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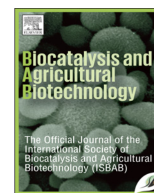

Valorization of passion fruit peel by-product: Xylanase production and its potential as bleaching agent for kraft pulp

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ABSTRACT

Passion fruit peel is a by-product of the fruit industry. Like other agroindustrial by-products, it is generated in large amounts, possesses low commercial value, and is discarded as waste. This study aimed to explore the potential of Araucaria forest fungi to produce xylanases, using passion fruit peel as the substrate. About 65 fungal lineages were isolated from Araucaria forest soil and screened for xylanase enzyme production. Among these, a fungal strain, identified as *Aspergillus flavus*, showed high potential for xylanase production by using passion fruit peel as the sole substrate. Process optimization resulted in a 7.89-fold increase in enzyme activity. The xylanases were stable at 55–60 °C, over a broad pH range; these are characteristics desired for pulp biobleaching. Therefore, the potential of using *A. flavus* crude xylanases for bleaching kraft pulp was examined; the results show that these enzymes are potential bleaching agents, with a Kappa efficiency of 35.93%. To the best of our knowledge, this is the first study to show impressive yields of xylanases by using passion fruit peel by-product as carbon source. This kind of "waste valorization" can help to reduce the production cost of xylanase, as well as the environmental problems posed by inappropriate disposal.

1. Introduction

Among Brazilian biomes, the Atlantic Forest is, in particular, one of 25 areas listed as global hotspots for wildlife conservation (Faoro et al., 2010). Because of its exceptional levels of species endemism and species richness and the loss of large areas of the original forest cover, this biome is considered one of the five biodiversity hotspots (Mittermeier et al., 2011; Myers et al., 2000). Their diversity of plants favors the development of numerous interactions with microorganisms (Joly et al., 2014), exerting selection on distinct phyllosphere microbiomes (Lambais et al., 2006). Thus, the soil microbiomes assembled may also be considered endemic, with respect to their components and particular assemblages (Lima-Perim et al., 2016).

Despite its importance, a huge amount of the Atlantic Forest biome microbial diversity remains unrevealed (Faoro et al., 2010; Lima-Perim et al., 2016). In addition, the real potential of some of its phytogeography units, such as the Araucaria forest, is unknown. Therefore, the Atlantic Forest biome can offer great opportunities for bioprospecting, including microbial xylanase producers.

Xylanases (endo-β-xylanase, EC 3.2.1.8) are enzymes that catalyze the hydrolysis of β-1,4 linkages in xylan, releasing xylooligosaccharides (Polizeli et al., 2005; Sharma and Kumar, 2013). In the last decade, special attention has been devoted to xylan-degrading enzymes because of their potential biotechnological applications, especially in the development of ecofriendly technologies in the bioconversion, food, and pulp and paper industries. In bleaching of pulp, xylanases are employed as alternatives to toxic chlorinated organic compounds, increasing pulp brightness and contributing to reduction of pollution (Khonzue et al., 2011; Walia et al., 2017).

The success of industrial biocatalysis depends on low-cost enzymes. Despite the progress, many enzyme production processes have certain drawbacks such as high substrate cost (Murugan et al., 2011). To overcome this issue, agroindustrial wastes have been explored for utilization as feedstock for the production of microbial enzymes such as xylanases, thereby conferring economic and environmental advantages (Knob et al., 2014; Walia et al., 2013).

Passion fruit (*Passiflora* sp.) is produced on a large scale in Brazil, which is the leading producer worldwide and is responsible for almost 60% of the global produce (Oliveira et al., 2016), with an estimated production of 900,000 t per annum (Corrêa et al., 2017). The passion

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fruit peel comprises about 50–60% of the weight of the entire fruit (Kulkarni and Vijayanand, 2010). Until now, only a little quantity of this waste has been used to supplement animal feed; this use is also limited owing to logistic- and storage-related problems, because of the high moisture content of the peel (Zilly et al., 2012). Therefore, a large quantity of waste is inappropriately discarded in the environment, resulting in a negative environmental impact. To date, studies addressing the reuse of passion fruit peel and those discussing interesting perspectives are lacking.

This agroindustrial by-product is rich in proteins, fiber, and carbohydrates, of which approximately 42% is cellulose, 25% are pectins, and 12% are hemicelluloses (Almeida et al., 2015a; Yapo and Kouassi, 2008; Zilly et al., 2012) to warrant its use as feedstock in the fermentation process, including xylanase production. Recently, we reported the potential use of this agroindustrial waste for fungal β-glucosidase production, which suggested new possibilities for its conversion into other value-added products (Almeida et al., 2015b). However, to the best of our knowledge, the production of xylanases using this waste, a major by-product of the fruit industry, has not been described so far. Therefore, the ability of microbial strains to utilize passion fruit peel to produce xylanases and other enzymes of industrial importance warrants evaluation.

In this study, we showed the feasibility of fungal xylanase production, using passion fruit peel as the substrate. Initially, distinct fungal lineages isolated from the Araucaria forest soil, Atlantic Forest biome, Brazil, were evaluated for xylanase production, specifically by using passion fruit peel as the substrate. Experimental design was adopted to optimize enzyme production by the best strain. We further biochemically characterized the xylanase produced and determined its potential application in enzymatic bleaching of pulp.

2. Materials and methods

2.1. Waste material

Passion fruit (*Passiflora edulis*) peel was obtained locally. The residue was washed with tap water then dried at 60 °C for 24–48 h and then milled (35 mesh). The chemical composition of passion fruit peel can be achieved in the work of Almeida et al. (2015a).

2.2. Fungal isolation and screening test

The fungal lineages were initially isolated from 20 composite soil samples collected in soil depths of 0–20 cm at different sites in two Araucaria Forest fragments, Atlantic Forest biome, localized in Votorões Park, C andói, Paraná, Brazil (25°34'07.4"S, 52°02'43.1"W) and Guarapuava, Paraná, Brazil (25°17'46.73"S, 51°26'20.71"W). Soil samples were introduced in sterile polythene bags and kept cool (< 4 °C) until they were brought to the laboratory for processing. The samples were subject to successive dilution technique. One gram of each sample was placed in test tubes with 10 mL of sterilized distilled water and homogenized using a mixer. Serial dilutions were obtained to 10^{-3} and 100 µL of each dilution was spread on the surface of Sabouraud agar plates supplemented with chloramphenicol (50 mg L^{-1}). Growth in Petri dishes was performed at 28 °C for 5–10 days. Fungal colonies with distinct morphological aspects were isolated by repeated streaking. Pure cultures were cultivated on Sabouraud Agar slants for subsequent identification and storage.

Two step screening assay was used to determine the fungal xylanase production, using passion fruit peel by-product as substrate. Initially, the isolates were grown on solid Vogel medium (Vogel, 1956) supplemented with 1% beechwood xylan, pH 6.5, for 96 h. After cultivation, the plates were stained with 0.5% Congo red dye for an hour and were then washed with 1 M NaCl solution at room temperature for counterstaining the plates. Xylanase production was indicated by zones of clearance. The halos were measured for subsequent calculation of the enzymatic index (EI).

After the initial screening, 10 fungal strains that exhibited an EI higher than 1.50 were selected to further examine the xylanase production through submerged fermentation (SmF) using passion fruit peel. For this, the fungi were grown on Vogel's solid medium with 1.5% (w/v) agar and 1.5% (w/v) glucose, at 28 °C, for 7 days for conidia production. After that, flasks containing 25 mL of Vogel's medium pH 6.5 supplemented with 1% passion fruit peel were inoculated with 1.0 mL of spore suspension of fungus (1.0×10^7 spores mL^{-1}). Cultivation was practiced for five days, at 28 °C. After that, cultures were vacuum filtered and the xylanase assay was done.

2.3. Strain identification

The target strain was identified through the analysis of the products amplified with primers specific for internal transcribed spacer region 1 (ITS1) in ribosomal genes (White et al., 1990). Total genomic fungal DNA was extracted using the pellets produced in Vogel's liquid medium with 1% (w/v) glucose after 30 h of culture, at 28 °C, under 100 rpm of stirring speed (Góes-Neto et al., 2005). The pellets were recovered by centrifugation and used for DNA extraction using the hexadecyltrimethylammonium bromide method.

The ITS1 region was amplified from the genomic DNA using the polymerase chain reaction (PCR) by using the ITS1 (5′ GAACCWGCGGARGGATCA 3′ forward) and ITS2 (5′ GCTGCGTTCTTCATCGATGC 3′ reverse) primers. The sequence obtained was deposited in GenBank under the accession number MF372379.

The sequence of amplified products was compared with others deposited in GenBank NCBI database, using the basic local alignment tool BLAST (www.blast.ddbj.nig.ac.jp/). A phylogenetic tree based on ITS1 region was generated by Neighbor-joining method with 1000 bootstrap replications, using MEGA 6.0. The strains were maintained on Sabouraud dextrose agar and then stored at 4 °C.

2.4. Quantitative assay of xylanase

The enzyme activity was measured spectrophotometrically at 50 °C, using 1.0% (w/v) beechwood xylan in McIlvaine buffer pH 6.5. After 5 min incubation at 50 °C, the reaction was terminated by adding of 3.5-dinitrosalicylic acid (DNS) and the reducing sugar content was determined according to Miller (1959), taking xylose as standard. One unit of xylanase was considered as the enzyme amount required to released one µmol of reducing sugar per min, under the test conditions. All measurements were run in triplicate and the mean value were calculated along with standard derivations.

2.5. Optimization of xylanase production

Response surface methodology (RSM) was applied to provide the optimum levels of some factors that influence the fungal xylanase production and their relationships. The substrate concentration, cultivation time and pH of medium were considered as independent variables and xylanase activity as dependent variable. The Vogel's nutrient medium was employed as basal medium, supplemented with passion fruit peel as feedstock for fermentation.

A 2^3 full factorial central composite rotational design (CCRD) consisting of three factors and five levels including six axial points and three central points was adopted for fitting a response surface (second order). The inoculum and the cultivation were performed as described previously, in different conditions according to experimental design. The ranges of the variables and the full experimental design with regard to their values in actual and coded form are shown in Table 1.

The runs were performed in an aleatory order, and the experimental data was examined by means of a second order model:

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