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Analysis of the biotransformation of (-) - cubebin in (-) - hinokinin by *Aspergillus Niger*

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ABSTRACT

Biotransformation of natural products by means of various microorganisms is a method for the production of new bioactive substances. The (-)- hinokinin has an important trypanocidal activity and can be obtained by the biotransformation of (-)- cubebin by the fungus Aspergillus niger. However, the ideal conditions in which the micro-organism is capable of promoting the reactions have not yet been established. Thus, this work proposes to establish ideal conditions of cultivation of this fungus in order to reach higher concentration and productivity of (-)-hinokinin in crops. For this, different culture media were analyzed for selection of the medium that produces high concentration of cells in suspension. In addition, the (-)- cubebin biotransformation process was performed to analyze whether the strain produces the compound of interest. The results showed that the PDB medium is the most indicated to be used. In addition, it was observed that the strain is capable of producing (-)- hinokinin in this culture medium after 8 days of cultivation. The results, although preliminary, are of great importance for the trials being carried out in order to understand the mechanisms that (-)- hinokinin are obtained from the biotransformation of (-)- cubebin for future production in bioreactors .

Key words: Biotransformation, Aspergillus niger, (-) - cubebin, (-) - hinokinin.

1. INTRODUCTION

Biotransformation is a process in which organic compounds are chemically altered through enzyme catalyzed reactions contained in cellular systems (Arakawa, 2007). Compared with chemical processes, it presents some relevant advantages of this, using sustainable methodologies, known as "Green Chemistry" (Meyer & Turner, 2009)

The biotransformation of substances isolated from plants has been highlighted in several works due to the relevant biological activities, such as (-) - cubebin (CB), a lignan that can be extracted mainly from the dried seeds of piper cubeba. From the prototypes already obtained from the CB stands out the (-)-hinokinin (HQ), which demonstrates important trypanocidal activity (De Souza et al., 2005).

HQ can be obtained by oxidation of CB, where a good yield is observed (Esperandim et al., 2012). However, there is the disadvantage in obtaining this molecule by this process due to the use of a selective oxidation catalyst, PCC (pyridinium chlorochromate), which is a toxic reagent containing Chromium and having carcinogenic potential (Andrade et al. 2009). In the context of "Green Chemistry", Arruda 2015 , demonstrated on a small scale that the HQ can be obtained from the biotransformation of CB using the *A. niger* fungus, reporting the capacity of this biotechnological process to obtain this molecule.

Considering the importance of this molecule and its relative potential, it is necessary: (i) to understand the biotransformation mechanism of the fungus; (ii) studying the culture conditions so as to be able to identify the ideal biotransformation conditions of the compound and finally (iii) improving the process in order to increase the concentration and productivity of the molecule of interest.

2. METODOLOGY

I- Growth curve and kinetic parameters in different culture media

In this step, assays were carried out to construct the kinetic growth curve in four different culture media pre-established in the literature for *A. niger* cultures. For this, periodic samples were taken and then the cell concentration was analyzed by dry mass measurement by the method described by Olsson and Nielsen (1997). The medium tested were: **Medium 1**: PDB (Potato Dextrose Broth) 24 g / L, **Medium 2** whose composition is: glucose 10g / L, peptone 5g / L, K_2PO_4 5g / L, yeast extract 5g / L, glycerol 5mL / L, NaCl 5g / L; **Medium 3**: 10g / L yeast extract, 10g / L malt extract and 20g / L glucose; **Medium 4**: Proposed by Jackson et al. (1993). These experiments were performed in shaker at 200 rpm and 30°C.

II - Tests with addition of (-)-cubebin

To verify if the strain used produces the compound of interest, the A. fungus was inoculated into the PDB culture medium under the above conditions of stirring and temperature, containing CB at concentrations of 0.2; 0.4; 0.6; 0.8 and 1 g / L (concentrations established on the basis of MIC assay, data not shown). For conversion monitoring, daily aliquots of 1 mL 3x partitioned with ethyl acetate were taken. Qualitative analyzes for the presence of HQ were performed on HPLC using a Phenomenex® C18 column of 250x460 mm diameter in a mobile phase of acetonitrile / Acetic acid + 1%, in the proportion 6: 4 at wavelength of 235 nm.

III- Trials in progress

In order to understand the mechanism of biotransformation by A. niger, an experiment is underway in different culture conditions regarding the addition of CB in the medium. For this, the addition of CB (0.2 g / L) was performed in four different ways: (i) in the stationary growth phase in PDB medium, in the same growth medium; (ii) in the culture medium (fermented wort) without the presence of the cells, which were removed after the onset of the decline phase. (iii) in the PDB culture medium with the dead biomass, also after the decline phase; (iv) In CZAPEK medium. In the latter, the cells were cultured in PDB medium until the decline phase. After, the biomass was filtered and transferred to this medium. All experiments are performed in duplicates and grown in shaker at 200 rpm and 30 ° C.

3. RESULTS AND DISCUSSION

In the assays for growth curve construction, the results showed that the best culture medium to be used is the PDB, because although it did not present the highest production of cells in comparison with the other media tested, it favored the growth of cells in suspension , An important factor for bioreactor trials **(Figure 1).**

In the tests for the verification of formation of the product of interest, the production of the HQ in all the tests was observed for all concentrations of CB analyzed after 8 days of culture, represented in Figure 2. For identification of the molecule, it was compared with The standard at the retention time (RT) (6.7 min). As a crude extract, without the purification of substances, other peaks were observed in the sample and may indicate the presence of fungal metabolites. **(Figure 2).**

The data obtained in this work support the basis for the tests being carried out in order to evaluate the conditions under which conditions the fungus is able to transform CB into HQ to increase production and develop a process system in bioreactors.

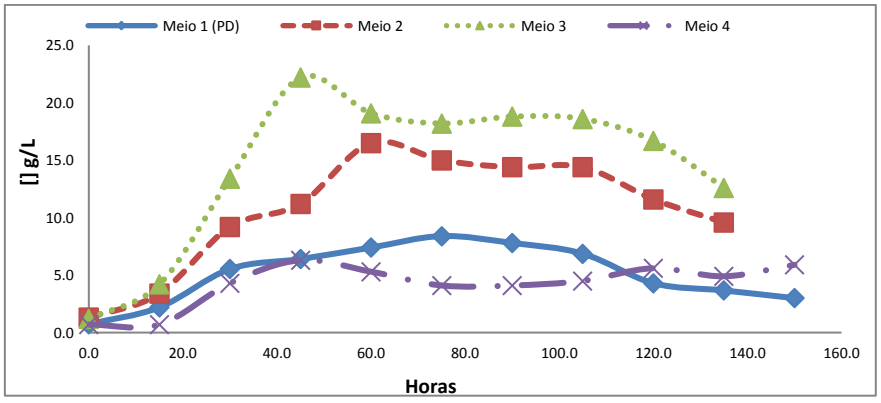

Figure 1: Growth curve in different culture media

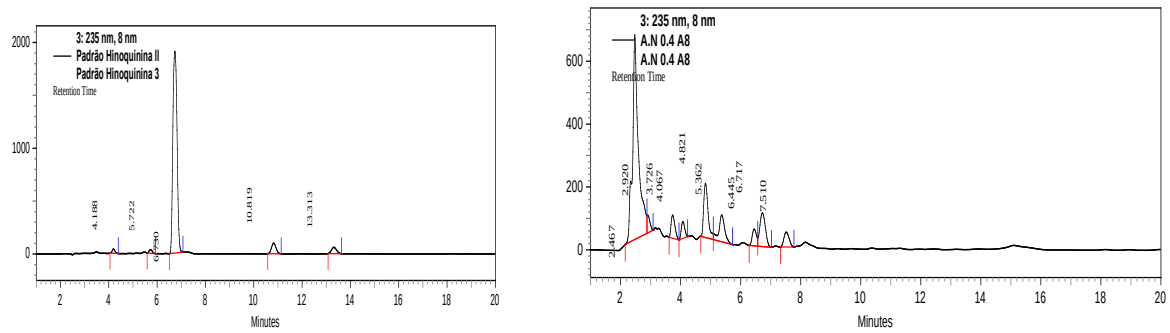

Figure 2: Chromatogram of the standard (-) - Hinokinin and Chromatogrma after 8 days of culture

4. CONCLUSION

Until now, it can be concluded that the PDB medium is suitable for the biotransformation experiments, since there was a high cell growth and also promoted the culture of the cells in suspension, an important factor for subsequent reactor culture. In addition, it can be concluded that the *A. Niger* strain adopted for this research is capable of biotransforming the (-) - cubebin into (-) - hinokinin, a fact observed after 8 days of cultivation through a qualitative HPLC analysis .

5. REFERENCES

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