BACTERIAL BIOCONVERSION OF *p*-COUMARIC ACID TO 4-VINYLPHENOL IN SYNTHETIC MEDIA

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ABSTRACT

Vegetal biomass is currently underutilized in Brazil, typically burned for electricity generation. However, this material is a renewable carbon source for generating various high-value chemical compounds, such as commodities and raw chemicals. Among the raw chemicals, phenolic compounds are a promising starting material. These phenolics are found in large quantities in tropical grass plants, such as sugarcane. One of the most common phenolic compounds found is *p*-coumaric acid, a precursor to 4-vinylphenol in microorganisms. 4-vinylphenol has high-value organic compound due to its broad applicability in many industrial processes. Therefore, this study aimed to evaluate the bacterial bioconversion capacity of *p*-coumaric acid to 4-vinylphenol in a model solution. Among the tests, the wild-type strain *Bacillus subtilis* TG 3.48 proved capable of tolerating a concentration of 300 mg L^{-1} of *p*-coumaric acid, converting 92% into 4-vinylphenol in 3 hours of cultivation, in mineral medium supplemented with glucose. The strain also demonstrated the ability to perform the abovementioned conversion in sugar-free medium, although with consumption and yield of 89%. In both assays, no other metabolites were observed.

Keywords: Phenolic compounds. Bacterial bioconversion. *p*-Coumaric acid. 4-Vinylphenol.

INTRODUCTION

The Brazilian agricultural industry produces a large amount of underutilized lignocellulosic waste. However, the increasing demand for renewable sources of energy and materials has spurred research into the utilization of this biomass. Therefore, the discovery of new chemical and biological processes is essential for economically viable conversion of biomass into chemicals, such as biofuels and raw chemicals widely used in industry [1].

Lignin is the second most abundant macromolecule on earth after cellulose, composed of phenolic subunits that are highly recalcitrant, making its deconstruction challenging. Among the compounds obtained from lignin is *p*-coumaric acid (CA), found associated with the cell wall of grass plants through ester bonds to lignin and polysaccharides [2 3].

The CA is a natural antioxidant that exhibits varied bactericidal effects in microorganisms, causing deleterious mutations in DNA and destabilizing the plasma membrane, leading to osmotic lysis. Therefore, the search for strains tolerant to significant concentrations of this compound is crucial for the industrial valorization of derived compounds. These strains typically possess metabolic pathways for bioconversion, transforming it into less toxic or assimilable intermediate metabolites, such as 4-vinylphenol (4VF) [4].

In previous work at the Laboratory of Biochemistry and Applied Microbiology, Ibilce, Unesp, it was found bacteria capable of tolerating and bioconverting ferulic acid into 4-vinylguaiacol [5]. These results suggest that these strains may also tolerate and bioconvert CA. Thus, the present study aimed to evaluate the tolerance and bioconversion of CA acid to 4VF by the wild-type strain *Bacillus subtilis* TG 3.48 in model solutions.

MATERIAL & METHODS

The culture media used in the assays were: liquid LB medium and adapted mineral medium (MLM) [6] composed of 1.1% ammonium sulfate, 0.02% magnesium sulfate, 0.002% calcium chloride, 0.1% monobasic potassium phosphate, 0.1% anhydrous dibasic potassium phosphate, supplemented with 0.0003% manganese sulfate, 0.0003% zinc sulfate, and 0.0001% cobalt chloride or solid media used for strain plating consisted of MLN with the addition of 1.5% agar for polymerization (mass:volume).

The pre-inoculum consisted of LB supplemented with CA at 300 mg L^{-1}, with the addition of *Bacillus subtilis* TG 3.48 cell suspension, incubated at 28.0 °C with agitation at 150 rpm, initiated at pH 6.0, and cultivated for 12 hours. The amount of biomass used in the assays was determined by optical density at 600 nm (OD600nm) analyzed by spectrophotometry, calculating the observed result to initiate fermentation with an initial biomass of ≅ 0.05 OD600nm [5].

The experimental unit consisted of 250 mL Erlenmeyer flasks containing 150 mL of mineral medium, initiated at pH 6.0 supplemented with 500 mg L^{-1} (0.05%) glucose and 300 mg L^{-1} (0.03%) CA. The experiments were conducted in triplicate, accompanied by abiotic and biotic controls. The culture was incubated at 28.0 °C with orbital agitation at 150 rpm.

In the laminar flow chamber, 3 aliquots of 2 mL were taken every 15 minutes until one hour of incubation was completed. The aliquots were used for bacterial growth measurement through OD (OD600nm), pH measurement, determination of glucose consumption by the reducing sugar technique, and quantification of CA biotransformation into metabolites by HPLC. The bioconversion of CA acid was also evaluated under the same experimental conditions described above, but without the addition of glucose to the medium.

RESULTS & DISCUSSION

The experiment in Fig. 1 shows that 80% of CA was consumed within 15 minutes of incubation, demonstrating a rapid assimilation of the compound. By the end of the 3-hour cultivation period, the concentration was reduced to 92% of the initial amount. Conversely, simultaneous with the consumption of CA, there was a gradual increase in 4VF, reaching a yield of 92% after 3 hours of cultivation. It is noteworthy that the two curves, for CA consumption and 4VF generation, are inversely proportional, indicating a highly efficient direct conversion. There was a continuous increase in biomass during fermentation, quadrupling by the end of the 3 hours.

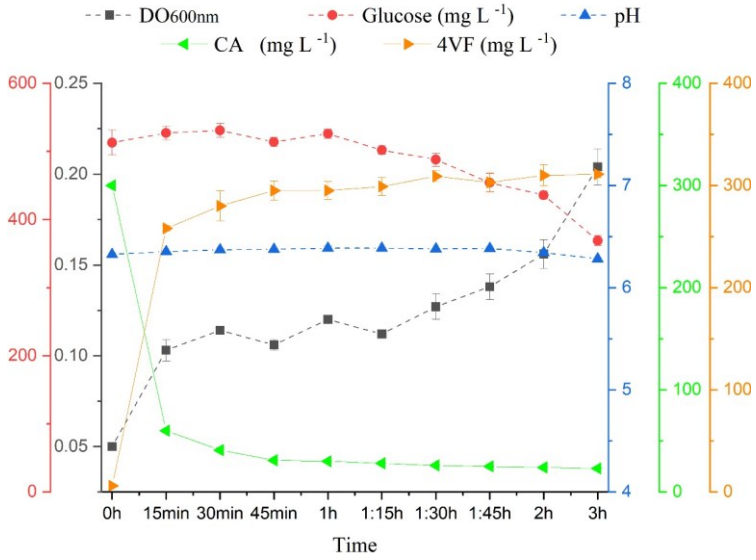

Figure 1 Bacterial growth profile of the *Bacillus subtilis* TG 3.48 strain, CA consumption, 4VP formation, and pH in culture medium with glucose.

Although there are studies in the literature on the bioconversion of CA at higher concentrations in synthetic and hydrolyzed media than reported in this work, the results of the *Bacillus subtilis* TG 3.48 strain remain quite promising [7] [8]. Most of the observed conversion occurs within the first 15 minutes of cultivation, with a high yield also in the production of 4VF.

Based on the results obtained in the previous assay, it was suggested that the bioconversion of CA to 4VF at 300 mg L^{-1} could occur independently of the presence of glucose in the culture medium. Therefore, seeking confirmation of the premise, another experiment was conducted under the standardized cultivation conditions of the previous assay, but without glucose.

The assay in Fig. 2 showed that bacterial growth was reduced by half, with the yield of 4VF in the bioconversion of CA being more affected only in the first hour of fermentation. However, in the end of the 3 hours, a yield of 89% was achieved, almost the 92% obtained in the medium containing glucose.

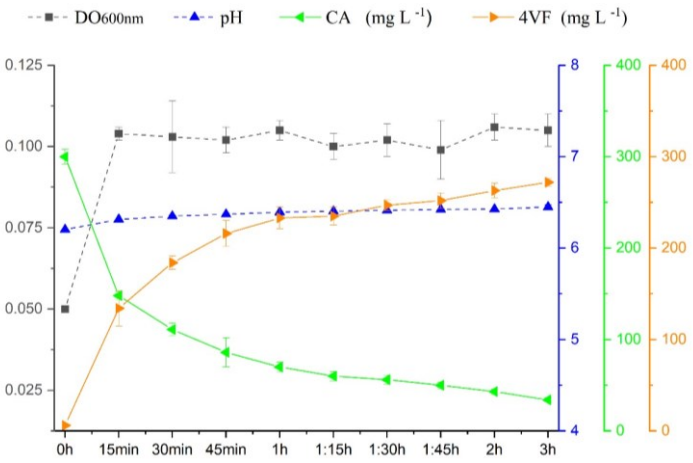

Figure 2 Bioconversion of CA to 4VP by the bacterial strain *Bacillus subtilis* TG 3.48 in culture medium without glucose.

Although there are no reports in the literature of bacterial biotransformation of CA to 4VF in synthetic media without additional carbon sources, there are reports of metabolic pathways of CA leading to the degradation of the aromatic compound under unfavorable conditions [9]. However, no other phenolic compounds were observed in HPLC. It is more likely, therefore, that the occurrence of these suggested metabolites would be observed in later stages of cultivation.

CONCLUSION

The experimental data indicated that among the initially selected bacterial strains, only the *Bacillus subtilis* TG 3.48 was able to tolerate CA at a concentration of 300 mg L^{-1} without significantly affecting its growth. The strain was capable of bioconverting CA acid into 4VF with a yield of 92% in 3 hours of cultivation. HPLC analyses, used for quantifying the consumption of CA and formation of 4VF, did not indicate the presence of other phenolic compound, suggesting that the conversion occurred without other intermediates. The bioconversion was also conducted in medium without glucose, confirming the ability of the mentioned strain to perform the bioconversion under this condition. However, there was a decrease in the capacity to consume CA and form 4VF reaching 89% of yield. The hypothesis is the bacteria may utilize CA as exclusive carbon source; however, it was not possible to observe the identification of any other metabolites besides 4VF in HPLC analysis.

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