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Enhanced production of pectinase by *Saccharomyces cerevisiae* isolate using fruit and agro-industrial wastes: Its application in fruit and fiber processing

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A B S T R A C T

The present study is focused on the utilization of fruit and agro-industrial wastes for the production of yeast pectinases using response surface optimization techniques. Orange peel (OP), groundnut oil cake (GC), $MnSO_4$ and incubation period (IP) were found as significant parameters for pectinase production. The central composite design of media optimization indicated that OP (5), GC (4), $MnSO_4$ (0.08%, w/v) and IP (48 h) increased the pectinase production by 9-fold. Crude pectinase promoted the enzymatic peeling of oranges and processing of raw banana fibers. Topographical changes of enzyme treated orange and mango peels were studied using AFM which provides a new tool to unravel the role of pectin aggregation in the dissolution of middle lamella during enzymatic hydrolysis. Changes in the fingerprint regions of enzyme treated fruit substrates were observed along with the disappearance of the impurities such as waxes and pectin using FTIR spectral analysis.

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1. Introduction

Microbial pectinolytic enzymes play a vital role in breakdown of pectin polymers for the nutritional purposes of microbes. They have a significant role in plant pathogenesis, symbiosis and decomposition of plant deposits (Lang and Dornenburg, 2000). Pectinases are mainly produced by higher plants and various microorganisms (Naidu and Panda, 1998). Polygalacturonase (PG) is a most common enzyme among a group of pectinases which plays a crucial role in converting protopectin in cell walls to a soluble form during fruit ripening (Yamaki et al., 1979). It is also involved in the hydrolysis of cell walls and softening of fruit during ripening by depolymerization of the middle lamella (Della Penna et al., 1990). Some other major industrial applications of pectinases include retting, bioscouring and degumming of fiber crops, treatment of pectic waste water, paper making, oil extraction, coffee and tea fermentation (Kashyap et al., 2001). Pectinases having a broad range of applications has attracted the industrial biotechnologists towards optimization of process variables as they are essential and prerequisite parameters for industrial scale production and utilization. *Saccharomyces cerevisiae* is one of the few yeast species in which PG production has been described. Gainvors et al. (1994) reported three types of pectinolytic enzymes (polygalacturonase, pectin lyase, and pectin esterase) secreted by a wild strain of *S. cerevisiae*.

Earlier studies reported that production cost including the carbon and nitrogen sources mainly affects the outlay of growth medium in the industrial production of enzymes (Laxman et al., 2005). Accordingly development of cost-effective, easily available media formulations containing organic carbon and nitrogen supplements from fruit and agro-industrial wastes have gained much attention. Media components in a growth medium greatly influence the synthesis of pectinolytic enzymes by microorganisms (Nair et al., 1995). The poly-galacturonase production in SMF depends on carbon and nitrogen sources, temperature, pH and inoculum size in the growth medium and has been optimized using submerged culture by (Leuchtenberger and Mayer, 1992). Fruit processing industries generate huge amount of waste material in the form of peel, pulp and seeds. According to Wilkins et al. (2007) approximately 50–60% of the citrus fruit is converted into citrus peel waste which leads to accumulation of large quantities of citrus peel waste as a by-product in the citrus-processing industry. Agro-industrial wastes also serve as suitable sources for industrial

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fermentations as they are mainly composed of complex carbohydrates and crude proteins that might serve as nutrients for microbial growth and production of enzymes. This waste generated by all these sources direct to proper disposal problems and ultimately leads to adverse environmental pollution. A variety of microbial transformations have been proposed for the utilization of food processing, fruit and agro-industrial wastes for producing valuable products like biogas, ethanol, citric acid, chemicals, various enzymes, volatile flavoring compounds, fatty acids and microbial biomass (Choi et al., 2002). A mixture of agro-industrial waste such as lemon peel, orange peel, apple pomace, deseeded sunflower heads, wheat bran, sugarcane bagasse, and coffee pulp, have been used as solid substrates for microbial pectinases production using solid state fermentation (Vivek et al., 2010).

The objective of the present study was to screen and study the effects of various fruit and agro-industrial wastes as suitable substrates for PG production by *S. cerevisiae* in statistically optimized fermentation conditions. Furthermore, prospective applications of the present enzyme in few industrial applications such as fiber processing, enzymatic hydrolysis and fruit peeling were studied.

2. Materials and methods

2.1. Yeast isolates

S. cerevisiae PVK4, a low temperature active pectinase producing strain was isolated in our laboratory and it is used in the present study (Vijaya kumar et al., 2015). Selected yeast isolate was maintained at 4 °C on agar slants containing (g/L): yeast extract (3.0), peptone (5.0), malt extract (3.0) and agar (20).

2.2. Analysis of substrates

Various substrates were selected like orange peel (OP), mango peel (MP), grape peel (GP), tamarind kernel (TK), sapota peel (SP), banana peel (BP), apple pomace (AP), papaya peel (PYP), pomegranate peel (PP), groundnut oil cake (GC), palm oil cake (PC), sunflower oil cake (SC), coconut oil cake (CC), rice bran (RB) and wheat bran (WB). All the raw materials were cleaned, dried at 50–60 °C, ground to fine powder, packed in airtight precleaned containers and stored at room temperature. All these processed substrates were used at 1% level in fermentation media directly instead of using of dextrose, and inoculated with 1% of 24 h culture (1×10^4 cells/mL) and the required physical parameters like temperature, pH and agitation were kept at 28 ± 2 °C, 6.0 pH and 200 rpm, respectively. All the substrates were analyzed for carbon and nitrogen contents using CHN analyzer (Perkin-Elmer 2400 Series), total sugars using anthrone method and the crude protein content using Micro-Kjeldahl method.

2.3. Production of pectinase in submerged fermentation (SMF)

The selected yeast isolate was grown in 1 L Erlenmeyer flasks containing 250 mL YEPD broth, wherein dextrose was replaced with respective carbon and nitrogen sources and inoculated with yeast cells (1×10^4 cells/mL). Enzyme production was carried out in SMF by incubating the flasks at 28 ± 2 °C for 48 h on a rotary shaker with a speed of 150 rpm. At the end of fermentation, samples were assayed for the enzyme activity.

2.4. Polygalacturonase (PG) assay

The PG activity was assayed by incubating a mixture of 1.5 mL of 1% polygalacturonic acid (dissolved in 0.05 M acetate buffer, pH 5.0), 8.0 mL of sodium acetate buffer and 0.5 mL of culture filtrate at 45 °C for 1 h in a shaker incubator. Activity was measured by quantifying the amount of reducing sugar groups which had been liberated after incubation with 1% polygalacturonic acid at 45 °C, by the method of DNS (Miller, 1959) using galacturonic acid as a standard. One unit of PG activity (U) was defined as the amount of enzyme that liberates 1 μM of galacturonic acid per min.

2.5. Plackett–Burman (PB) design

In order to formulate cost effective suitable medium components to enhance enzyme production, Nine different carbon sources i.e., OP, MP, GP, TK, SP, BP, AP, PYP and PP and 6 nitrogen sources i.e., GC, PC, SC, CC, RB and WB were evaluated for their effect on the PG production. PB design is widely employed when a large number of factors are available (Plackett and Burman, 1946).

Carbon and nitrogen sources were screened by conducting a series of 12 experiments at combinations of '+' (high 0.5%) and '−' (low 0.05%) values of the process variables in 250 mL fermentation medium (YEPD broth without dextrose) in 1 L Erlenmeyer flasks with varying concentrations as per the design in submerged fermentation. The results were evaluated for the effect of variables on the response (PG yield). The main effect was calculated as the difference between the average of measurements made at the high level (+1) and low level (−1) of each factor. Plackett–Burman (PB) design was based on the first order model:

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

where, Y is the response (PG yield), βo is the model intercept, β_i is the variable estimates. PB design matrix was developed and the results were analyzed using MINITAB-17 statistical software.

2.6. Effect of mineral nutrients

Six different mineral nutrients ($CoCl_2$, $CaCl_2$, KH_2PO_4, $MgSO_4$, $MnSO_4$ and ZnSO4) were evaluated for their effect on PG production in media containing selected agro-industrial wastes as carbon and nitrogen sources. The carbon and nitrogen sources were taken at 1% w/v concentrations and mineral nutrients were taken at a concentration of 0.04%. Media containing only carbon, nitrogen sources and the combination of selected carbon and nitrogen sources without the addition of any mineral nutrients were also tested for PG production.

2.7. Experimental design

Most significant factors affecting PG production were identified using PB design. Selected factors were optimized in response surface methodology by changing the concentrations of substrates and mineral nutrients along with incubation period to maximize the PG production. Four variables orange peel powder (OP), groundnut oil cake (GC), $MnSO_4$ and incubation period were selected for optimization. Central composite design (CCD) with five coded levels (−2, −1, 0, +1, +2) was performed in a design of 30 experiments with six replicates at the central point. The following equation was used for coding the actual experimental values of the variables**:**

$$\chi_i = (X_i - X_0)/\Delta X \quad (2)$$

where χ_i is dimensionless coded level of the variable, X_i is actual value of that variable, X_0 is average of the high and low level values of that variable, and ΔX is high value minus low the value of that variable. A second order polynomial equation was used to analyze the response (PG production, U/mL) and the data were fitted into the equation by multiple regression methods. The three

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