrone method and the crude protein content was estimated by micro-kjeldahl method (N × 6.25).

2.4. Enzyme assay

The reaction mixture contained 1 mL of 1% sucrose in 0.1 M phosphate buffer (pH 6.5), 0.1 mL of culture supernatant (as enzyme source) obtained by centrifugation at 3750*g* and 0.9 mL of 0.1 M phosphate buffer (pH 6.5). Incubation was done for 30 min at 30 °C. Release of reducing sugars was quantified by dinitro salicylic acid method (Miller, 1959). One unit (U) of enzyme is defined as the amount of enzyme required to release 1 μmole of reducing sugar (glucose equivalent) per milliliter per minute under assay conditions.

Table 1
Uni-dimensional screening of different agro-industrial wastes as carbon and nitrogen source for fructofuranosidase production by *Saccharomyces cerevisiae* GVT263.

Type of agro-industrial waste	Substrate source as carbon/nitrogen	Enzyme produced (U mL^{-1})
Banana peel*	Carbon	879
Banana leaf*	Carbon	929
Mango peel*	Carbon	731
Pineapple peel*	Carbon	733
Cabbage leaf*	Carbon	628
Sweet lime peel*	Carbon	533
Orange peel*	Carbon	485
Sugarcane leaf*	Carbon	506
Cauliflower stalks*	Carbon	828
Corn cob	Carbon	432
Corn leaf	Carbon	458
Potato peel	Carbon	382
Spent grain	Carbon	480
Jack fruit peel	Carbon	248
Coconut oil cake*	Nitrogen	982
Sunflower oil cake*	Nitrogen	967
Soyabean oil cake*	Nitrogen	867
Cotton seed oil cake*	Nitrogen	929
Groundnut oil cake*	Nitrogen	1406
Green gram husk	Nitrogen	355
Red lentil powder*	Nitrogen	406
Yeast cells	Nitrogen	179

* Selected for second level screening.

2.5. Selection of agro-industrial wastes as suitable carbon and nitrogen sources by unidimentional approach

Initially 15 different carbohydrate rich substrates (Table 1) were screened as carbon source for FFase production. These substrates were directly used in fermentation medium (YEME broth) at 1% level in place of sucrose, inoculated with 1% of 24 h culture (1×10^8 CFU mL^{-1}) and the physical parameters temperature, pH and agitation were held constant at 30 °C, 6.0 rpm and 200 rpm, respectively. After selecting the best carbon source, 8 different protein rich substrates (Table 1) were screened as nitrogen source for FFase production. Medium was designed to contain 1% pre-selected carbon source and 1% different nitrogen sources one at a time.

2.6. Evaluation of most suitable carbon and nitrogen sources using Plackett–Burman design

Based on the results obtained from the preliminary study on FFase production by *S. cerevisiae* GVT263, 10 different carbon substrates i.e., BP, BL, MP, PP, SPM, CL, SLP, OP, SCL and CFS and 6 nitrogen substrates i.e., SOC, CSOC, GOC, COC, SBOC, and RL were selected. They were further screened to identify the most significant variables affecting the enzyme production by Plackett–Burman design. PB design is a two-level factorial design and allows the investigation of $n-1$ variables in n experiments (Plackett and Burman, 1946). This design requires that the frequency of each level of a variable should be equal and that in each test the number of high and low variables should be equal. Then the effects of changing the other variables cancel out while determining the effect of particular variable.

For screening carbon sources, a set of 16 experiments were performed at combinations of '+' (high-0.3%) and '−' (low-0.03%) values of the process variables in 50 mL sterile medium (YEME broth without sucrose) (in 250 mL Ehrlenmayer flasks) with varying concentrations of selected variables as per the design (Table 2), inoculated with 1% of 24 h culture (1×10^8 CFU mL^{-1}) and incubated for 24 h at above conditions. For screening nitrogen sources, a set of 12 experiments were performed at combinations of '+' (high-0.5%) and '−' (low-0.05%) values of the process variables in 50 mL sterile medium containing pre-selected carbon source at 1% level (in 250 mL Ehrlenmayer flasks) with varying concentrations of selected variables as per the design (Table 3), inoculated with 1% of 24 h culture (1×10^8 CFU mL^{-1}) and incubated for 24 h at above conditions.

The results were analysed for the effect of variables on the response (FFase production). In the experimental design, each row represents an experiment and each column represents an independent variable (Tables 2 and 3). The concentrations of variables were fixed based on previous experiments (data not shown). The main effect was calculated as the difference between the average of measurements made at the high level setting (+1) and low level setting (−1) of each factor. This design is practical especially when the investigator is faced with large number of factors and is unsure of which settings are likely to produce optimal or near optimal responses. Plackett–Burman experimental design was based on the first order model:

$$Y = \beta_o + \sum \beta_i X_i \quad (1)$$

where, Y is the response (FFase productivity), β_o is the model intercept, β_i is the variable estimates. Plackett–Burman design