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Isolation of native *Aspergillus niger* from Ecuadorian Amazon to produce citric acid from sugarcane bagasse.

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Abstract: Amazonian fungal identification is crucial to unravel their biodiversity and to elucidate their potential use in several industrial and biotechnological processes. This research aims to isolate and identify *Aspergillus niger* from Ecuadorian Amazon region in order to assess their capability to produce citric acid from sugar cane bagasse fermentation. Sampling point were settled to perform the distribution pattern of the fungi in the main areas of sugar cane production in Puyo municipality. For isolation, raw material was placed under humid chambers to produce sporulation. After growth, isolates were plated in Potato Dextrose Agar media for purification. MorpHo-cultural characterization was assessed for isolates identification. A complete randomized experimental design was conducted under controlled conditions to search out the capability of isolates to produce citric acid. Six days fermentation at pH 2 and three levels of substrates loading (20, 30 and 40 mg bagasse L^{-1}) were the experimental variables. Bagasse was cut into small pieces to homogenization and a concentration of $1x10^7$ spores ml^{-1} was inoculated. Morpho-cultural analysis threw four isolates with features related to *A. niger*. Black aerial mycelia, fast growth and copious sporulation matched with the main characteristics of *the fungus*. The citric acid assay showed the most favourable conditions were provided by the substrate loaded with 30 mg L^{-1} of bagasse, which yielded 9.9 g of citric acid per kilogram of bagasse. These results show the potential of native Amazonian *A. niger* to produce citric acid and to perform another trials with other raw material under different conditions.

Keywords: Inoculum; Substratum; Fermentation

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1. Introduction

Organic acids are products derived from microbiological processes that are used for various applications. One of the most widely used organic acids is citric acid because of its low toxicity compared to other acidulants (Mostafa & Alamri, 2012).

The *Aspergillus niger* filament fungus plays an important role in the field of biotechnology, is outstanding at the industrial level both in the exploitation of organic acids as well as for hydrolytic enzymes(Andersen et al., 2011). It effectively degrades the major polysaccharides found in cell wall plant cellulose, hemicellulose and pectin (De Souza et al., 2013), And is one of the leading producers of commercial enzymes for the conversion of plant biomass due to its high capacity for enzyme secretion (Tamayo-Ramos & Orejas, 2014). *A. niger* is the preferred fermenting microorganism in the production of citric acid because of its high yield per unit time even at low pH, with the ability to ferment a wide range of cheap substrates (Narasimha, Kumar, Srilakshmi, & Hariveeran Goud, 2012). The use of new biotechnological processes using fungi has gained great interest at the present time in the production of citric acid as compared to the chemical synthesis that is an expensive procedure. Submerged fermentation is the most widely used method worldwide, however, new studies in solid state fermentation generated significant amounts of citric acid (Javed, Asgher, Sheikh, Nawaz, & Jamil, 2011; Narasimha et al., 2012).

In 2008, the production of citric acid was approximately 1.6 tons and is expected to increase in the coming years (Radwan et al., 2010). At present, due to the demanding demand for citric acid has increased the tendency for the use of agroindustrial residues, the obtaining of citric acid by means of by-products generates an important combination of reutilization of waste materials (Husseiny, Helemish, Younis, & Farag, 2010).

The objective of this research is to isolate and identify *A. niger* from the Ecuadorian Amazon to produce citric acid from bagasse.

2. Results and Discussion

The fungus isolated from cane bagasse samples was identified as *A. niger* by its macroscopic and microscopic characteristics (Figure 1).

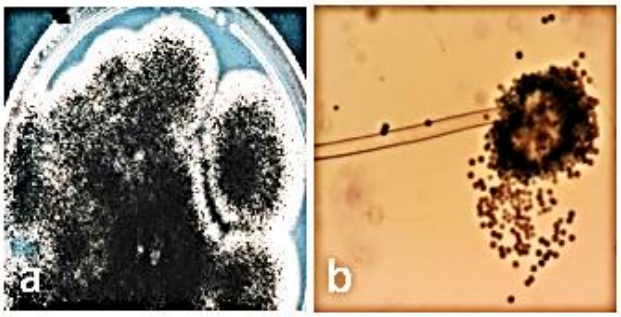

Figure 1. Macroscopic characteristics (a) and microscopic (b) *A. niger*

Macroscopic characteristics: the color of the obverse is identified black to grayish black and colorless reverse, velvety texture, filamentous and with a speed of growth of 11,25 mm d^{-1} (Abarca, 2000).
Microscopic characteristics: length of conidiophores 3.2 mm, diameter of conidiophores 2.96 mm and conidia globose to ovoid shape, smooth or slightly rough (Table 1) (Abarca, 2000).

Table 1. Characteristics de *A. niger*

Macroscopic characteristics		
Color	*Texture*	*Growth rate (mm d^{-1})*
Black	Filamentous	11,25
Microscopic characteristics		
Conidiophores lenght (µm)	*Conidiophores diameter (µm)*	*Conidia shape*
3,2	2,96	Globose

On the sixth day the citric acid was identified and quantified by the consumption of NaOH and then the slope calculation of each of the substrate loading values (Table 2).

Table 2. Concentration of citric acid

Substrate loading (mg L^{-1})	Acid citric concentrate (mg Kg^{-1})
20	0
30	9,6
40	9,5

In the present study the maximum production was observed in the conditions established from pH 2 until the sixth day of evaluation with a substrate load of 30 g L -1 resulting in the production of citric acid of 9.9 g of citric acid per kg Bagasse (Amenaghawon, Areguamen, Agbroko, Ogbeide, & Okieimen, 2013).

3. Material and Methods

Isolation and identification of the citric acid-producing fungus

The culture of the fungus was isolated from cane bagasse samples by means of humid chambers allowing development of the fungus, later isolated and purified in potato Dextrose Agar culture medium to determine their morpho-cultural characters, thus allowing the identification according to The macroscopic and microscopic characteristics of the fungus (Luna, Lozada, & Trigos, 2010).

Substrate and pretreatment

The cane bagasse for the fermentation process was obtained from sectors that are engaged in the production of panela in the Province of Pastaza. Drying was performed in a stove to determine the drying curve, milled and sieved to a particular size of 0.5 mm. The acid hydrolysis was performed with H_2SO_4 2N for 24 hours, washed with sterile water and then dried

Inoculum

The spore suspension of *A. niger* had a concentration of 2 x 107 ml mL^{-1} by adding 25 mL of sterile water with two drops of 20% Tween onto a Petri dish, shaking vigorously to allow release of the spores (Amenaghawon et al., 2013).

Fermentation in solid state

The solid state fermentation was carried out in 250 ml Erlenmeyer with cane bagasse, which was enriched with sucrose medium (g/L) (sucrose, 310, $CuSO_4$, 0.04 and methanol 4% w/v) in such a way that the moisture containing up to 75% of the flask containing the fermentation medium with an inoculum volume of 0.5 mL at a spore concentration of $2x10^7$ and then incubated at 30°C (Amenaghawon et al., 2013).

Design of the experiment

A three-factor design was performed on the sixth fermentation day with a pH of 2 and the substrate loading (20, 30 and 40 mg bagasse L^{-1}). As a result of this design, 3 experimental runs were performed according to the conditions shown in the table 3.

Table 3. Design and experimental runs

N°	pH	Timer (d)	Substrate loading (mg L^{-1})
1	2	6	20
2	2	6	30
3	2	6	40

Finally, the methodology used for the quantification of citric acid is based on the titratable total acidity and the neutralization curve, the results are represented in g of anhydrous citric acid per kg of bagasse (Pando, Hérdez, & Jacques, 1978).

4. Conclusions

The Ecuadorian Amazon is home to numerous microorganisms that are used for biotechnological processes, the fungus from bagasse samples was identified as *A. niger* and used as a fermentor fungus to obtain citric acid.

Cane bagasse is an excellent substrate for obtaining citric acid from *A. niger* as a fermenting microorganism. The production of citric acid is directly related by pH, fermentation time and substrate loading.

The best citric acid yield was observed at pH 2, fermentation time of 6 days and a substrate load of 30 mg L-1, under these conditions the citric acid concentration was 9.9 g citric acid per Kg of bagasse.

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Conflicts of Interest

The authors declare no conflict of interest.

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