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Taxonomic Approach to *Aspergillus* sp. Isolated from Caatinga Soil and Potential to Amylase Production

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A B S T R A C T

Filamentous fungi are very promising for the productions of many products in special amylases are used in several industrial areas. The aim of this work was filamentous fungus isolation from Caatinga characterized soil, and evaluation of the biotechnological potential to amylase production. The Caatinga soil was characterized containing high level of Calcium, followed Magnesium, Potassium, Sodium and Phosphorus, and absence of Aluminium. The fungus isolated from Caatinga soil was identified as *Aspergillus* sp., code UCP 1261. The diffusion test to amylase production showed influence of pH 6 and temperature on the production of amylase production in solid by *A. tamarii*. The results showed that the sample identified was *Aspergillus tamarii* UCP 1261 showed a higher halo (68mm) of amylase at 37ºC, pH 6 and 72h.These results described the biotechnological potential of filamentous fungi *A. tamarii* isolated from Caatinga biome soil isolated from Caatinga biome soil as promising strain for the production of amylase.

Introduction

Filamentous fungi are very promising for the production of many compounds of biotechnological and industrial interest, due to the great variety of catalytic activities, easy adaptation and possibility of enzymatic production.

There are several enzymatic productions described in the literature through biotechnological processes with the use of filamentous fungi of the genus *Aspergillus* (Moreira, 2013; Cunha, 2016).

The wide diversity in the characteristics of the enzymes potentiates their application in different processes in the industry, thus, faced with the demand for new amylases with prospect of applicability in the sector, there is a natural stimulus for the exploration of the microbial biodiversity, with the isolation and selection of new strains enzyme producers (Baratto, 2012).

The use of amylases in several areas of industry causes microorganisms to be

increasingly studied and their enzymes characterized, providing a range of knowledge. Thus, the study of the amylolytic capacity of these microorganisms represents an important advance in the knowledge of the possibilities of applying their potential for the production of enzymes of industrial interest, such as amylases (Bastos *et al.,* 2015).

Materials and Methods

Microorganisms

This study were used three samples of *Aspergillus* UCP 1392, *Aspergillus flavus* UCP 1383 and *Aspergillus niger* UCP 1382) isolated from Caatinga soil at Pernambuco, Brazil, and they strains were deposited in Culture Collection UCP (UniversidadeCatólica de Pernambuco), in the Nucleus of Research in Environmental Sciences and Biotechnology, Catholic University of Pernambuco, Recife, Brazil.

Soil sample, chemical and granulometric analysis

For the isolation of filamentous fungus, soil samples were collected from an area of the Caatinga located in the municipality of Serra Talhada, Pernambuco, Brazil. Different points of the site were previously demarcated and the area separated into homogeneous areas. Soil samples will be collected in zigzags, every 20 points of the field at 20 cm depth and taken to the Laboratory of the Nucleus of Research in Environmental Sciences and Biotechnology (NPCIAMB), Catholic University of Pernambuco.

Obtaining monosporic culture

Lineage spores were transferred from the test tube to a screw cap glass containing 2.0 mL of 0.1% Tween 80 solution 100 mL of water. The suspension was shaken to disaggregate the spores. The spores were counted in hematimeter chamber and appropriate dilutions were done in order to obtain from 20 to 100 colonies each Petri dish containing BDA culture medium (4.0 g potato, 20.0 g dextrose, 15.0 g Agar, 1000 mL of distilled water, pH 5.6 + 0.2 at 25°C). Plates were maintained at room temperature. Spore germination was observed from 12 to 72 hours of growth. After germination of the spores with the aid of the magnifying glass, a single spore was transferred to a test tube, containing BDA, for the development of the colony.

Fungus identification

The strain was identified according to the *Aspergillus* identification manual described by Klich (2002) and Samsom *et al.,* (2005). The isolate was inoculated in the following culture media: Czapek yeast autolysate (CYA) agar, (Czapek concentrate 10 mL, sucrose 30g, yeast extract 5g, K_2HPO_4 1g, agar 20g and distilled water 1000 mL) and Malt Extract Agar (MEA) (malt extract 20g, peptone 1g, glucose 20g, agar 20g and distilled water 1000 mL). The media were adjusted to pH 6 and autoclaved at 121°C for 15 minutes. Inoculations were made from spore suspension in a semi-solid agar solution containing 0.2% and 0.05% Tween 80, using a micropipette for inoculation at three equidistant points and incubated for seven days at 25 and 37°C. After 7 days, the diameters of the colonies were measured and the macroscopic characteristics were observed: colony texture, sporulation degree, conidia color, texture and color of the mycelium, presence of pigment colors and exudate.

For the microscopic observations, the mycelium was removed from the areas where the adjacent colonies were closer, from the area of the colony where the conidia were

beginning to develop. The slides were assembled with lactic acid (85%) and drops of 70% ethanol to disperse the conidia and to prevent air bubbles when mounted on lactic acid. Microscopic structures, such as length, width and texture of conidiophores, conidial head shape, vesicle diameter, length of metulae (if present) and phialides, diameter, shape and texture of ascospores and conidia (if present), form and color of cleistothecia/ sclerotia (if present).

Evaluation of amylase production

To determine the amylase-producing *Aspergillus*spp sample, tests were performed to determine the amylase activity in solid medium according to the Hankin and Anagnostakis (1979) methodology, adding 2% soluble starch to the medium. The culture medium was dispensed into Petri dishes, and after solidification holes were drilled in the center of the plates and inoculated with 100 µL of the fungal spore suspension. The plates were incubated at different temperatures (28, 37 and 45°C) and pH (6; 7; 8) for 96 h. After the enzymatic detection period, they were developed with a 0.1 N solution of iodine for 5 minutes. The formation of a transparent halo around the grown colonies evidenced the presence of amylase. All assays were performed in triplicate.

Results and Discussion

Caatinga soil chemical analysis

The analysis of the physical characteristics of the Caatinga soil was during the summer season that showed in its composition the presence of sand between 33 and 23g Kg^{-1} and of clay between 21 and 23 g Kg^{-1} according to granulometry test. For the gravimetric test, 9.68% moisture was detected, pH around 5.9. The study of the chemical composition of the soil was detected of high level of calcium, followed magnesium, sodium, potassium, and phosphorus, and absence of aluminum according to Table 1.

Filamentous fungus identification

According to classical identification, based on the macroscopic and microscopic characteristics, the isolate belongs to the species *Aspergillus tamari* Kita. The colonies grown in MEA showed white mycelium, colorless reverse, conidiophore of irregular lengths that guarantee a coarse texture and conidia were yellowish-brown with aging of the colony. In CYE, they had a diameter of 70 mm at 25 and 37°C showing white mycelium, colorless to yellowish gray; Colony floccose to flat, conidia of olivaceous brown color with the aging of the colony.

Microscopic observations showed radial to columnar conidial heads, conidiophores with a rough and colorless surface, globular pyriform vesicles between 20 and 45 µm wide, predominantly biseriate (presence of metulae and phalides), as shown in Figure 1A, rarely was found uniseriate (only phialides), metulae/phialides in most cases covering the whole surface of the vesicle, metulae reaching 4 to 8µm and phialides between 4 and 6 mm in length.

Globose conidia, roughly rough with thick walls, as shown in Figure 1B presenting roughly 5µm to 8µm in diameter, rarely 13µm in diameter.

The data reported correspond to the studied literature Klich (2002), which mentions being a species originally isolated from the tamari sauce and also considered a common fungus of the soil, especially in tropical soils. The Caatinga region is one of the most threatened biome in Brazil, where much of its area has been desertified, mainly due to extreme weather conditions.

Table.1 Chemical characteristics of the Caatinga soil of the state of Pernambuco, Brazil

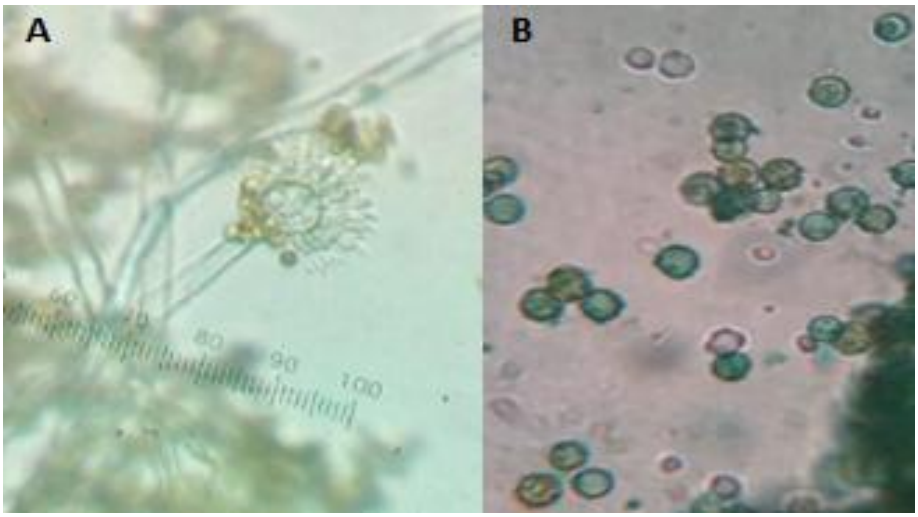
Chemical elements	Content
Calcium	8.6 cmolc / dm 3
Magnesium	3.55 cmolc / dm 3
Sodium	0.01 cmolc / dm 3
Potassium	0.15 cmolc / dm 3
Aluminum	Negative
Phosphorus	2.0 mg / dm3

Table.2 Influence of temperature and pH for detection of amylase production by *Aspergillus* strains for 72 hours of culture

		Temperature (ºC)/Amylase Halo (mm)		
Fungi	pH	28	37	45
Aspergillus tamari UCP 1261	6	43	68	53
	7	41	65	35
	8	40	37	51
Aspergillus UCP 1392	6	44	42	51
	7	42	31	51
	8	46	43	40
Aspergillus flavus UCP 1383	6	-	-	-
	7	-	-	-
	8	-	-	-
Aspergillus niger UCP 1382	6	-	-	-
	7	35	30	29
	8	29	-	-

(-) No formation of the characteristic halo

Fig.1 *Aspergillus tamarii*. (A) Conidia head (400x); (B) conidia (1000x)



Despite adverse weather conditions there, such as solar radiation, low rainfall, etc., many fungal species are isolated from the soil of the semi-arid region. Cavalcanti and Maia (1994) isolated cellulolytic fungi in the Caatinga semi-arid soil. Costa *et al.,* (2006), studied *Hiphomycetes* of soil contaminated by ores in the semi-arid region of the Northeast, Santiago and Souza-Motta (2006) that isolated Mucorales from the Caatinga soil. Several species of the genus *Aspergillus* and among other genera were isolated from the Xingó region in the Northeast by Queiroz *et al.,* (2006), among them 28 *A. tamarii* strains were found in the three Alagoan municipalities studied. *Aspergillus* species showed influence of temperature and pH on amylase production. According to the data, *Aspergillus tamarii* (UCP 1261) showed higher potential to produce amylase extracellularly after 72 hours of incubation at pH 6 at 37ºC, resulting in the formation of characteristic halo with a diameter of 68 mm (Table 2). Results considered inferior were obtained by Souza *et al.,* (2008), after testing the potential of *Basidiomycetes* samples in the amylase production, which resulted in the characteristic halo formation with dimensions between 6.20 and 22.30 mm after growth at 28ºC. Moreover, Alves *et al.,* (2002) carried out the study of amylase production with *Mucor* spp. and observed results similar to those obtained in this study, demonstrating the formation of the characteristic halo with dimensions between 46-85 mm after 72 h.

Conflict of Interest

The authors confirm that this article content has no conflict of interest.

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