SCREENING OF MUCOR SPP. FOR THE PRODUCTION OF AMYLASE, LIPASE, POLYGALACTURONASE AND PROTEASE

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ABSTRACT

Fungi are well known by their ability to excrete enzymes into the environment. Among them, representatives of Mucor Fresen. have important biotechnological potential and some of them produce industrial enzymes. This work studied amylase, lipase, polygalacturonase and protease production by fifty-six isolates of Mucor belonging to 11 different taxa, selected from herbivores dung using solid media. The results showed that the majority of the isolates presented several enzymatic activities with predominance of polygalacturonase (96%), followed by amylase (84%), protease (82%) and lipase (66%).

Key words: Fungal enzymes, Mucor, amylase, lipase, polygalacturonase, protease, herbivorous dung

The enzymes are essential proteins for the metabolic system of all living organisms and have an important role in the degradation of organic matter, in host infection and food spoilage. In the metabolic pathways, they act in organized sequences of catabolic and anabolic routes (12). Enzymes may also act in the control of biochemical processes in the living cells. They may be isolated from animals, plants and microorganisms. The last ones are considered good sources of industrial enzymes for the great diversity of enzymes that have been found (13). The enzymes are used in large scale in the textile (amylase, cellulase, oxidoreductase); detergents (protease, lipase, cellulase, oxidoreductase); food (pectinase, protease, cellulase, oxidoreductase); paper (xylanase, oxidoreductase and lipase) and leather (protease, lipase) industries (14).

Extracellular enzymes may be produced in liquid or solid media. The use of solid media permits a fast screening of large populations of fungi, allowing the detection of specific enzymes (3,8,10,17,19,20) and helping in the chemotaxonomical differentiation of many microorganisms (10). The production of enzymes by microorganisms assures a potential and unlimited supply and also makes it possible the genesis of new enzymatic systems that cannot be obtained from plant or animal sources (2,13).

The species of the genus Mucor constitute a group of microorganisms responsible for the production of several enzymes such as amylases, lipases, pectinases and proteases (5,16,20). Mucor hiemalis, M. racemosus (15), M. bacilliformis (7) and M. miehei (6) present protease activity of commercial value and M. miehei is the most studied specie concerning the production of lipase (14).

The objective of this work was to detect the presence of the aforementioned enzymes by taxa of Mucor isolated from herbivores dung, using solid culture media.

The 11 taxa of Mucor isolated from dung of herbivores animals, from two locations in Recife, PE (1), are shown in the Table 1. These microorganisms have been preserved by the Castelani method (4) in culture collections of the following institutions: Catholic University of Pernambuco (UCP-
Table 1. Taxa and identification number of the isolates in the Culture Collections of UCP, URM and SPC, indicating the herbivores animal origin.

| TAXA                        | Culture Collection | Dung/Animal          | Origin/Animal |
|-----------------------------|--------------------|----------------------|---------------|
|                             | UCP    | URM    | SPC    |                     |                     |
| *Mucor circinelloides* f. circinelloides | 6      | 4136   | 1768   | *Bos indicus* Linnaeus | ZOO                  |
|                             | 36     | 4140   |        | *Bison bonasus* H. Smith | PDI                 |
|                             | 37     |        |        | *Bison bonasus* H. Smith | PDI                 |
|                             | 53     |        |        | *Bison bonasus* H. Smith | PDI                 |
| *M. circinelloides* f. griseo-cyanus | 1      | 4183   | 1769   | *Bison bonasus* H. Smith | PDI                 |
|                             | 20     | 4182   |        | *Mazama gouazoubira* Fischer | PDI                |
|                             | 42     |        |        | *Mazama gouazoubira* Fischer | PDI                |
|                             | 47     | 4192   |        | *Ovis aries* Linnaeus | ZOO                  |
|                             | 46     |        |        | *Bison bonasus* H. Smith | PDI                 |
|                             | 54     | 4184   |        | *Capra hircus* Linnaeus | ZOO                  |
|                             | 55     | 4185   |        | *Oryctolagus cuniculus* Lilljeborg | ZOO |
|                             | 57     | 4160   |        | *Taurotragus oryx* Wagner | PDI                |
|                             | 58     |        |        | *Ovis aries* Linnaeus | ZOO                  |
| *M. circinelloides* f. janssenii | 8      | 4139   |        | *Taurotragus oryx* Wagner | PDI                |
|                             | 9      | 4148   |        | *Mazama gouazoubira* Fischer | PDI                |
|                             | 10     |        |        | *Oryctolagus cuniculus* Lilljeborg | ZOO |
|                             | 19     | 4141   | 1770   | *Oryctolagus cuniculus* Lilljeborg | ZOO |
| *M. circinelloides* f. lusitanicus | 51     | 4137   | 1771   | *Mazama gouazoubira* Fischer | PDI                |
| *M. genevensis*             | 7      | 4188   | 1772   | *Dasyprocta fuliginosa* Wagler | PDI |
|                             | 15     |        |        | *Dasyprocta fuliginosa* Wagler | PDI |
|                             | 23     | 4187   |        | *Ovis aries* Linnaeus | ZOO                  |
|                             | 24     |        |        | *Ovis aries* Linnaeus | ZOO                  |
| *M. hiemalis* f. hiemalis   | 12     |        |        | *Equus caballus* Linnaeus | ZOO                  |
|                             | 13     |        |        | *Capra hircus* Linnaeus | ZOO                  |
|                             | 14     |        |        | *Oryctolagus cuniculus* Lilljeborg | ZOO |
|                             | 18     | 4190   |        | *Mazama gouazoubira* Fischer | PDI                |
|                             | 28     | 4193   | 1773   | *Dasyprocta fuliginosa* Wagler | PDI |
|                             | 30     |        |        | *Mazama gouazoubira* Fischer | PDI                |
|                             | 31     |        |        | *Mazama gouazoubira* Fischer | PDI                |
|                             | 52     |        |        | *Bison bonasus* H. Smith | PDI                 |
| *M. hiemalis* f. luteus     | 5      | 4186   | 1774   | *Ovis aries* Linnaeus | ZOO                  |
|                             | 11     | 4191   |        | *Dasyprocta fuliginosa* Wagler | PDI |
|                             | 17     |        |        | *Dasyprocta fuliginosa* Wagler | PDI |
|                             | 2      | 4142   |        | *Oryctolagus cuniculus* Lilljeborg | ZOO |
|                             | 35     |        |        | *Ovis aries* Linnaeus | ZOO                  |
|                             | 44     | 4144   |        | *Equus caballus* Linnaeus | ZOO                  |
|                             | 45     |        |        | *Ovis aries* Linnaeus | ZOO                  |
|                             | 48     | 4147   |        | *Capra hircus* Linnaeus | ZOO                  |
|                             | 50     |        |        | *Ovis aries* Linnaeus | ZOO                  |
| *M. piriformis*             | 41     | 4145   | 1775   | *Equus caballus* Linnaeus | ZOO                  |
| *M. racemosus* f. chibinensis | 2      | 4149   | 1777   | *Capra hircus* Linnaeus | ZOO                  |
|                             | 3      |        |        | *Dasyprocta fuliginosa* Wagler | PDI |
|                             | 4      |        |        | *Dasyprocta fuliginosa* Wagler | PDI |
|                             | 16     |        |        | *Dasyprocta fuliginosa* Wagler | PDI |
|                             | 21     |        |        | *Dasyprocta fuliginosa* Wagler | PDI |
|                             | 26     | 4135   |        | *Bison bonasus* H. Smith | PDI                 |
|                             | 27     |        |        | *Dasyprocta fuliginosa* Wagler | PDI |
|                             | 33     |        |        | *Dasyprocta fuliginosa* Wagler | PDI |
|                             | 34     |        |        | *Dasyprocta fuliginosa* Wagler | PDI |
|                             | 39     |        |        | *Oryctolagus cuniculus* Lilljeborg | ZOO |
|                             | 40     |        |        | *Oryctolagus cuniculus* Lilljeborg | ZOO |
|                             | 43     | 4143   |        | *Dasyprocta fuliginosa* Wagler | ZOO                  |
|                             | 49     |        |        | *Dasyprocta fuliginosa* Wagler | PDI                 |
|                             | 56     |        |        | *Dasyprocta fuliginosa* Wagler | PDI                 |
| *M. subtilissimus*          | 29     | 4133   | 1778   | *Equus caballus* Linnaeus | ZOO                  |
| *M. varyiosporus*           | 25     | 4219   | 1779   | *Mazama gouazoubira* Fischer | PDI |

PDI=Parque Dois Irmãos, Recife, Pernambuco; Zoo=Department of Zootecny of the University Federal Rural of Pernambuco. Font: (1) modified.
The isolates were reactivated in Petri dishes containing Synthetic Mucor Agar (SMA) (Hesseltine and Anderson (11) and/or Potato Dextrose Agar (PDA) and submitted to monosporic cultivation according to the methodology proposed by Gams et al. (9) in order to obtain pure cultures and to diminish variability at the morpho-physiologic, biochemical and genetic levels when submitted to successive cultivations.

A suspension of spores was prepared from the monosporic colonies. The mycelium was washed with sterilized distilled water with the aid of glass beads, and the suspension aseptically transferred to a test tube. An aliquot of this suspension was removed for counting in a Newbauer Camera, under optical microscope, and diluted whenever necessary to obtain 10^6 spores/mL.

For detection of enzymes, the methodology used was the one proposed by Hankin and Anagnostakis (10), modified with the substitution of the “hexadecyltrimethylammonium bromide” by hydrochloric acid (HCl) 5N. To verify the activity of the enzymes amylases, lipases, pectinases (polygalacturonases) and proteases, soluble starch (Merck), Tween 20 (Merck), citric enzyme amylases, lipases, pectinases (polygalacturonases) and hydrochloric acid (HCl) 5N. To verify the activity of the proteases within 96 hours.

The isolates numbered 1, 46 and 47 (M. circinelloides f. griseo-cyanus) with halo 47 cm were the ones that presented the highest lipases activity (halo > 8 cm). Except for M. subtilissimus, all taxa presented polygalacturonase activity. M. circinelloides f. lusitanicus, followed by M. hiemalis f. luteus, M. piriformis and M. genevensis (average end of halo > 7 cm) showed the bests halo average for this enzyme (Fig. 1C). These data were similar to the ones presented by Thompson and Eribo (17) (18) that detected lipase activity in 27 isolates of 10 different species of Mucor. They do not agree with the results obtained by Thompson and Eribo (17) that did not detect lipase activity in M. hiemalis, M. mucedo, M. piriformis and M. racemosus f. racemosus.

In Table 2 it is shown that a large number of isolates (54) presented polygalacturonase activity, mainly the ones numbered 5, 32 and 44 (M. hiemalis f. luteus), 15 and 24 (M. genevensis), 34, 39 and 56 (M. racemosus f. chibinensis) and 53 (M. circinelloides f. circinelloides) with halo > 8 cm. Except for M. subtilissimus, all taxa presented polygalacturonase activity. M. circinelloides f. lusitanicus, followed by M. hiemalis f. luteus, M. piriformis and M. genevensis (average end of halo > 7 cm) showed the bests halo average for this enzyme (Fig. 1C). These data were similar to the ones presented by Thompson and Eribo (17) that observed polygalacturonase activity in three of the four species of Mucor studied. In this work, among the 56 isolates studied, only two isolates did not produce polygalacturonase. These results agree with Petrucioli and Federici (16) that detected polygalacturonase in M. genevensis, M. racemosus and M. ramannianus, but the degradation halo (1-9.5 mm and 2-8 mm) produced by M. genevensis and M. racemosus, respectively, were smaller than the ones obtained in this study.

In Table 2 it can be observed that the majority of the isolates presented protease activity and that the isolates numbered 3, 4, 26, 33 and 40 (M. racemosus f. chibinensis), 6 (M. circinelloides f. circinelloides), 7 and 23 (M. genevensis), 8 (M. circinelloides f. janssennii), 11 and 32 (M. hiemalis f. luteus), 18 (M. hiemalis f. luteus) and 57, 58 (M. circinelloides f. griseo-cyanus) were the ones that demonstrated the biggest halo (> 5.5 cm). It may be observed that the majority showed proteases activity without significant differences among them (Fig. 1D).

The results obtained with the proteases activity agree with the data observed by Thompson and Eribo (17) that obtained this enzymatic activity in four species of Mucor. Petrucioli and Federici (16) also detected protease activity in M. racemosus and M. ramannianus. Hankin and Anagnostakis (10) observed...
Table 2. Averages diameters halo (cm) for the enzymatic activities in taxa of *Mucor* and respective isolates.

| TAXA ISOLATE | ENZYMATIC ATIVITY | AMYLASE | LIPASE | PECTINASE (POLYGALACTURONASE) | PROTEASE |
|--------------|--------------------|---------|--------|-------------------------------|----------|
| *Mucor circinelloides f. circinelloides* | | | | | |
| 6 | 7.2c | 0.0 | 0.0 | 6.1c | 5.7a |
| 36 | 7.8b | 0.0 | 5.6d | | 5.2ab |
| 37 | 8.4a | 6.5a | 6.8b | | 4.0b |
| 53 | 8.5a | 5.3a | 8.8a | | 0.0 |
| *M. circinelloides f. griseo-cyanus* | | | | | |
| 1 | 5.7d | 7.9a | 6.3b | | 5.0a |
| 20 | 5.9d | 0.0 | 0.0 | 6.1bc | 3.8b |
| 42 | 7.2bc | 5.5b | 5.8bed | | 4.7ab |
| 46 | 6.8c | 7.0a | 0.0 | | 0.0 |
| 47 | 8.2a | 7.1a | 5.4de | | 5.0a |
| 54 | 6.2d | 0.0 | 5.2e | | 5.0a |
| 55 | 7.5b | 4.7bc | 7.9a | | 5.3a |
| 57 | 7.6ab | 4.3c | 5.6cde | | 5.7a |
| 58 | 7.7ab | 6.8a | 5.3de | | 5.7a |
| *M. circinelloides f. janssenii* | | | | | |
| 8 | 5.7b | 4.6c | 6.5a | | 6.2a |
| 9 | 4.6c | 5.7b | 7.0a | | 4.1c |
| 10 | 6.2a | 7.3a | 6.5a | | 4.9b |
| 19 | 5.7b | 0.0 | 6.5a | | 4.2c |
| *M. circinelloides f. lusitanicus* | | | | | |
| 51 | 0.0 | 2.3 | 7.9 | | 0.0 |
| *M. genevensis* | | | | | |
| 7 | 6.4 | 5.3b | 5.0c | | 6.8a |
| 15 | 0.0 | 0.0 | 8.8a | | 2.7c |
| 23 | 0.0 | 8.1a | 7.2b | | 5.6b |
| 24 | 0.0 | 0.0 | 8a | | 0.0 |
| *M. hiemalis f. hiemalis* | | | | | |
| 12 | 5.0e | 4.8ab | 5.7a | | 4.5cd |
| 13 | 6.4d | 5.7ab | 6.2a | | 4.9bc |
| 14 | 6.6d | 0.0 | 6.2a | | 4.2de |
| 18 | 5.5e | 4.3b | 5.5ab | | 5.5a |
| 28 | 7.7b | 0.0 | 3.7b | | 3.8e |
| 30 | 8.4a | 6.1a | 6.6a | | 4.7bc |
| 31 | 8.4a | 4.3b | 6.7a | | 5.0ab |
| 52 | 7.2c | 4.9ab | 6.7a | | 0.0 |
| *M. hiemalis f. luteus* | | | | | |
| 5 | 4.9c | 0.0 | 8.0b | | 3.6e |
| 11 | 5.7b | 2.3c | 7.5b | | 6.2a |
| 17 | 5.7b | 5.9a | 6.6c | | 4.8bced |
| 32 | 0.0 | 0.0 | 8.5a | | 5.5ab |
| 35 | 5.9b | 0.0 | 7.4b | | 5.2bc |
| 44 | 0.0 | 5.5a | 8.1a | | 0.0 |
| 45 | 0.0 | 0.0 | 7.3b | | 0.0 |
| 48 | 8.0a | 4.2b | 6.8c | | 4.2de |
| 50 | 0.0 | 5.0ab | 7.6b | | 4.5cd |
| *M. piriformis* | | | | | |
| 41 | 0.0 | 3.6 | 7.4 | | 0.0 |
| *M. racemosus f. chibinensis* | | | | | |
| 2 | 5.3e | 5.0ab | 6.6c | | 4.6de |
| 3 | 6.6cd | 6.0a | 6.7c | | 6.3a |
| 4 | 6.0de | 4.2b | 6.6c | | 6.0ab |
| 16 | 6.9c | 5.8a | 6.6c | | 3.2f |
| 21 | 8.1ab | 3.0c | 5.1e | | 4.1e |
| 26 | 7.3c | 2.0c | 5.6de | | 5.9ab |
| 27 | 6.6cd | 5.0ab | 6.1cd | | 4.4de |
| 33 | 7.0c | 0.0 | 6.3c | | 6.4a |
| 34 | 7.1c | 2.7c | 8.5a | | 4.9cd |
| 39 | 7.3bc | 0.0 | 8.5a | | 3.0f |
| 40 | 7.1c | 0.0 | 5.3de | | 5.6bc |
| 43 | 8.2a | 0.0 | 7.7b | | 0.0 |
| 49 | 7.2c | 0.0 | 7.8b | | 4.1e |
| 56 | 7.4bc | 0.0 | 8.4ab | | 5.0cd |
| *M. subtilissimus* | | | | | |
| 29 | 4.7 | 2.5 | 0.0 | | 0.0 |
| *M. variosporus* | | | | | |
| 25 | 8.3 | 2.9 | 5.6 | | 5.3 |

Averages equal to zero were not included in the statistical analysis. Numbers followed by the same letter among the isolates group of each species were not significantly different according to Fischer’s protected LSD test.
protease activity in *Mucor* sp, although the degradation halo diameters mentioned by these authors were smaller than the ones obtained in this work.

The results show that all *Mucor* isolates possessed a high potential for enzyme production, especially lipase, which was present in the majority of the taxa studied. It was observed that enzymatic activity does not establish true standards for separation of the taxa at a specific level since it varied in different isolates belonging to the same taxon.

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**RESUMO**

**Screening de Mucor spp. para produção de amilase, lipase, poligalacturonase e protease**

Os fungos apresentam a capacidade de produzir e secretar enzimas para o meio ambiente. Entre esses, representantes de *Mucor* Frezen constituem um grupo de microrganismos com importante potencial biotecnológico, sendo responsáveis pela produção de várias enzimas usadas em processos industriais. Foi observado que 56 isolados do gênero *Mucor*, totalizando 11 táxons, obtidos de fezes de herbívoros são capazes de produzir amilase, lipase, poligalacturonase e protease em meios sólidos. Os resultados demonstraram que 96% dos isolados produziram poligalacturonase, (84%) amilase, (82%) protease e (66%) lipase.

**Palavras-chave:** Enzimas fúngicas, *Mucor*, amilase, lipase, poligalacturonase, protease, fezes de herbívoros.

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**Figure 1.** Average diameter of halo (cm) for the activities of amylase (A), lipase (B), polygalacturonase (C) and protease (D) in species of *Mucor*: 1 - *M. circinelloides* f. *circinelloides*; 2 - *M. circinelloides* f. *griseo-cyanus*; 3 - *M. circinelloides* f. *janssenii*; 4 - *M. circinelloides* f. *lusitanicus*; 5 - *M. genevensis*; 6 - *M. hiemalis* f. *hiemalis*; 7 - *M. hiemalis* f. *luteus*; 8 - *M. piriformis*; 9 - *M. racemosus* f. *chibinensis*; 10 - *M. subtilissimus* and 11 - *M. variosporus*. Numbers followed by the same letter are not significantly different according to Fischer’s protected LSD test.
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