PRODUCTION OF TANNASE BY *Trichoderma stromaticum* AM7 IN SOLID-STATE FERMENTATION USING AGROINDUSTRIAL WASTES AS SUBSTRATE

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ABSTRACT

Trichoderma stromaticum is a notable enzyme producer and a significant fungus in the natural world. The tannase enzyme is widely utilized in a number of sectors, particularly in the food industry. The objective of the present study is to produce tannase by *T. stromaticum* AM7using agro-industrial residues as a carbon source. Of the residues tested, coffee grain (0.40 U/g) and mangosteen peel (0.30 U/g) were identified as the most effective inducers. Furthermore, the incorporation of 1% tannic acid with mangosteen residue, malt residue, and cocoa shell led to a notable enhancement in enzyme production, reaching 20 U/g. Following the analysis of the interaction between different parameters using the Box-Behnken statistical design, a tannase activity of 32.1 U/g was observed when mangosteen peel was used, in 6 days, with 80% moistening agent (sodium acetate) at pH 6.0. This demonstrated that the fungus *T. stromaticum* is a promising producer of tannase with potential biotechnological applications.

Keywords: *Trichoderma*. Enzyme. Tannase. Waste. Solid-state fermentation.

1 INTRODUCTION

The importance of enzymatic catalytic power is on the rise, becoming increasingly crucial to meet the growing demands of industrial productivity [1]. As a result, non-biological catalysts are becoming obsolete, paving the way for the growing use of enzymes in industrial processes [2]. The search for enzymes with catalytic potentials above market standards has led to increased enzymatic prospecting in biotechnological and agronomic areas [3]. In this context, species of the genus *Trichoderma* have become frequent targets of this bioprospecting [4].

Some *Trichoderma* species have been characterized as excellent producers of various enzymes. For example, *T. reesei* is recognized for its high production of cellulase and hemicellulase, both used in the food industry [5]. Another widely recognized species applied in various biotechnological fields is *T. harzianum*, known for its production of chitinase [6]. *Trichoderma stromaticum* AM7 has already been reported as a producer of xylanase [7], cellulase and amylase enzymes by our research group, however there are still no reports in the literature on the production of tannase by this specie.

Tannase (E.C: 3.1.1.20), or Tannin Acyl Hydrolase, is an extracellular enzyme capable of hydrolyzing phenolic compounds found in plant tissue with hydrolysable tannins, producing glucose and gallic acid [8]. Tanase is naturally produced by filamentous fungi, yeasts, bacteria, and even plants, but among these organisms, filamentous fungi are the main producers. Species of the *Aspergillus* and *Penicillium* genera are the primary producers of this enzyme [9]. Therefore, the present work aims to produce tannase by *T. stromaticum* using agro-industrial residues as a carbon source.

2 MATERIAL & METHODS

The strain *T. stromaticum* AM7 (CCMB617P) [7], used in this research is preserved in the Laboratory of Applied Microbiology in Agroindustry (LABMA) at the State University of Santa Cruz. The fungus will be reactivated on Potato Dextrose Agar medium and incubated in a BOD at 28ºC for 5 days, afterward, a spore suspension in agar-water (1.5 mL) will be prepared and stored at 4ºC for continuous use.

The solid-steate fermentation (SSF) experiments were initially conducted unifactorial and simplex-centroid to determine the best residue for tannase production. Erlenmeyer flasks of 125 mL containing 8.0 g of dehydrated residue with 80% moisture content were used. The residues used were malt residue, cocoa shell, coffee grounds, mangosteen peel, tamarind and peach-palm waste. All components were autoclaved at 120ºC for 20 minutes. Subsequently, the cultures were incubated in a BOD at 28 ºC for 6 days. The same cultures supplemented with TA 1% were carried out. Then, the culture was suspended with 5 mL/g using sterile distilled water and homogenized at 150 rpm at 25 ºC for 30 minutes. The resulting suspensions were filtered using sterile gauze and then centrifuged for 15 minutes at a speed of 15,000 *g* at 4 ºC. The resulting supernatants were considered as the crude enzymatic extract and subjected to further analysis.

A Simplex-centroid experiment (100, 50, 33.33 %) with the best residues was applied to seek greater production of tannase. To delineate the abiotic production parameters, such as pH (4, 6 and 8), Time (days: 2, 6 and 10) and Humectant (70, 80, 90 %), a Box-Behnken experiment was carried out with 3 factors (1, 0 and -1), totaling 15 experiments with three central points. To delineate the abiotic production parameters, such as pH (4, 6 and 8), Time (days: 2, 6 and 10) and Humectant (70, 80, 90 %), a Box-Behnken experiment was carried out with 3 factors (1, 0 and -1), totaling 15 experiments with three central points.

Tannase activity was confirmed using the methanolic rhodanine method. The substrate used was methyl gallate at 100 mM in a 100 mM sodium acetate buffer, pH 5.0, as described by Sharma et al. [11]. The reaction consisted of 500 µL, of which 250 µL were substrate and 250 µL were crude enzyme sample, carried out at 37°C for 10 minutes. The reaction was then stopped by adding 150 µL of methanolic rhodanine (0.667% (m/v)). After 5 minutes, 100 µL of 0.5M potassium hydroxide (m/v) was added, resulting in a violet coloration. Subsequently, each volume was diluted in 2 mL of autoclaved distilled water. After 10 minutes, readings were taken on a spectrophotometer with a wavelength of λ=520 nm.The activity unit (U) was defined as the amount of enzyme required to produce 1 mole of gallic acid per minute under assay conditions.

3 RESULTS & DISCUSSION

Six agricultural residues were tested as substrates for tannase production, with and without the application of 1% tannic acid (TA 1%) (p/v). The data shown in Table 1 revealed that mangosteen and coffe Grounds was the ideal substrate for tannase production without application of TA 1% compared to other substrates.

Table 1 Effect of different tannin-rich residues on tannase production by *T. stromaticum* AM7.

Residues	Tannase activity (U/g)
Malt residue	0,04
Cocoa shell	0,23
Mangosteen shell	0,30
Peach-palm waste	0,14
Tamarind	0,13
Coffee grounds	0,40

Following these results, one-factor tests were carried out with the residues considered most advantageous for the production of tannase, taking into account the annual disposal or the richness of tannins in their already known and documented composition [7]. Thus, the residues supplemented with TA 1% were: Coffe ground (CA), Mixture 1 (MA), Mixture and Coffe (CMA), Peach-palm (PA). Thus, our data demonstrate that there is no statistical difference between the means of tansylic enzyme activity between the different residues tested (Figure 1).

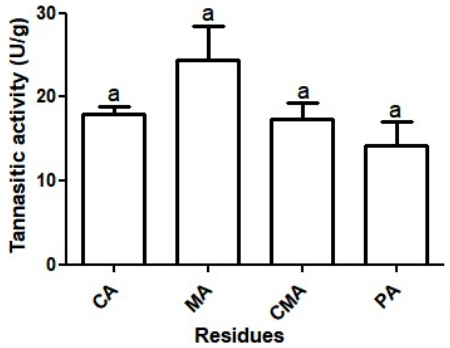

Figure 1 Comparison of the average enzyme activities of tannase produced by different residues supplemented with 1% TA, by *T. stromaticum* AM7. The results presented are not significant p>0.05 and there are no differences between the means. The sampled data has a normal distribution according to the Shapiro-Wilk W test (CA = 0.9902; MA = 0.887; CMA = 0.848 and PA = 0.9628). Means with different letters are significantly different

T. stromaticum AM7 in this study achieved a maximum production value of 32.1 U/g using mangosteen peel, in 6 days, with 80% moistening agent (Sodium Acetate) at pH 6.0. However, since mangosteen is a seasonal fruit with regional demand, the use of peach palm was chosen for process optimization, achieving a production of 19.5 U/g of activity under the same conditions. Tannase exhibits similar production activity in different studies, with optimal production time between 5 and 6 days, optimal pH 5.0 or 6.0, varying only in the moistening agent (ΔS), nitrogen source, or sugars when present [9]. In this work, we corroborate with these parameters, having optimal production points in 6 days and pH 6.0, differing in the moistening agent and its supplements (Figure 2).

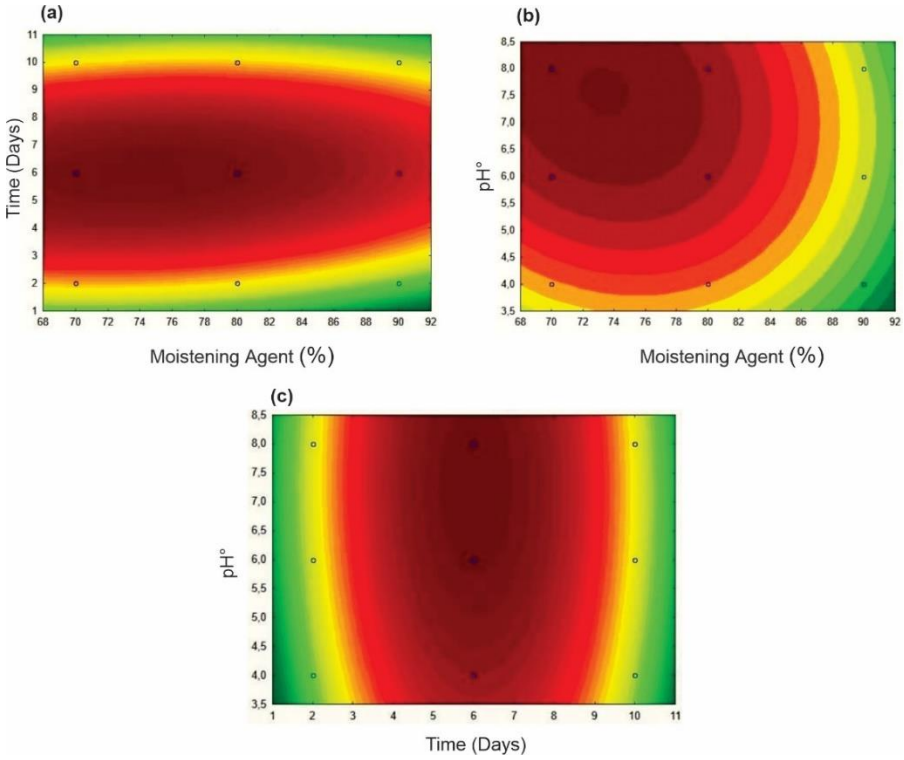

Figure 2 Response surface plots of the effects of interactions between different variables on tannase production by *T. stromaticum* AM7.

4. CONCLUSION

Here we reveal that *Trichoderma stromaticum* AM7 is capable of producing this tannase using different residues, with a production of 32.1 U/g. These data, together with the previous taxonomic and biotechnological knowledge surrounding this species, make it clear that it is necessary to exhaust all the biotechnological potential present in species that already have a demonstrated value. Therefore, further studies on the enzymatic production of tannase by *T. stromaticum* using agro-industrial residues should be conducted, in order to increase production levels on an industrial scale, as well as to characterize this enzyme for potential application in some biotechnological sector.

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