



# Valorization of wheat bran for the production of polygalacturonase in SSF of *Aspergillus sojae*



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## ABSTRACT

Wheat bran, among various agro industrial by products, screened for the production of polygalacturonase (PG) in solid-state fermentation of *Aspergillus sojae* mutant strain, was found to be the most suitable substrate without the addition of any nutritive or inducing supplement. It was further characterized for its physicochemical composition and particle size distribution. The process conditions that favored the PG production using this substrate were determined as;  $10^7$  spore/g substrate inoculum concentration, 4 days of fermentation, 37 °C of incubation temperature, 62% initial moisture content, water as the moistening agent, 100–250 µm particle size of wheat bran, 3 times/day agitation and spore solution as the inoculum type which resulted into maximum PG activity of 535.4 U/g substrate. Overall, this optimization process resulted in 7.3 and 3.9 fold of significant enhancement in the PG activity and productivity, respectively.

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## 1. Introduction

Industrial market for pectinases approached 70 million dollars around the world that corresponded approximately to 5% of global enzyme sales in the year 2011 (Alimardani-Theuil et al., 2011). Commercial pectinases are commonly used in juice extraction and clarification processes of the sparkling clear juices (apple, pear and grape juices), cloudy juices (citrus juices, prune juices, tomato juice and nectars), and unicellular products where the intent is to preserve the integrity of the plant cells by selectively hydrolyzing the polysaccharides of the middle lamella (Kashyap et al., 2001). Polygalacturonase (PG, EC 3.2.1.67) is one of the most important pectinolytic enzyme classified under depolymerases class of pectinases. Polygalacturonase catalyzes the hydrolysis of α-1,4-glycosidic linkages in pectic acid with the introduction of water across the oxygen bridge (Jayani et al., 2005).

Pectinases are mainly produced from *Aspergillus niger* (polygalacturonase, pectinesterase, pectinlyase producing strains) for industrial uses (Alimardani-Theuil et al., 2011). However, the potential of *Aspergillus sojae* as a PG-producing strain was initially revealed by our group. The first study with *A. sojae* ATCC 20235 (wild type) was carried out by Göğüş et al. (2006) on the production of the PG by submerged fermentation (SmF) technique, which

indicated that this organism holds a good potential for PG synthesis with the desired pellet morphology. Additionally, a solid-state fermentation (SSF) study with the same strain was performed by Ustok et al. in 2007 using corncob, maize meal and crushed maize as substrates in which inoculum size and incubation time factors were optimized using response surface methodology techniques. Ustok et al. (2007) have also concluded that *A. sojae* ATCC 20235 could be a good candidate for the production of polygalacturonase by SSF. These previous studies constituted a basis for the idea of using mutant strains of *Aspergillus sojae* and solid-state fermentation technique for the improvement of the enzymatic activity of polygalacturonase in order to check the possible industrial potential of this process. In a recent SSF study, Demir et al. (2012) improved the PG activity and spore production potential of a mutant *Aspergillus sojae* strain (*A. sojae* M3) using response surface methodology in a fermentation medium lacking the support of synthetic compounds, but including natural components such as orange peel and wheat bran.

Solid-state fermentation is defined as the growth of microbes without free flowing aqueous phase. (Bhargav et al., 2008). The industrially important enzymes can be produced by SSF techniques especially using the fungal metabolisms and agro-industrial residues as low-cost and available solid substrates. However, there are some important factors that should be optimized in order to maintain microbial growth and enzyme synthesis.

Wheat bran is one of the most popular agro-industrial residue preferred by many researchers to produce value-added metabolites from various microorganisms using SSF (Balkan and Ertan,

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2010; Corona et al., 2005; Das et al., 2013; Freitas et al., 2006). According to the statistics of United States Department of Agriculture (USDA), a total of 655,270,000 tons of wheat were produced and 680,419,000 tons of wheat were consumed in the period of 2012–2013 in the world (USDA, 2013). Since, about 15–20% (in weight) of wheat bran was reported to be discarded in the wheat flour production process (Dobrev et al., 2007), wheat bran can be accepted as a sustainable by-product for the microbial production of industrially important enzymes applying SSF. The wheat flour manufacturers often dispose wheat bran because the cost of transportation is more than its worth, and such waste also causes potential environmental concerns (Xie et al., 2008). Therefore, utilization of this by-product to produce a value-added enzyme will also help to solve the pollution problem of wheat flour manufacturers.

There are many studies in the literature using wheat bran for the production of industrially important enzymes by SSF, however all of them achieved to produce the enzymes with the aid of nutritive supplementary liquids added to the residues (Delabona et al., 2013; Fontana et al., 2005; Meena et al., 2013; Taşkın and Eltem, 2008). The motivation of the current study is the production of PG having reasonably high activities with the use of only wheat bran and distilled water by an *Aspergillus sojae* mutant strain. For this purpose, the most important factors inoculum size, incubation period, incubation temperature, initial moisture content, moisturizing agent (pH), particle size, agitation and type of inoculum were optimized by one-at-a-time method. Many responses such as PG activity, final pH, spore count, specific PG activity, consumption of carbohydrate etc. were monitored to be able to state, present and discuss the metabolic activities of the fermentation process and decide on the optimum parameters.

## 2. Materials and methods

### 2.1. Microorganism and propagation

*Aspergillus sojae* ATCC 20235 (wild type) was purchased in the lyophilized form from Procochem Inc., an international distributor of ATCC (American Type of Culture Collection) in Europe. This wild type culture was randomly mutated using ultraviolet light exposure according to the modified method of Nicolás-Santiago et al. (2006) by Jacobs University gGmbH, Bremen and used as the mutant strain in this study.

### 2.2. Preparation of inoculum for SSF and SmF experiments

After the propagation step on YME plates, the spore suspensions used as inoculum were obtained on molasses agar slants given by Göğüş et al. (2006). The inoculum preparation procedure for SSF was performed according to Demir et al. (2012). Besides, spore/g substrate was estimated by dividing the total number of obtained spores to the amount of substrate used. The submerged seed culture (referred as SmF) was prepared in 250 ml Erlenmeyer flasks with 50 ml medium consisting of; maltrin (120 g/l), glucose (25 g/l), peptone (2.5 g/l), disodium phosphate (3.2 g/l) and monosodium phosphate (3.3 g/l). Each flask was inoculated with  $1.7 \times 10^7$  total spore/50 ml inoculum concentration which was optimized by Tari et al. (2007) and incubated by shaking at 150 rpm, 30 °C for 0, 24, 48 and 72 h.

### 2.3. Enzyme production by solid-state fermentation

Appropriate amount of solid substrate was placed in the 250 ml Erlenmeyer flask. Half of the appropriate amount of liquid calculated to maintain the desired initial moisture content was added to the medium before sterilization. Other half of the liquid was

sterilized and used for the inoculation of the medium including the sufficient inoculum concentration. The content of the flask was mixed well until the homogenous spread of the inoculum on the solid substrate was obtained. The prepared flask was placed in the static incubator at the desired temperature until the end of the fermentation.

### 2.4. Enzyme extraction

Appropriate amount of fermented sample was placed in a 250 ml Erlenmeyer flask and mixed with Tween 80 (0.02%) solution in the ratio of 1:10 (sample amount: Tween 80 solution) and shaken at 150 rpm and 25 °C for 30 min. Afterwards, the pH of the flask content was measured with a pH meter and the content filtered through the cheese cloth and centrifuged at 4 °C, 5000 rcf for 15 min. The supernatant was separated and used for enzyme, protein and total sugar assays, immediately.

### 2.5. Enzyme activity, total sugar and protein determination

Polygalacturonase (PG) activity was assayed according to the modified procedure of Panda et al. (1999) using 2.4 g/l of polygalacturonic acid as substrate. One unit of enzyme activity was defined as the amount of enzyme that catalyses the release of 1  $\mu$ mol of galacturonic acid per unit volume of culture filtrate per unit time at standard assay conditions (pH 4.8 and 40 °C). Galacturonic acid (Sigma, St. Louis, MO) was used as standard for the calibration curve of PG activity. Total sugar content of the crude enzyme was determined with the phenol–sulphuric acid method of Dubois et al. (1956). Protein content of the crude enzyme was analyzed according to the Bradford method (Bradford, 1976).

### 2.6. Characterization of wheat bran

Wheat bran was supplied from Hazal Flour and Feed Manufacturing Company, Turgutlu, Manisa, Turkey. For pH measurement, Mettler Toledo SevenEasy pHmeter (Mettler-Toledo AG, Switzerland) was used after a homogenization step at Heidolph, SilentCrusher M (Heidolph Instruments GmbH & Co. KG, Germany). Water activity of the samples was determined using a Rotronic HygroLab benchtop humidity temperature indicator (Rotronic AG, Bassersdorf, Germany). Moisture content was determined with a Precisa XM-60 moisture content analyzer (Precisa Instruments, Diekinton, Germany). Data were reported on a wet basis and were averages of two determinations. For reducing sugar content determination, 100 ml of distilled water was added into 5 g of wheat bran and autoclaved at 105 °C for 5 min. After this process, the reducing sugar content of the soluble fraction of wheat bran was analyzed according to modified Nelson–Somogyi method. Ash content of wheat bran was determined with the help of an ash oven (Protherm, Turkey). The crucibles with the dried samples were burned in the ash oven at 650 °C for 12 h in order to get fully white ash. Dietary fiber content of the wheat bran was determined using a Total Dietary Fiber Assay Kit (Sigma–Aldrich) and estimated by subtracting the weight of the residue from the total weight of the protein and ash. Protein content of the wheat bran was determined with the Kjeldahl method using fully-automated nitrogen-protein digestion and distillation system (Gerhardt, Germany). The coefficient of 6.25 was taken as the protein conversion factor. Water holding capacity was measured by the modified method of Camilios-Neto et al. (2011). Retsch AS 200 Basic (Germany) sieve shaker and 850, 500, 250, 150 and 75  $\mu$ m mesh size stainless steel sieves (20 cm diameter) and a receiver were employed for the determination of particle size distribution. Sieving was performed for 15 min for each

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