Journal of Biosciences and Medicines, 2020, 8, 152-164
https://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

Isolation of Filamentous Fungi from the Caatinga Region and Production of Amylolytic Enzymes of Great Industrial Interest

Ingrid Cristina Soares Amorim[1], Gessica Oliveira Marinho[2], Tarcisio Michael Ferreira Soares de Oliveira[1], Juan Pedro Bretas Roa[1], Arlete Barbosa dos Reis[1], David Lee Nelson[2], Thiago Machado Pasin[3*], Vivian Machado Benassi[1]

[1]Instituto de Ciência e Tecnologia, Universidade Federal dos Vales dos Jequitinhonha e Mucuri, Campus JK, Diamantina, Brasil
[2]Programa de Pós-Graduação em Biocombustíveis, Universidade Federal dos Vales dos Jequitinhonha e Mucuri, Campus JK, Diamantina, Brasil
[3]Departamento de Bioquímica e Imunologia, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, Brasil
Email: *thiagopasin@hotmail.com

How to cite this paper: Amorim, I.C.S., Marinho, G.O., de Oliveira, T.M.F.S., Roa, J.P.B., dos Reis, A.B., Nelson, D.L., Pasin, T.M. and Benassi, V.M. (2020) Isolation of Filamentous Fungi from the Caatinga Region and Production of Amylolytic Enzymes of Great Industrial Interest. *Journal of Biosciences and Medicines*, **8**, 152-164.
https://doi.org/10.4236/jbm.2020.811014

Received: October 14, 2020
Accepted: November 16, 2020
Published: November 19, 2020

Copyright © 2020 by author(s) and Scientific Research Publishing Inc.
This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).
http://creativecommons.org/licenses/by/4.0/
Open Access

Abstract

The industrial importance and the high cost of the commercial amylase require the study of microorganisms that produce these enzymes. For this reason, the objective of this work was to isolate filamentous fungi from a region of the caatinga and evaluate their potential for the production of amylase. Four soil samples were collected from a deactivated dump located in the city of Diamantina, MG, in a region of the caatinga. The analysis of amylolytic production in a submerged medium at the ideal temperature of each microorganism was performed using the saccharification method, and the reducing sugars formed were quantified by DNS. Fourteen filamentous fungi were isolated, which had different morphological aspects. Regarding amylase production, a mean activity of 0.477 $U \cdot mL^{-1}$ was obtained with the isolates I 1.2.1 and I 4.4.1. These results bring important information regarding the biodiversity of the caatinga, in addition to the isolation of microorganisms that can be used as biological machinery to obtain metabolites with high biotechnological and industrial potential.

Keywords

Amylolytic Enzyme, Bioprospecting, Filamentous Fungi, Industrial Interest

1. Introduction

Brazil is one of the countries with the greatest diversity of living organisms, including fungi that are found in abundance in different habitats [1]. The prospecting of these organisms is considered important because they represent a rich source of genetic resources for application in numerous biotechnological processes and products [2].

Enzymes are pre-eminent among the metabolites of technological importance. They can be obtained from the most diverse organisms, such as vegetables, animals and microorganisms such as bacteria, yeasts and filamentous fungi. However, according to Freitas *et al.* [3], it is a less costly and simpler process to obtain enzymes from fungi because they require easy maintenance and they excrete the enzymes to the external environment as a natural process that allows fungi to obtain their nutrients.

Microorganisms are considered be the largest biological source for the production of enzymes, and filamentous fungi are responsible for 60% of the total industrial enzymes, followed by bacteria and yeasts, which together total 40% [4]. Enzymes are biological catalysts, involved in the hydrolysis of different biomasses because they perform specific actions in the degradation of the substrate of interest [5]. Of the enzymes used in industry, approximately 80% correspond to hydrolases, including proteases and amylases.

Amylases are enzymes that act synergistically in the hydrolysis of the glycosidic bonds of the starch molecule, hydrolyzing it into maltooligosaccharides. These enzymes lead the world production on an industrial scale because they are widely used in industry in the most varied sectors [6] [7]. However, the high cost of production requires the search for good amylolytic producers, as well as the standardization of the cultivation of these organisms using less expensive carbon sources, such as agro-industrial waste.

Enzyme production by fungi is a natural process that can be affected by different parameters, and these parameters must be investigated because different microorganisms have different optimum conditions for the production of enzymes. Thus, the composition of the media directly influences enzyme synthesis [8].

This fact was confirmed by Oliveira *et al.* [9] when they showed that the enzymatic potential of microorganisms is directly related to the media and temperature conditions in which they develop. For this reason, our work sought to isolate filamentous fungi from four different samples, to analyze the macroscopic morphological characteristics of the isolates, and to verify the potential for amylase production by these microorganisms.

2. Material and Methods

The tests were conducted at the Laboratório de Biocombustíveis of the Instituto de Ciência e Tecnologia (ICT) of the Federal University of the Valleys of Jequitinhonha and Mucuri (UFVJM), JK campus, Diamantina, Minas Gerais, Brazil

and in the Laboratório Integrado de Pesquisa e Ensino Multiuso (LIPEMVALE). The microorganisms were registered in the Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado (SisGen), number A64AD93.

2.1. Collection, Isolation and Maintenance of Filamentous Fungi

Four different soil samples were collected with characteristics of the caatinga located in Diamantina, MG, at coordinates 18˚12'19.5"S and 43˚34'13.3"W, altitude 1296.1 m. The collection was achieved aseptically using previously autoclaved materials. The isolation was performed in a culture medium proposed by Emerson [10] and composed of 4% Quaker® oats and 2% bacteriological agar. The samples were maintained in a bacteriological incubator at 30˚C for four days, and the growth of the filamentous fungi was monitored every 24 hours.

As the specimens grew, point isolation was performed in the center of another Petri dish containing the same culture medium, and the samples was maintained at 30˚C. The isolation was achieved by macroscopic observation, and the isolated fungi were identified with the letter I and three digits. The first number referred to the number of the collected sample, the second was related to the day of isolation, and the third number was related to the microorganism isolated from the specific sample.

The isolated strains were kept on silica gel, where a spore suspension was prepared in 3 ml of powdered milk (200 $g{\cdot}L^{-1}$ of distilled water). Approximately 1 mL of this suspension was added to test tubes containing 7 g of silica gel, stirred, sealed and stored at 4˚C, as previously reported by Pasin *et al.* [7].

2.2. Analysis of Macroscopic Morphological Characteristics of Isolated Fungi

The isolated fungi were placed punctually in the center of the Petri dish in solid oat culture medium [10] and incubated at 30˚C for four days. The macroscopic morphological characteristics were analyzed for texture, pigmentation, appearance, border, and colony color [11].

2.3. Analysis of Growth Temperature of Isolated Microorganisms

The organisms were cultured in a solid Potato-Dextrose-Agar (BDA, Himedia®) medium in a bacteriological oven at temperatures that varied from 30˚C to 50˚C, with intervals of 5˚C. The measurement of the radii of the filamentous fungus colonies was performed after 48 hours of growth of the microorganism, and the growth rate was calculated in centimeters per hour ($cm{\cdot}h^{-1}$).

2.4. Analysis of Amylase Production in Submerged Culture Medium

Four isolated fungi that presented a higher growth rate per hour in solid BDA were selected to verify their amylolytic potential. The microorganisms were cultured in 50 mL of CP submerged culture medium [12] contained in a 250 mL

Erlenmeyer flask that was maintained at the ideal temperature for each isolate for seven days, without stirring in a bacteriological incubator.

For the inoculation, the cultures of the fungi were suspended in inclined test tubes containing oat medium [10] with 10 mL of sterile distilled water. A 2-mL aliquot of the spore suspension (7.5×10^{-3} spores·mL^{-1}) was inoculated in the medium. After the growth of the fungi, vacuum filtration was performed with the aid of a Büchner funnel and Unifil® filter paper for the separation of the fungal mycelial mass and the extracellular crude enzymatic extract containing the amylolytic enzymes.

2.5. Determination of Amylolytic Activity by the Saccharification Method

The determination of amylase activity was achieved by the formation of reducing sugars during incubation of the enzyme with the 1% (m/v) starch substrate in 100 mM sodium acetate buffer, pH 5.5, at 55˚C using 3',5'-dinitrosalicylic acid (DNS) [13]. The enzyme-catalyzed reaction consisted of the incubation of 500 µL of the substrate with 500 µL of the extracellular crude extract for 5 minutes in a water bath at 55˚C. After the termination of the reaction, 200-µL aliquots were removed and added to tubes containing 200 µL of DNS. A 200-µL aliquot was also removed from the reaction mixture immediately after the enzyme extract was added to the substrate, and this aliquot was transferred to a tube containing 200 µL of DNS reagent. This sample was used as the control the for reaction (time zero). Subsequently, the tubes were boiled for 5 minutes and, after cooling, 2 ml of distilled water were added. The readings were performed at 540 nm in a RayLeigh UV-2601® spectrophotometer against the zero-reaction time, in which spontaneous hydrolysis of the substrate was minimal. The method was previously standardized using a standard glucose curve (0.1 to 1.0 mg·mL^{-1}), and the enzyme activity was expressed in U·mL^{-1}, where U is defined as the amount of enzyme that hydrolyzes a µmol of substrate per minute under the test conditions.

3. Results and Discussion

3.1. Isolation of the Filamentous Fungi and Analysis of the Macroscopic Morphological Characteristics of the Isolates

Fourteen filamentous fungi were isolated from the soil samples collected, the microorganisms being morphologically distinct. Among the isolates, there was a variation in the color of the fungi including white, green, black, white with lilac, white with green and white with pink colorations. Regarding the texture of the microorganisms, 78.6% were classified as cottony; 14.3% as powdery, and only one of them, I 2.3.3, had a velvety texture (**Figure 1** and **Table 1**).

With regard to the topography of the microorganisms, 42.8% had circular borders, and the other 57.2% of the isolated organisms had irregular and regular borders. Regarding pigmentation, only 21% had pigments in the solid culture medium containing oats, and 79% did not develop any pigmentation when placed

in this culture medium. As for appearance, 28.6% were determined to be opaque, 35.7% were dry and another 35.7% were humid (Figure 1 and Table 1).

This variety of fungus with distinct characteristics is similar to the a fungus

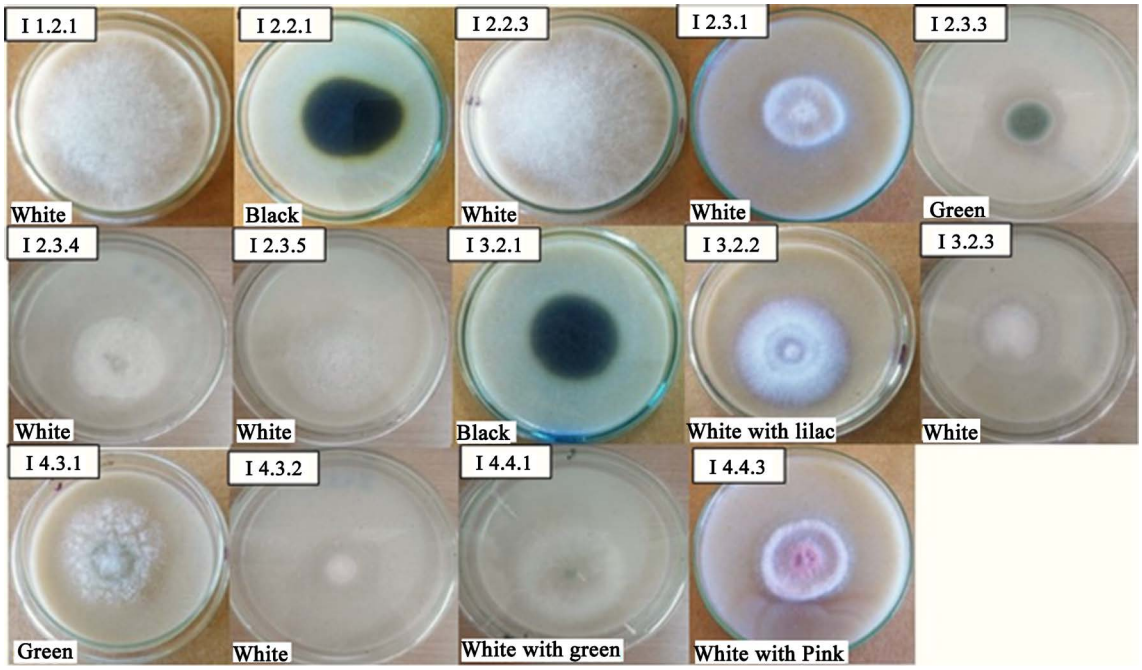

Figure 1. Representative images of the filamentous fungi isolated in solid culture medium containing oatmeal, at 30˚C.

Table 1. Macroscopic morphological characteristics of the filamentous fungi isoladed in solid culture medium containing oats after four days of growth at 30˚C.

Fungi	Macroscopic morphological characteristics			
	Color	Aspect	Border	Texture
I 1.2.1	White	Dry	Irregular	Powdery
I 2.2.1	Black	Opaque	Irregular	Powdery
I 2.2.3	White	Dry	Regular	Cottony
I 2.3.1	White	Opaque	Regular	Cottony
I 2.3.3	Green	Opaque	Circular	Velvety
I 2.3.4	White	Dry	Irregular	Cottony
I 2.3.5	White	Moist	Regular	Cottony
I 3.2.1	Black	Opaque	Circular	Cottony
I 3.2.2	White/Lilac	Moist	Irregular	Cottony
I 3.2.3	White	Dry	Circular	Cottony
I 4.3.1	Green	Moist	Regular	Cottony
I 4.3.2	White	Moist	Circular	Cottony
I 4.4.1	White /Green	Dry	Circular	Cottony
I 4.4.3	White /pink	Moist	Circular	Cottony

obtained by Benassi and Almeida [14], where they isolated forty-eight filamentous fungi from different samples collected from the areas of conserved ferruginous rupestrian field; a degraded ferruginous rock field; and a quartzitic rock field, in addition to two fractions of cassava in an advanced stage o deterioration, all within the perimeter of the UFVJM. Among the microorganisms, 52% had a cottony texture; 14.6% were powdery; 12.5% were granular; 8.3% were butyrous and velvety, whereas 4.2% had a suede texture. There is a predominance of fungi with similar morphological characteristics in both studies. However, even though they were obtained from areas in close proximity, the microorganisms were completely different. This fact emphasized the importance of prospecting process so that microorganisms with possible biotechnological and industrial potentials can be isolated.

In the work performed by Inforsato and Sette [15], the importance of seeking remote environments in search of more resistant microorganisms and often never-before-studied species is underscored. The authors explored the fungal diversity in regions that have extreme environments, such as marine sediments from Antarctica, and obtained nineteen filamentous fungi with different characteristics. This result is similar to that found in the present study.

It is known that microorganisms from extreme regions, as well as those found in the soil or in materials undergoing biodegradation, are of great commercial interest because they can produce amylases with greater stability at high temperatures and extreme pH. These characteristics are fundamental for the applications of these enzymes and justify the need for research in these locations [2] [15] [16]. In addition, prospecting furnishes new fungus strains with high amylolytic production, which is of great biotechnological interest, as well as revealing the microbial diversity of the region [17].

On the other hand, Santos *et al.* [18] isolated fifty-two fungi with characteristics similar to one another when analyzing Restinga de Guaibim in Bahia to evaluate the biotechnological potential of the region. These fungi were of the Penicillium genus. However, only 26.92% of the isolates were characterized as strong producers of amylases. Thus, microorganisms of the same genus and even with the same morphological characteristics (same species) can present different metabolisms because they come from different microenvironments and regions, and a great genetic variability can exist among them.

According to Pasin *et al.* [7], possible metabolic differences observed in the same genus and species of fungi can be explained by the fact that each fungus was obtained from a different location, so they presented a natural genetic variation. Therefore, this result reinforces the need to prospect and develop research to become familiar with the microorganisms of each region, as well as to compare their metabolisms and obtain interesting metabolites from the biotechnological and industrial point of view. It should also be noted that the plant communities found in the place where the work by Santos *et al.* [18] was performed are made up of species from ecosystems such as the Atlantic Forest, Tabuleiros

and Caatinga, as well as that observed in the present study.

A study by Almeida [19] proved that the prospecting environment directly influences the diversity and quantity of isolated microorganisms. The action of fungi on the mass of residues and the identification of these microorganisms in terms of genus and species can generate important information to improve the degradation system of Urban Solid Waste (USW) in landfills. At the end of the study by Almeida [19], a total of eighty-one filamentous fungi with different characteristics were obtained after collections performed at 12 different points of a land provided by the Universidade Federal de Campina Grande (UFCG) in the state of Paraíba.

Finally, the quantity and diversity of isolated microorganisms are influenced by several factors, such as the sample collected, the amount of sample, the time of year, and the climate, among other factors [20]. The data obtained in this work is essential to reveal the microbial diversity of the caatinga region in which they were collected, in addition to bringing to light new amylases with characteristics of commercial interest, given the possibility of being highly stable under adverse conditions.

3.2. Growth Temperature Analysis of Filamentous Fungi Isolated from Caatinga

Observing the relationship of fungal growth temperature allows us to differentiate the growth and survival states of the organisms because temperature is a crucial factor in their development [21]. Each microorganism has a temperature below which it is unable to grow. This is called the minimum temperature. Each microorganism also has a maximum temperature, where the death of the microorganism occurs. Between these two temperatures, there is the optimum temperature for growth, which is closer to the maximum temperature than the minimum [22] [23] [24] [25].

Among the fourteen isolated fungi, all the microorganisms grew when subjected to 30˚C, whereas only two microorganisms, I 3.2.3 and I 4.3.2, did not grow at 35˚C. The organisms that obtained the highest growth rate at 30˚C were identified as I 1.2.1 and I 2.2.3, both with 0.156 $cm \cdot h^{-1}$, and a small increase in the growth rate (0.160 and 0.171 $cm \cdot h^{-1}$) occurred at 35˚C (**Figure 2**).

Only four organisms grew at 40 ˚ C, and the growth rates of fungi I 1.2.1 and I 2.2.3 were 0.114 and 0.113 $cm \cdot h^{-1}$, respectively, followed by isolates I 2.3.3, with a rate of 0.010 $cm \cdot h^{-1}$, and I 4.4.1 with a rate of 0.006 $cm \cdot h^{-1}$. Growth was observed at 45˚C for only one isolate, I 2.3.3, which grew at 0.007 $cm \cdot h^{-1}$. At 50˚C, no fungus grew (**Figure 2**).

Pelczar *et al.* [26] divided the organisms into three classes according to the ideal growth temperature: psychrophils, mesophiles and thermophiles. Psychrophilic organisms preferentially grow in the range of 15˚C to 20˚C, and can develop at lower temperatures, whereas mesophiles, a group that involves most microorganisms, are those that grow best at temperatures between 25˚C and 40˚C. Some of these micoorganisms can grow at higher temperatures, so they are

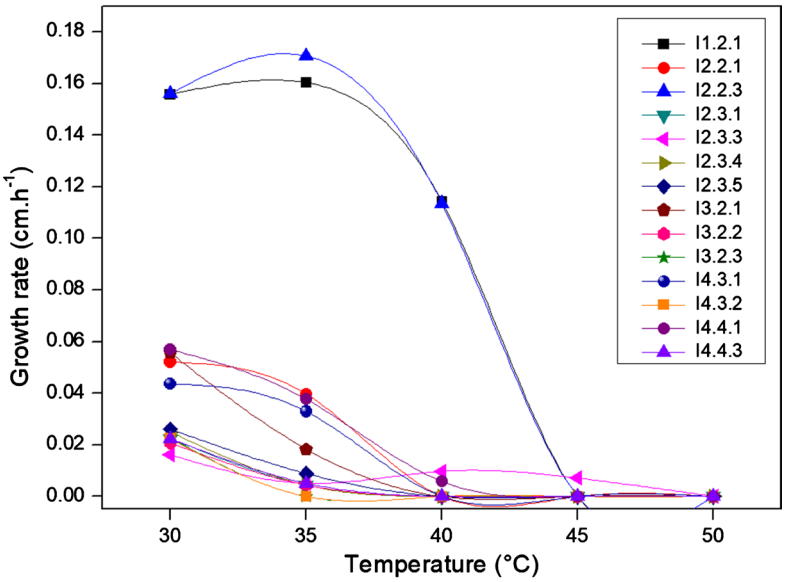

Figure 2. Growth rate of filamentous fungi in solid BDA culture medium at different temperatures.

called thermotolerant. There are also those species that develop only at elevated temperatures (40˚C to 85˚C), where the range of greatest growth is 50˚C to 60˚C, and they are called thermophiles.

Thus, it can be inferred that the filamentous fungi isolated from this work are mesophilic organisms, which have growing temperatures of 30˚C and 35˚C, are preferential for development. In addition, the results obtained can be linked to the environment of origin of these microorganisms.

According to Pasin *et al.* [27], the temperature, humidity and atmosphere of the environment of origin of the fungi are the main determinants for their development. Pasin *et al.* [2] isolated twenty filamentous fungi from a specific rural area in the state of Bahia, Brazil and observed that all the fungi developed at 30 ºC, results similar to those obtained in this study, where the preferred temperature for the growth of all fourteen microorganisms was this same temperature.

However, Izidoro and Knob [28] observed that the best growth temperature of the fungus Aspergillus niger was at 25˚C, with a decrease in the growth rate from 30˚C, a result that differs from that observed in the present study. Other studies have shown that the optimum temperature range for growth was between 25˚C and 30˚C. Some isolates grew well in the range of 30˚C to 37˚C, and only a few species were able to tolerate temperatures equal to or greater than 40˚C [29].

3.3. Screening of Amylase-Producing Filamentous Fungi

Four fungi were selected that exhibited the highest growth rate at 30˚C in the BDA solid culture medium, these being identified as I 1.2.1, I 2.2.3, I 3.2.1 and I 4.4.1. This BDA culture medium contains starch, which, according to Orlandelli *et al.* [4], is a carbon source that induces the production of amylase. Therefore, because the microorganisms grew, it can be concluded that there was an induc-

tion of the amylolytic enzyme that catalyzed the cleavage of this polysaccharide into smaller sugars for its metabolism.

These four filamentous fungi were grown in submerged CP for seven days at 30˚C, with starch as a carbon source for the production and quantification of amylolytic activity. Most of the fungal amylases described are produced by mesophilic organisms, whose growth temperatures vary from 25˚C to 37˚C [30].

Normally, submerged fermentation is the most widely used for the production of enzymes. The medium is composed of more than 95% water. In addition, it has several advantages, such as an ease of growth of filamentous fungi, better control in the culture pattern, ease of recovery of extracellular enzymes and greater homogeneity of the medium [4].

According to Pelczar *et al.* [26], the time for cultivation of a fungus influences enzyme production directly and expressively. If incubated for a short period of time, the enzymatic activity might be much lower than the maximum activity because it will be in the vegetative phase. Likewise, incubation for prolonged periods can deplete nutrients and cause a decrease in production and enzyme activity.

As a result, the fungi that produced amylases with the highest activities were identified as I 1.2.1 and I 4.4.1, whose enzymatic activities were 0.560 and 0.414 $U \cdot mL^{-1}$, respectively, followed by the microorganisms I 2.2.3 and I 3.2.1, whose activities were 0.363 and 0.225 $U \cdot mL^{-1}$, respectively. These results corroborate those observed by Silva [31], who isolated nineteen filamentous fungi and performed the screening of amylases by cultivating five of these fungi in a submerged medium with starch as the sole carbon source.

In the work by Luz *et al.* [32], ten samples were collected in mining regions, and 36 different microorganisms were obtained, of which 53% of the isolates exhibited amylolytic activity, a result different from that observed in the present study. However, it is important to note that the amylolytic activity observed so far could be optimized because it is influenced by the source of carbon, nitrogen and minerals used in the culture media, as well as the condition of aeration and incubation time [33].

Finally, the production of amylases by filamentous fungi varies according to the genus and the species involved [34]. Many species of *Aspergillus*, *Fusarium*, *Lipomycetes*, *Mucor*, *Penicillium*, *Rhizopus* and *Rhizomu* express genes for *α*-amylase and glucoamylases [35]. However, most studies on fungi that produce *α*-amylases are limited to some mesophilic species [14]. Therefore, prospecting studies for microorganisms with a potential for industrial application have an important role in the economic scenario because the production of enzymes of microbial origin is one of the main sectors of current industrial biotechnology, and amylases occupy the second place in the world market for industrially applied microbial enzymes, second only to proteases [36].

4. Conclusion

Bioprospecting is a viable alternative that allows one to discover the organisms

of a certain region, enabling the knowledge of the existing species and their respective biotechnological potentials. Investments in research that promote the exploration of soils can bring scientific advances of extreme importance to economic and ecological areas because it is a complex environment with enormous biodiversity. The prospecting of fungal species from the caatinga can furnish important information regarding biodiversity and soil quality, in addition to discovering new species that make up this rich Brazilian biome. Thus, fourteen filamentous mesophilic fungi from four different soil samples, were isolated. They had different morphological characteristics, and some were different from species already known. In addition, four of these isolated fungi proved to be good producers of enzymes in the amylolytic system, being identified as I 1.2.1, I 2.2.3, I 3.2.1 and I 4.4.1. These results can represent a discovery of new microorganisms with great biotechnological and industrial potential.

Acknowledgements

The authors express their gratitude for the support of the Research Laboratory in Biofuels of the Institute of Science and Technology (ICT), and the Integrated Laboratory for Research and Multipurpose Teaching (LIPEMVALE), both of the Federal University of the Vales do Jequitinhonha and Mucuri (UFVJM).

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Pereira, J.O., De souza, A.Q.L., de Souza, A.D.L., De castro, F.S. and Oliveira, L.A. (2017) Overview on Biodiversity, Chemistry, and Biotechnological Potential of Microorganisms from the Brazilian Amazon. In: Azevedo J. and Quecine M., Eds., *Diversity and Benefits of Microorganisms from the Tropics*, Springer, Cham, 71-103. https://doi.org/10.1007/978-3-319-55804-2_5

[2] Pasin, T.M., Benassi, V.M., Moreira, E.A., Jorge, J.A. and Polizeli, M.L.T.M. (2014) Prospecting Filamentous Fungi for Amylase Production: Standardization of Aspergillus Japonicus Culture Conditions. *British Biotechnology Journal*, **4**, 482-498. https://doi.org/10.9734/BBJ/2014/7659

[3] Freitas, F., Torres, C.A.V. and Reis, M.A.M. (2017) Engineering Aspects of Microbial Exopolysaccharide Production. *Bioresource Technology*, **245**, 1674-1683. https://doi.org/10.1016/j.biortech.2017.05.092

[4] Orlandelli, R.C., Specian, V., Felber, A.C. and Pamphile, J.A. (2012) Enzimas de Interesse Industrial: Produção por fungos e aplicações. *SaBios-Revista de Saúde e Biologia*, **7**, 97-109.

[5] Silva, C.F. (2018) Isolamento e seleção de microrganismos produtores de enzimas de interesse comercial. *Scientia Plena*, **14**, 1-10. https://doi.org/10.14808/sci.plena.2018.024201

[6] Benassi, V.M., Lucas, R.C., Michelin, M., Jorge, J.A., Terenzi, H.F. and Polizeli, M.L.T.M. (2012) Production and Action of an *Aspergillus phoenicis* Enzymatic

Pool Using Different Carbon Sources. *Brazilian Journal of Food Technology*, **15**, 253-260. https://doi.org/10.1590/S1981-67232012005000019

[7] Pasin, T.M., Moreira, E.A., Lucas, R.C., Benassi, V.M., Ziotti, L.S., Cereia, M. and Polizeli, M.L.T.M. (2020) Novel Amylase-Producing Fungus Hydrolyzing Wheat and Brewing Residues, *Aspergillus carbonarius*, Discovered in Tropical Forest Remnant. *Folia Microbiologica*, **65**, 173-184. https://doi.org/10.1007/s12223-019-00720-4

[8] Rosa, T.M.F., Marinho, B.M. and Benassi, V.M. (2017) Produção de celulases e formação de açúcares fermentescíveis pela degradação do bagaço de cana-de-açúcar. *Revista Ciência & Tecnologia*, **9**, 38-43.

[9] Oliveira, T.B., Gomes, E. and Rodrigues, A. (2015) Thermophilic Fungi in the New Age of Fungal Taxonomy. *Extremophiles*, **19**, 31-37. https://doi.org/10.1007/s00792-014-0707-0

[10] Emerson, R. (1941) An Experimental Study of the Life Cycles and Taxonomy of Allomyces. *Lloydia*, **4**, 77-144.

[11] Dias, D.R. and Schwan, R.F. (2010) Isolamento e identificação de leveduras. In: Moreira, F.M.S., Huising, E.J. and Bignell, D.E., Eds., *Manual de Biologia dos solos tropicais*: *amostragem e caracterização da biodiversidade*, MG Universidade Federal de Lavras, Lavras, 368 p.

[12] Peixoto, S.C., Jorge. J.A., Terenzi, H.F. and Polizeli, M.L.T.M. (2003) *Rhizopus Microsporus* Var. Rhizopodiformis: A Thermotolerant Fungus with Potential for Production of Thermostable Amylases. *International Microbiology*, **6**, 269-273. https://doi.org/10.1007/s10123-003-0140-1

[13] Miller, G.L. (1959) Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. *Analytical Chemistry*, **31**, 426-428. https://doi.org/10.1021/ac60147a030

[14] Benassi, V.M. and Almeida, A. (2018) Prospecção de fungos filamentosos termotolerantes e termofílicos de distintos materiais coletados no estado de minas gerais e análise de potenciais produtores de amilases. *Revista Unimontes Científica*, **20**, 150-169.

[15] Inforsato, F.J. and Sette, L.D. (2016) Prospecção de lipase e protease adaptadas ao frio de microrganismos da antártica. *Revista Ciência & Tecnologia*, **8**.

[16] Sleator, R.D.C., Shortall, C. and Hill, C. (2008) Metagenomics. *Letters in Applied Microbiology*, **47**, 361-366.

[17] Rosa, I.Z. (2014) Isolamento e seleção de fungos filamentosos termofílicos produtores de celulases, xilanases e celobiose desidrogenase com potencial para sacarificação do bagaço de cana-de-açúcar. Master dissertation, Paulista State University Júlio de Mesquita Filho-São José do Rio Preto-SP, Brazil, 78 p.

[18] Santos, N.S.C., Nunes, V.J., Andrade, J.P., Nascimento, R.P., De souza, J.T. and Marbach, P.A.S. (2016) Produção de amilase por espécies do gênero Penicillium. *I Congresso Brasileiro de Microbiologia Agropecuária*, *Agrícola e Ambiental* (CBMAAA), Jaboticabal, 9-12 May 2016, Vol. 8.

[19] Almeida, M.V.A. (2015) Identificação de fungos filamentosos presentes em um biorreator de resíduos sólidos urbanos. Federal University of Campina Grande, PB, Brazil.

[20] Magalhães, F.E.A., Freire, F.C.O., Moura, L.F.W.G., Oliveira, M.V., Mota, J.G.S.M., Lô, M.M. and Ribeiro, A.R.C. (2016) Isolamento e identificação de fungos associados às plantas medicinais nativas da caatinga da região de Inhamus, Tauá, Ceará, Brasil. *Essentia*, **17**, 43-63.

[21] Madan, M. and Thind, K.S. (1998) Physiology of Fungi. A.P.H. Publishing Corpora-

tion, New Delhi, 213.

[22] Madigan, M., Martinko, J.M. and Parker, J. (2003) Brock Biology of Microorganisms. 10th Edition, Prentice Hall, Upper Saddle River, NJ.

[23] Akinyele, B.I. and Adetuyi, F.C. (2005) Effect of Agrowastes, pH and Temperature Variation on the Growth of *Volvariella volvacea*. *African Journal of Biotechnology*, **4**, 1390-1395.

[24] Gomes, E., Guez, M.A.U., Martin, N. and Silva, R. (2007) Enzimas termoestáveis: Fontes, produção e aplicação industrial. *Química Nova*, **30**, 136-145. http://dx.doi.org/10.1590/S0100-40422007000100025

[25] Pereira, J.C. (2013) Isolamento e seleção de fungos filamentosos termofílicos/ termotolerantes produtores de celulases e xilanases e aplicação dos extratos enzimáticos na sacarificação do bagaço da cana-de-açucar. Paulista State University Júlio de Mesquita Filho-São José do Rio Preto-SP, Brazil, 121 p.

[26] Pelczar, M.J., Chan, E.C.S. and Krieg, N.R. (1997) Microbiologia: Conceitos e Aplicações. MAKRON Books, São Paulo, Vol. 1, 524.

[27] Pasin, T.M., Benassi, V.M., Heinen, P.R., Damasio, A.R.L., Cereia, M., Jorge, J.A. and Polizeli, M.L.T.M. (2017) Purification and Functional Properties of a Novel Glucoamylase Activated by Manganese and Lead Produced by *Aspergillus japonicus*. *International Journal of Biological Macromolecules*, **102**, 779-788. https://doi.org/10.1016/j.ijbiomac.2017.04.016

[28] Izidoro, S.C. and Knob, A. (2014) Production of Xylanases by an *Aspergillus niger* strain in Wastes Grain. *Acta Scientiarum Biological Sciences*, **36**, 313-319. https://doi.org/10.4025/actascibiolsci.v36i3.20567

[29] Bellí, N., Pardo, E., Marín, S., Farré, G., Ramos, A.J. and Sanchis, V. (2004) Occurrence of Ochratoxin A and Toxigenic Potential of Fungal Isolates from Spanish Grapes. *Journal of the Science of Food and Agriculture*, **84**, 541-546.

[30] Gupta, R., Gigras, P., Mohapatra, H., Kumar, V.G. and Chauhan, B. (2003) Microbiol α-Amylases: A Biotecnological Perspective. *Process Biochemistry*, **38**, 1599-1616. https://doi.org/10.1016/S0032-9592(03)00053-0

[31] Silva, T.M. (2009) Produção e determinação das propriedades funcionais das amilases de Aspergillus niveus. University of São Paulo, Ribeirão Preto, SP, Brazil, 171 p.

[32] Luz, B.D.S., Bicas, J.L., Sarrouh, B. and Lofrano, R.C.Z. (2016) Bioprospecção de microrganismos produtores de enzimas de interesse industrial realizada no Parque Estadual Serra do Ouro Branco, Brasil. *Interbio*, **10**, 13-14.

[33] Nwagu, T.N. and Okolo, B.N. (2011) Extracellular Amylase Production of a Thermotolerant *Fusarium* sp. Isolated from Eastern Nigerian Soil. *Brazilian Archives of Biology and Technology*, **54**, 649-658. https://doi.org/10.1590/S1516-89132011000400002

[34] Pasin, T.M., Scarcella, A.S.A., Oliveira, T.B., de Lucas, R.C., Cereia, M., Betini, J.H.A. and Polizeli, M.L.T.M. (2020) Paper Industry Wastes as Carbon Sources for Aspergillus Species Cultivation and Production of an Enzymatic Cocktail for Biotechnological Applications. *Industrial Biotechnology*, **16**, 56-60. https://doi.org/10.1089/ind.2020.29201.tmp

[35] Viktor, M.J., Rose, S.H., Van-Zyl, W.H. and Viljoen-Bloom, M. (2013) Raw Starch Conversion by *Saccharomyces cerevisiae* Expressing *Aspergillus tubingensis* Amylases. *Biotechnology for Biofuels*, **6**, Article No. 167. https://doi.org/10.1186/1754-6834-6-167

[36] Pasin, T.M., Salgado, J.C.S., Scarcella, A.S.A., Oliveira, T.B., de Lucas, R.C., Cereia,

M., Rosa, J.C., Ward, R.J., Buckeridge, M.S. and Polizeli, M.L.T.M. (2020) A Halotolerant Endo-1,4-β-Xylanase from *Aspergillus clavatus* with Potential Application for Agroindustrial Residues Saccharification. *Applied Biochemistry and Biotechnology*, **191**, 1111-1126. https://doi.org/10.1007/s12010-020-03232-x