agriculture

MDPI

Article

Prospection of Cellulolytic Fungi from Composted Samples of Saturated Horse Litter

Ana Gabriela C.R. do Nascimento [1,*], Alessandra M. de Paula [2], Jader G. Busato [2], Samia G. da Silva [2] and Antonio Raphael T. Neto [2]

[1] Post-Graduation Program in Animal Health, Faculdade de Agronomia e Veterinária da Universidade de Brasília, Campus Universitário Darcy Ribeiro,Brasília-DF 70910-900, Brazil
[2] Campus Universitário Darcy Ribeiro, Faculdade de Agronomia e MedicinaVeterinária, University of Brasília, Brasília-DF 70910-900, Brazil; alessandramp@unb.br (A.M.d.P.); jaderbusato@unb.br (J.G.B.); raphaeltx@gmail.com (S.G.d.S.); samia.gomes@unb.br (A.R.T.N.)
* Correspondence: gabyvett@ifto.edu.br; Tel.: +55-(61)-98315-2860

Abstract: The treatment of saturated horse beds before their final destination is necessary to avoid the risk of animal and environmental contamination. For this purpose, the composting process has great functionality due to its low cost, effectiveness, and operational ease. However, because of the nature of the materials used, this process can be long, and it is necessary to improve it to optimize the composting cycles. This work aimed to isolate and identify fungi present in compost piles of saturated equine bedding made with shavings and rice straw, selecting those with the greatest potential for cellulase production. Using specific cellulolytic media containing shavings or rice straw, seven strains were isolated. The total cellulase enzymatic activity of the isolates from the beds made with shavings was lower than that obtained from rice straw beds. Four strains showed high enzymatic potential for use in the shavings substrate (MA6.2.1, MA6. 2.2, MA7. 9, and MA 7. 10) and three had potential for use in the rice straw substrate (PA7.5, PA7.7, and PA7.10). The isolate PA -7 5 reached 0.376 IU mL^{-1}, which was the best index among all the isolates. These isolates were identified as belonging to the *Aspergillus fumigatus* species.

Keywords: horse waste; composting process; microorganisms; beneficial; enzymes; cellulase; *Aspergillus sp.*

Citation: Nascimento, A.G.C.R.; Paula, A.M.; Busato, J.G.; da Silva, S.G.; Texeira Neto, A.R. The Evaluation of Cellulolytic Fungi in Composted Samples of Saturated Horse Litter. *Agriculture* **2023**, *13*, x. https://doi.org/10.3390/xxxxx

Academic Editor(s):

Received: 20 April 2023
Revised: 1 June 2023
Accepted: 2 June 2023
Published: date

1. Introduction

The bioconversion of organic waste in processes such as composting enables the safe recycling of such materials and produces a stabilized product that is enriched with humic substances and plant nutrients [1]. Because of its simplified operability, low cost, and effectiveness, composting has been the main strategy for treating organic waste. Through this practice, the circular bioeconomy is fostered [2] and the negative environmental impacts from the inadequate disposal of these materials are minimized. Composting is also a tool recognized by the United Nations in Goal 12 (sustainable consumption and production), which was proposed in the 2030 Agenda [3] that was formulated to encourage countries to adopt actions for sustainable development. Horses that are bred and treated in feedlots require bedding formed from organic materials such as shavings and rice straw, as it is used to absorb moisture from the animal excreta, ensuring greater sanitation in the environment [4]. On average, between 8 to 9 kg of integral materials are required per day to make the beds, resulting in up to 25 kg of bedding saturated with feces and urine [5]. Annually, each adult horse can generate up to 10 tons of saturated bedding [6], which is usually directly applied in pastures without treatment and can potentially produce unpleasant odors, increase the emission of greenhouse gases, contaminate the soil, and increase the incidence of pathogenic microorganisms in the area [6,7]. The disposal of these

materials without proper treatment can also cause an accumulation of antibiotic residues and antimicrobial resistance genes (ARGs) [8]. The search for economically viable, attractive, and sustainable alternatives is still a major challenge for production systems. Composting, despite being an old methodology of waste recycling, is effective for the correct reuse of organic waste, as it is capable of producing biological and fully acceptable fertilizer [9,10].

Some chemical characteristics of the materials used in the composition of horse litter (e.g., a high C/N ratio) may negatively affect composting. In this sense, adjustments to the nitrogen availability, temperature, aeration, and humidity of the compost piles can improve the process. This can possibly reduce the time needed for the completion of the process and ensure that the product (compost) is stable and free of pathogens [6]. The enrichment of the compost piles with microorganisms with specific degradation abilities can also favor the composting process since some of these microorganisms produce a high number of enzymes responsible for the degradation of biomolecules, such as cellulose. Thus, the microbial inoculation of these compost piles can be a promising biotechnological tool for improving composting [11–13]. The microorganisms present in the composting process are responsible for the generation of heat, the conversion of nutrients, and the depolymerization of the compounds through the production of specific enzymes. The fungi naturally present in the compound degrade most of the organic polymers. However, if there is not enough diversity, if the environmental parameters are not ideal, and depending on the characteristics of the raw material, they may require multiple degradation cycles, which can result in an increased composting time and a lower efficiency [14].

The isolation of degrading fungi during the thermophilic phase of composting (temperatures above 45 °C) potentiates the selection of strains capable of surviving the high temperatures of the process when inoculated in the compost piles. Besides the ability to tolerate high temperatures, the isolated fungi produce considerable amounts of enzymes responsible for the degradation of cellulose and lignin, as well as compounds of a secondary metabolism that assist in the degradation of carbohydrates [15].

The inoculation of preselected thermophilic fungi, according to their capacity to produce ligninolytic enzymes, may be a strategy capable of influencing the degradation process in composting lines, and thus reducing the period of time required for the stabilization of the material. In addition, it is possible that some of these microorganisms interfere with the cycle of some plant nutrients, such as nitrogen and phosphorus, increasing their availability in the final product obtained [1]. Research that applied mixed microbial inoculants belonging to the genera *Bacillus* and *Aspergillus*, which were applied in the initial phase of composting residues from milk mixed with sugarcane leaves, resulted in a more accelerated succession of the microbial population, increasing the participation of microorganisms that degrade lignocellulosic compounds [16]. Similarly, fungi belonging to the genus *Trichoderma*, which were isolated from cattle manure compost, as well as those belonging to *Trichoderma* and *Aspergillus*, which were obtained from a mixture of chicken manure with corn straw, were applied in the composting process of animal and vegetable waste and consistently accelerated the stabilization of the material, making the necessary cycle shorter and enabling the safe use of the compost [17,18]. Fungi belonging to the genera *Trichoderma* (*T. harzianum, T. viride*) and *Aspergillus* (*A. niger*) isolated from the composting of municipal solid waste also showed high enzymatic activities associated with the cellulose cycle [13]. Wu et al., 2019 [19], observed that the application of microbial inoculants potentiated the transformation of lignocellulose into straw–manure compounds during the thermophilic phase of the process.

However, the range of waste used in composting is large and the microbial groups employed as process accelerators may present selectivity. In this sense, studies involving the isolation of fungi with the potential for degradation directly from equine litter were not found at the time of the preparation of the present study; thus, this topic deserves to be evaluated. With this in mind, the main objective of this study was to isolate fungi with a high potential for the production of enzymes associated with the cellulose cycle in

compost samples (during the thermophilic phase) from the saturated bedding in equine stalls that are formed by shavings and rice straw, with the aim of reusing them in compost piles.

2. Materials and Methods

2.1. Place of the Experiment, Assembly of the Piles and Obtaining the Samples

The composting process was performed at the Large Animal Veterinary Hospital of the Faculty of Agronomy and Veterinary Medicine, University of Brasilia, Brasilia-DF (latitude −15°74″93′, longitude −47°87″64′ an altitude of 1028 m). The residues used in the composting were obtained from saturated equine bedding formed with shavings and rice straw from the treatment of two adult male crossbred horses subjected to a 12% crude protein concentrate diet (4 kg divided into two daily portions) and tifton hay-based volume. The animals stayed in the stalls for 20 days, with the manure mixed with the bedding collected daily. At the end of this period, two compost piles were made with the bedding remaining in the stalls in a covered shed with a cement floor. The material was homogenized to make pyramidal piles with dimensions of 1.80 × 0.90 × 2.10 m, for shavings, and 1.70 × 0.98 × 2.40 m, for rice straw. The temperature was monitored daily using a digital thermometer with a 15 cm stem at 7 different points. The humidity was maintained between 40% and 50% throughout the process to ensure sufficient water content for the activities of the microorganisms. The piles were turned with the help of hand tools every three days over a period of 30 days. Samples from each pile were collected in the thermophilic phase, when the piles reached temperatures higher than 60 °C, which occurred on day 5 for shavings and day 13 for rice straw. Single samples were obtained at 5 different points in the piles, and these were homogenized to form the final sample. Each composite sample was subdivided into 4 replicates and kept under refrigeration at the temperature of 4 °C until the isolation of fungi.

2.2. Isolation of Fungi with Cellulolytic Ability

For the isolation of fungi, the samples were submitted to serial dilution (up to 10^{-8}), in 0.9% NaCl saline solution and inoculated in selective medium for cellulolytic fungi, according to Parkinson et al. [20], with adaptations. In substitution of the carboxymethyl cellulose, used as the carbon source, compounds based on shavings and rice straw were used. Each substrate was grounded in a Willy's type knife mill, macerated, passed through a 0.25 mm of mesh, and then decontaminated by autoclaving in 3 cycles at 121 °C and 1 atm. After sterilization, streptomycin antibiotic (30 mg L^{-1}) was added to prevent bacterial growth. Subsequently, the plates were conditioned in germination chambers at 28 °C for 7 days.

The amount of substrate (shavings or rice straw) equivalent to the same amount of carbon supplied to an atmosphere with the use of carboxymethyl cellulose was calculated from the determination of the total organic carbon content (TOC) present in the substrates, according to Tedesco [21]. The distinct fungal colonies observed from the morphological characteristics were isolated using the streak depletion technique [22] on potato-dextrose-agar (BDA) medium and incubated in a growth chamber at 28 °C for 3 days. The isolates were deposited in test tubes containing slanted BDA medium and filled with autoclaved water for preservation [23].

2.3. Total Cellulase Activity

To evaluate the cellulase enzyme activity, the isolates obtained were inoculated in liquid basal medium containing the original substrate of the beds as carbon sources (shavings or rice straw). In a laminar flow chamber, flasks containing 100 mL of sterile basal medium composed of KH_2PO_4, $CaCl_2$, $MgSO_4.7H_2O$, NH_4NO_3, $FeSO_4.7H_2O$, $MnSO_4.4H_2O$, $ZnSO_4.7H_2O$ and $CoCl_2$. $6H_2O$, at pH 5.5 [24], enriched with shavings or rice straw (100 g L^{-1} of substrate). Discs with 0.6 cm diameter containing mycelia of the selected fungi were

inoculated in the flasks in triplicate. The flasks were incubated in a shaker incubator with orbital motion for a period of 21 days at 45 °C under continuous 180 rpm agitation.

The total cellulase activity was evaluated after 14 and 21 days of incubation from the amount of reducing sugars formed by the enzymatic hydrolysis of cellulose, using the 3,5 dinitrosalicylic acid (DNS) method [25,26]. Strips of Whatman filter paper rolled into a spiral shape were placed in a test tube containing 1 mL of sodium citrate buffer solution (0.05 mol L^{-1}, pH 4.8) to which 0.5 mL of fungal enzyme broth was added. The solution was homogenized and kept in a water bath at 50 °C for 60 min. Afterward, the tubes were cooled at room temperature and 0.5 mL of DNS reagent was added. The tubes were shaken vigorously, and the resulting suspension was heated in a water bath at 100 °C for 15 min. The tubes were cooled at room temperature, with the subsequent addition of 5 mL of distilled water, and then the absorbance at 540 nm was read in a spectrophotometer. The enzyme activity of total cellulase was expressed by the enzyme activity unit (UI), defined as the amount of enzyme capable of releasing 1 μmol of reducing sugar per minute, being UI mL^{-1} the concentration of enzyme activity of the sample.

2.4. Morphological Characterization and Molecular Identification of Isolates

The morphological characterization and molecular identification were performed for the isolates that stood out in the evaluation of the enzymatic activity of total cellulase. The molecular characterization was performed using 1 cm^2 blocks of potato agar placed on a sterile slide contained in a sterile Petri dish supported on a glass. Each isolate was inoculated with a needle, from colonies grown for 7 days on the 4 vertices of the upper side of the agar blocks, which were covered with sterile coverslip. The plates were sealed and incubated for up to 7 days, until evidence of sporulation was observed. The coverslips were carefully removed with tweezers and transferred to slides containing lactophenol cotton blue. The characteristics of the hyphae and the sporulation pattern were evaluated by light microscopy using a 40X objective.

Molecular identification was performed from fungal DNA extraction using the FavorPrepTM Soil DNA Isolation Mini Kit, followed by amplification of genetic material by the polymerase chain reaction (PCR) technique, using the primers ITS1 and ITS4 (Internal transcribed spacer) [27]. Amplicons were sequenced from the capillary electrophoresis (Sanger) sequencing method using the BigDyeTM Terminator v3.1 Cycle Sequencing Kit from Applied Biosystems. The sequences were processed using the CLC SequenceViewer 8.0.0 Software and submitted for analysis in the nBLAST tool.

The data related to enzyme activity were submitted to analysis of variance and the means were compared by Tukey's test at a significance level of 1% probability when significant differences were found. The statistical analyses were performed by the software SISVAR 4.0 [28]. The morphological characterization and molecular identification of the isolates were discussed in a descriptive way.

3. Results

3.1. Isolation of Fungi with Cellulolytic Ability

From the fungal cultures maintained in media enriched with rice straw as the sole carbon source, five fungal isolates with the potential to hydrolyze cellulosic material were obtained, thus evidencing cellulolytic activity, and six with media enriched with shavings, allowing the elimination of strains that do not show satisfactory growth in these media.

3.2. Total Cellulase Activity

Among the eleven thermophilic fungal isolates, seven were selected for evaluation regarding total cellulase activity because they showed better development, characterizing a capacity to produce cellulolytic enzymes. From the isolated fungi, three belong to those that developed in the culture medium obtained with rice straw and four with the shavings.

The selected isolates were analyzed for their ability to produce cellulase during the 21 days incubation period using filter paper as substrate. Considering the average of the total cellulolytic enzyme activities in the two culture times (14 and 21 days), all isolates showed cellulase production. The isolates PA-5 field 1 (PA5.1) and PA-7 field 7 (PA7.7), obtained from rice straw, had the highest values, 0.376 and 0.358 IU mL^{-1}, respectively (Table 1). The other isolates MA^{-6} field F1 (MA6.F1), MA^{-6} 2 field F2 (MA6.F2), MA^{-7} field 9 (MA7.9), MA^{-7} field 10 (MA7.10) and PA^{-7} field 10 (PA7.10) showed the lowest activities, and the medians did not differ.

Table 1. Average total cellulase (FPase) enzyme activities during 21 days of cultivation expressed in UI/mL-1 for the selected colonies.

Treatments	Average Value (UI mL^{-1})
MA6.2 F1	0.099 ± 0.03 a1
MA6.2 F2	0.081 ± 0.05 a1
MA7.9	0.072 ± 0.04 a1
MA7.10	0.057 ± 0.08 a1
PA5.1	0.376 ± 0.05 b2
PA7.7	0.358 ± 0.06 b2
PA7.10	0.055 ± 0.01 a1

PA: colonies of fungi with thermophilic growth in composted horsemeat bedding using rice straw substrate; MA: colonies of fungi with thermophilic growth in composted horsemeat bedding using shavings substrate. Averages followed by numbers and equal letters do not differ by the *t* test (Student) at 1% probability.

When evaluating the performance of the total cellulolytic enzyme activity of the fungal isolates over time after 14 and 21 days, it was observed that 4 isolates (MA6.F1, MA6.F2, PA5.1 and PA7.7) had the highest enzyme activity after 14 days of culture growth in selective medium containing shavings and rice straw as carbon source (Figure 1). The isolate MA7.9 showed the lowest enzymatic activity among the evaluated colonies, without the influence of the evaluation time. The isolates MA7.10 and PA 7.10 showed the highest enzymatic activity after 14 days of culture, with no activity detected in the isolate PA7.10 after this period.

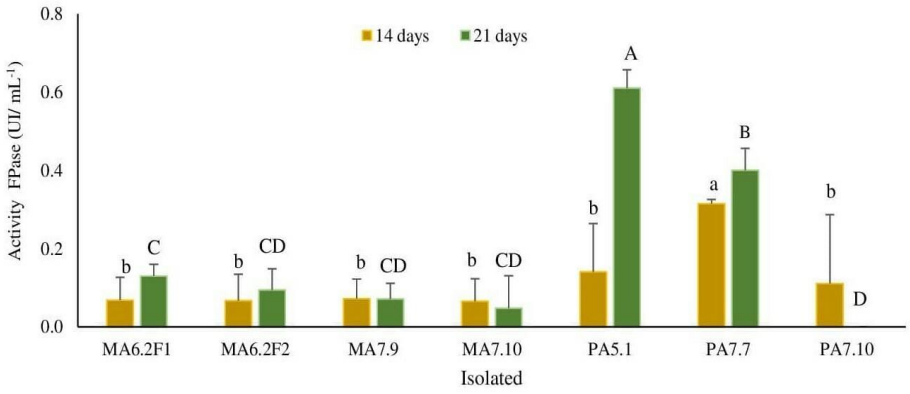

Figure 1. Average of enzymatic activities of the total cellulase (FPase) in 14 days and 21 days of culture expressed in UI mL-. MA: fungi isolated from horse bed compost made with shavings; PA: fungi isolated from horse bed compost made with rice straw. Lower case letters compare the enzymatic activity of the isolates in the incubation period of 14 days. Capital letters compare the enzymatic activity of cellulase of the isolates in the incubation period of 21 days. Different letters indicate difference by *t* test (Student) at 1% probability.

3.3. Morphological Characterization and Molecular Identification of Isolates

The fungal isolates that presented the best rates in relation to the total cellulolytic activity were conducted for morphological characterization and molecular identification. The microbiological characteristics of the isolates showed morphological structures, such as: conidia, spores, conidiophores and vesicles with characteristics belonging to the genus *Aspergillus* sp. in the microculture test on slides, shown in Figure 2.

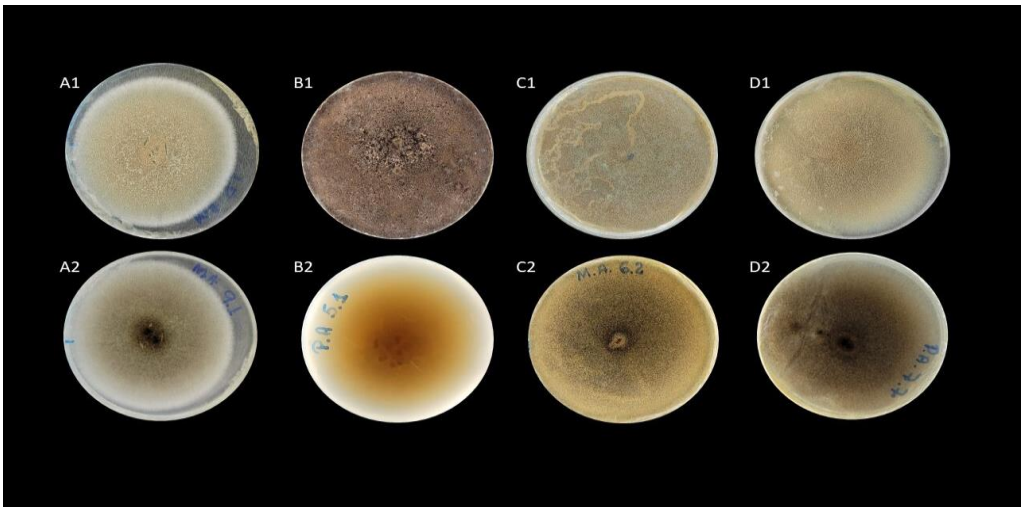

Figure 2. Macromorphological characteristics of the selected fungi. (**A**): MA7.9; (**B**): PA5.1; (**C**): MA6.F1; (**D**): PA7.7. The plates were photographed from the top (1) and bottom (2).

Converging with the microbiological results, the molecular identification of the seven selected isolates performed through the amplification of the ITS1 and ITS4 region, defined with fungal DNA barcodes, obtained amplicons for the genus Aspergillus sp, presenting the sequencing for the species identified as *Aspergillus fumigatus*. The seven isolates presented the same molecular identification (Table 2).

Table 2. Results of the identification process of the selected colonies by molecular analysis of the colonies using the barcoding primers ITS and amplicons Kit Big DyeTM Terminator v3.1 using Cycle Sequencing Kit from Applied Biosystems.

Treatments	Sequence Code	Molecular Identification
PA^{-7} 7	ITS1 01	*Aspergillus fumigatus*
PA^{-7} 7	ITS4 01	*Aspergillus fumigatus*
PA^{-7} 5	ITS1 02	*Aspergillus fumigatus*
PA^{-7} 5	ITS1 02	*Aspergillus fumigatus*
MA^{-6} 2 F2	ITS1 03	*Aspergillus fumigatus*
MA^{-6} 2 F2	ITS4 03	*Aspergillus fumigatus*
MA^{-7} 9.1	ITS1 04	*Aspergillus fumigatus*
MA^{-7} 9.1	ITS4 04	*Aspergillus fumigatus*

4. Discussion

The isolation and selection of microorganisms with abilities to decompose complex organic compounds, such as the most recalcitrant plant polymers, are among the main studies that aim for the use of microorganisms to accelerate the stability and enrich the compounds [29,30]. In this sense, recent works that focused on the isolation of fungi with the ability to produce lignocellulolytic enzymes pointed out that some species such as *Phanerochaete chrysosporium* [29,31], *Gloeophyllum trabeum* [32], *Trichoderma harzianum*, *T. viride*, *Aspergillus niger* [11], *A. nidulans* [24] and *A. fumigatus* [33–35], have played an

important part. Similarly, the use of microbial consortium has been successfully carried out in studies where each species was chosen based on specific ability has been successfully carried out [36].

The inoculation of fungal cultures isolated and selected from the compost heaps themselves in order to maximize the existing colonies can promote improvements in all the phases of the process and decrease adverse effects such as competition from exogenous microorganisms during the various phases of the composting process. The results presented in this research are preliminary; however, it is already evident that the studied compound itself can present lignocellulite microorganisms capable of degrading the recalcitrant material existing in the equine bedding, through the production of specific enzymes, capable of using a wide source of carbon such as: rice straw and shavings. Similar conclusions were presented by Li et al., 2019 [37] when analyzing the composting process of pig manure and corn straw with the application of bacterial inoculants selected from the pig manure compost. It was observed that these native inoculants prolonged the thermophilic phase of the process by two days and increased the germination rate.

Although the compound process of maturation presents several variables that will influence the quality of the product, the analysis of the effects of the enzymatic activities, produced by the organisms that degrade high complexity substrates, can be a way to boost decomposition cycles.

The total cell activity analyzed in this research shows that the isolates presented a satisfactory result in the first 14 days, the isolates PA5.1 and PA7.7 obtained the best index and showed a tendency to increase the values over time. These indices can be explained by the identification of the isolates as fungal strains A. *fumigatus.*

This species is recognized among the dominant species in the thermophilic phase of composting [33,34]. *A. fumigatus* are thermophilic, saprophytic and pathogenic fungi, being one of the most abundant species in decomposing materials [38]. They can survive in environments whose temperatures range from 12 to 65 °C and pH ranges from 2.1 to 8.8. [39]. Isolates of *A. fumigatus* have also been described for their ability to produce gibberellins and other plant hormone regulators [40], in addition to their ability to produce biosurfactants, which assist the degradation of crude oil [41], reinforcing the versatility and potential of this fungal species in various bioprocesses.

The dominance of the genus *Aspergillus* spp. among the fungal isolates corroborates the results of other studies on the same topic [13,35]. In a study conducted to evaluate the diversity of fungi in organic compostig of fruit waste, the species *A. fumigatus* was the most frequent [30], a result similar to what was observed in this study.

Investigation of secretomes of *A. fumigatus* grown on 3 carbon sources (glucose, avicel and rice straw) observed an increased production of lignocellulose in the medium containing rice straw, leading to the conclusion that specific enzyme mixtures are produced by the fungus under specific conditions [42].

The high cellulolytic activity of *A. fumigatus* isolates has also been recognized in research isolating fungi from organic composts of fruit waste [35] and municipal solid waste [13]. Isolates of *A. fumigatus*, inoculated separately or in consortium with other fungi or bacteria, have efficiently contributed to reduce composting time and humification of composted organic waste [30,43]. Promising results were observed in the joint inoculation of *A. fumigatus* Z5 and *Geobacillus stearothermophilus* B5 in rice straw waste, with prolongation of the thermophilic phase and greater degradation of cellulose and hemicellulose [30]. The use of a consortium of 3 *Aspergillus* species (*A. fumigatus*, *A. flavus* and *A. terreus*) reduced the compost stabilization time of rice straw waste and poultry manure [43].

The results found provided information of different responses of *A. fumigatus* strains regarding the enzymatic production indexes using the same carbon source and in different carbon sources. This fact can be explained by the fact that *A. fumigatus* are constituted by multiple sets of important genes for cellulolytic enzymes and their gene expression depends among other factors on the carbon source. This species employs important strategy and versatility to compound degradation, diversifying secretory enzyme production,

suggesting differentiated patterns of enzyme expression activated by different carbon sources [44–46].

A. fumigatus can grow in the presence of complex biomasses as its transcriptional profile can be completely distinct when subjected to distinct carbon sources, varying the profile of genes encoding hydrolytic enzymes [47].

5. Conclusions

Biotechnological tools such as the isolation of microorganisms with specific degradation abilities of certain organic compounds are important to optimize waste recycling through composting. The isolation of fungi with potential cellulolytic ability, obtained from residues of saturated bed of horses, formed by shavings and rice straw, allowed the selection of seven isolates identified as belonging to the species *A. fumigatus*.

The degree of complexity of this fungus in producing enzymes amidst the available carbon source was identified. *A. fumigatus* showed excellent rates of total cell activity in environments containing rice straw as a carbon source. The isolates PA7.5 and PA7.7 presented the highest enzymatic activity of total cellulase, standing out for inoculation in composting processes of saturated bedding of horses, with potential for maintaining the thermophilic phase of the process, as well as the acceleration of maturation of the compounds.

From the analysis of the enzymatic activities, the present study demonstrated that *A. fumigatus* has cellulolytic capacity with carbon sources from the studied compost, indicating its potential for the degradation of lignocellulosic substrates present in saturated horse litter, which can be used as an inoculant to optimize the maturation process of this material. The reapplication of these microorganisms can be important to optimize the time required for the stabilization of residues through composting.

Author Contributions: Conceptualization, A.G.C.R.d.N., A.M.d.P., J.G.B. and A.R.T.N.; methodology, A.G.C.R.d.N. and A.M.d.P.; formal analysis A.G.C.R.d.N. and A.M.d.P.; investigation, A.G.C.R.d.N., A.M.d.P., J.G.B. and A.R.T.N.; resources, A.G.C.R.d.N., and A.R.T.N.; data curation A.G.C.R.d.N., A.M.d.P., J.G.B. and A.R.T.N.; writing—original draft preparation, A.G.C.R.d.N. and S.G.d.S.; writing—review and editing, A.G.C.R.d.N. and S.G.d.S.; visualization, A.G.C.R.d.N. and S.G.d.S.; supervision, A.G.C.R.d.N., A.M.d.P., J.G.B. and A.R.T.N.; project administration, A.G.C.R.d.N. and A.M.d.P.; funding acquisition, A.G.C.R.d.N. and A.R.T.N. All authors have read and agreed to the published version of the manuscript.

Funding:

Institutional Review Board Statement: No applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

References

[1] BUSATO, J.G., PAULA, A. M., FERRARI, L. H. Enriquecimento microbiano visando otimizar o processo de compostagem. In: Severiano, E.C., Moraes, M.F.,Paula, A.M. (Org.). Tópicos em Ciência do Solo. **2019**, Volume X. pp 522-576 1ªed.Viçosa: SBCS.

[2] KHAM, F., ALI, Y. Moving towards a sustainable circular bio-economy in the agriculture sector of a developing country. Ecological Economics. **2022**. v. 196, 107402. doi:10.1016/j.ecolecon.2022.107402

[3] ONU. Organização das Nações Unidas. Objetivos de Desenvolvimento Sustentável. Disponível em https://nacoesunidas.org/pos. **2015**. Acesso 15/05/2022.

[4] DE SOUZA, G. H. R.; RODRIGUES, G. A. O tratamento da cama de equinos através do processo de compostagem. Revista Interface Tecnológica, **2017**, v.14, n.2, p. 100-110. doi: 10.31510/infa.v14i2.193

[5] BÖSKE J.; WIRTH B.; GARLIPP F.; MUMME J.; VAN DEN W. H. Anaerobic digestion of horse dung mixed with different bedding materials in an upflow solid-state (UASS) reactor at mesophilic conditions. Bioresource Technology, **2014**, v. 158, p.111-118. doi:10.1016/j.biortech.2014.02.034

[6] WESTENFORD, M., WILLIAN, C.A., MURPHY, S., KENNY, L., HASHEMI, M. Generation and Management of Manure from Horses and Oter Equids. In: WALDRIP, H.M., PAGLIARI P.H., HE, Z.: Animal Manure: Production, Characteristics, Environmental Concerns, and Management., Inc. Soil Science Society of America. **2020**. doi: 10.2134/asaspecpub67.c8

[7] FUJII, K. Y.; DITTRICH, J. R.; CASTRO, E. A. D.; SILVEIRA, E. O. C. Processos de tratamento de resíduos de cocheira e a redução ou eliminação de ovos e larvas infectantes do gênero Strongylus spp. Arquivos do Instituto Biológico, **2014**, v. 81, p. 226-231. doi 10.1590/1808-1657000482012

[8] GREFF, B.; SZIGETIJ.; NAGY A.; LAKATOS E.; VARGA L. Influence of microbial inoculants on co-composting of lignocellulosic crop residues with farm animal manure: A review. Journal of Environmental Management, **2022**, v. 302, p. 114088. doi: 10.1016/j.jenvman.2021.114088.

[9] HARINDINTWALI, J.D., ZHOU, J.L., YU, X.B. Lignocellulosic crop residue composting by cellulolytic nitrogen-fixing bacteria: a novel tool for environmental sustainability. Sci. Total Environ. **2020** 715, 136912. doi.org/10.1016/j.scitotenv.2020.136912.

[10] JIMENEZ-LOPEZ, C., FRAGA-CORRAL, M., CARPENA, M., GARCÍA-OLIVEIRA, P., ECHAVE, J., PEREIRA, A.G., LOURENÇO-LOPES, C., PRIETO, M.A., SIMAL-GANDARA, J. Agriculture wastes valorisation as a source of antioxidant phenolic compounds within a circular and sustainable bioeconomy. Food Funct, **2020**, 11, 4853–4877. doi.org/10.1039/d0fo00937g.[11] DU G.; FENG W.; CAI H.; MA Z.; LIU X.; YUAN C.; ZHANG B. Exogenous enzyme amendment accelerates maturity and changes microflora succession in horse and wildlife animal manure co-composting. Environmental Science and Pollution Research, **2021** v.28 p. 1-11. doi: 10.1007/s11356-020-11568-4

[12] DOS SANTOS PEREIRA, T.; PAULA, A.M.; FERRARI, L.H.; SILVA, J.; PINHEIRO, J.B.; NAVAS CAJAMARCA, S.M.; JINDO, K. ; SANTOS, M.P.; ZANDONADI, D.B.; BUSATO, J.G. Trichoderma-Enriched Vermicompost Extracts Reduces Nematode Biotic Stress in Tomato and Bell Pepper Crops. Agronomy-Basel, **2021**, v. 11, p. 1655. doi: 10.3390/agronomy11081655

[13] AWASTHI, A K., YUAN Z., AWASTHI. M.K., LI, M., MISHRA S., PANDEY A.K. Bioprocess potential of Eco-friendly fungal isolates converting organic waste to bioresource. Bioresource Technology, **2022** v. 346. doi: 10.1016/j.biortech.2021.126586

[14] GREFF, B., SZIGETI, J.;VARGA, A.; ĺLAKATOS, E.; SAHʹO, A., VARGA, L. Effect of bacterial inoculation on co-composting of lavender (Lavandula angustifolia Mill.) waste and cattle manure. 3 Biotech, **2021**, 11, 306. doi.org/10.1007/s13205-021-02860-2.

[15] SHARMA, P.; GAUR V.K.; KIM SH.; PANDEY A. Microbial strategies for bio-transforming food waste into resources. Bioresource technology, 2020, v. 299, p. 122. doi: 10.1016/j.biortech.2019.122580

[16] XU, J.Q., LU, Y.Y., SHAN, G.C., HE, X.S., HUANG, J.H., LI, Q.L., Inoculation with compost-born thermophilic complex microbial consortium induced organic matters degradation while reduced nitrogen loss during co-composting of dairy manure and sugarcane leaves. Waste Biomass Valor, **2019a**, 10, 2467–2477. doi.org/10.1007/s12649-018-0293-y

[17] BUSATO, J. G.; FERRARI, L. H.; CHAGAS JUNIOR, A. F.; SILVA, D. B.; PEREIRA, T. S.; PAULA, A. M. Trichoderma strains accelerate maturation and increase available phosphorus during vermicomposting enriched with rock phosphate. Journal of Applied Microbiology, **2021**, v. 130, p. 1208-121. doi: 10.1111/jam.14847

[18] WANG, X.; KONG, Z; WANG, Y.; WANG, M.; LIU, D.; SHEN, Q. Insights into the functionality of fungal community during the large scale aerobic co-composting process of swine manure and rice straw. Journal of Environmental Management, **2020**, v. 270, p. 110958 .doi 10.1016/j.jenvman.2020.110958.

[19] WU, Y.P., CHEN, Y.X., SHAABAN, M., ZHU, D.W., HU, C.X., CHEN, Z.B., WANG, Y. Evaluation of microbial inoculants pretreatment in straw and manure co-composting process enhancement. J. Clean. Prod., **2019**, 239, 118078. https://doi.org/10.1016/j. jclepro.2019.118078.

[20] PARKINSON, D.T., GRAY, R.G., WILLIAMS, S.T. Methods for studying the ecology of soil microorganisms. Melbourne. 19° ed. Blackwell Scientifi, California, **1997**, p. 465.

[21] TEDESCO, M.J.; GIANELLO, C.; BISSANI, C. A.; BOHNEN, H.; VOLKWEISS, S. J. Análises de solo, plantas e outros materiais. 2.Ed. Porto Alegre: Dpto. de solos da UFRGS, 174 p. 1995. In: TIAGO, P. V.; MELZ, E. M.; SCHIEDECK, G. Comunidade de bactérias e fungos de estercos antes e após vermicompostagem e no substrato hortícola após uso de vermicomposto. Revista Ciencia Agronomica, **2008,** v.39, n. 2, p. 187-192.

[22] RIBEIRO, M.C.; SOARES, M.M.S.R.: Microbiologia prática: roteiro e manual. Atheneu, São Paulo, (2002).

[23] CASTELLANI, E. D.; SILVA, A.; BARRETO, M. Influência do tratamento químico na população de fungos e na germinação de sementes de Bauhinia variegata L. VAR. Variegata. Revista Brasileira de Sementes, Brasília, **1996**, v.8, n.1, p.41-44.

[24] MANDELS. M.; WEBER, J. Production of cellulases. Advances in Chemistry Series. Whashington, **1996**, v.95, p.391-414.

[25] MILLER, G.L. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry, **1959**, v.31, p.426-428.

[26] GHOSE, T.K. Measurement of cellulase activities. Pure and Applied Chemistry, **1987**, v.59, 257-268.

[27] WHITE, T.J., BRUNS, T., LEE, S., TAYLOR, J. Amplifcation and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M.A., Gelfand D.H., Sninsky J.J., White T.J.: PCR Protocols **1990**.

[28] FERREIRA, D. Análises estatísticas por meio do Sisvar para Windows versão 4.0. Análises estatísticas por meio do Sisvar para Windows versão 4.0. 255-258, **2000.**

[29] SAJID, S.; KUDAKWASHE O.; RESCO DE DIOS V.; NABI F.; KYUNG Y.; KALERI A. R.; MA L.; ZHOO L.; ZHANG W.; DONG F.; HAN Y. Pretreatment of rice straw by newly isolated fungal consortium enhanced lignocellulose degradation and humification during composting. Bioresource Technology,**2022**, v.354,p.127. doi:10.1016/j.biortech.2022.127150.

[30] WANG, M. WANG, X. YUNCHENG, W. XIAOSONG, W. JIAVIN, Z. YU, L. ZHE, C. ZHONGKUN, J. WEI, T. JIBING Z. Effects of thermophiles inoculation on the efficiency and maturity of rice straw composting. Bioresource Technology, **2022**, v. 354, p. 127-195. doi: 10.1016/j.biortech.2022.127195.

[31] ZENG, G., YU, M., CHEN, Y., HUANG, D., ZHANG, J., HUANG, H., YU, Z. Effects of inoculation with Phanerochaete chrysosporium at various time points on enzyme activities during agricultural waste composting. Bioresource Technology, **2010**, v. 101, n. 1, p. 222-227. doi: 10.1016/j.biortech.2009.08.013

[32] ZHU, N., ZHU, Y., KAN, Z., LI, B., CAO, Y. JIN, H. Effects of two-stage microbial inoculation on organic carbon turnover and fungal community succession during co-composting of cattle manure and rice straw. Bioresource Technology, **2021**, v. 341, p. 125842. doi: 10.1016/j.biortech.2021.125842

[33] HEFNAWY, M.; GHARIEB, M.; NAGDI. O.M.: Microbial diversity during composting cycles of rice straw. International Journal of Advanced Biological and Biomedical Research. **2019**, v.7, p.30-43. doi: 10.3390/biology11040569

[34] NOREEN, N.; RAMZAN, N.; PARVEEN, Z.; SHAHZAD, S. A comparative study of cow dung compost, goat pellets, poultry waste manure and plant debris for thermophilic, thermotolerant and mesophilic microflora with some new reports from Pakistan. Pakistan Journal of Botany, **2019**, v.42, p.1155-1159. doi: 10.30848/PJB2019-3(42)

[35] DANISH, S.A.; HAQ, T.; PAULA, A.M.; FERRARI, L.H.; SILVA, J.; ZAFAR, U. Succession and Catabolic Properties of Fungal Community During Composting of Fruit Waste at Sub-Tropical Environment Waste Biomass (2022). doi:10.1007/s12649-021-01653-1.

[36] XU, J.Q., J. IANG, Z.W., LI, M.Q., LI, Q.L. A compost-derived thermophilic microbial consortium enhances the humification process and alters the microbial diversity during composting. J. Environ. Manag. **2019b**, 243, 240–249. doi.org/10.1016/j.jenvman.2019.05.008.

[37] LI, S.Y., LI, J.J., YUAN, J., LI, G.X., ZANG, B., LI, Y.Y.2. The influences of inoculants from municipal sludge and solid waste on compost stability, maturity and enzyme activities during chicken manure composting. Environ. Technol., 2017 38, 1770–1778. doi.org/10.1080/09593330.2017.1291755

[38] ROCHA, V.A.L; MAEDA, N. R.; PEREIRA JR, N.; KERN, F.M.; ELIAS, L.; SIMISTER, R.; MCQUEEN-MASON, S. J. Characterization of the cellulolytic secretome of Trichoderma harzianum during growth on sugarcane bagasse and analysis of the activity boosting effects of swollenin. Biotechnology progress, **2016**, v.32(2), p. 327-336. doi:10.1002/btpr.2217

[39] KWON-CHUNG, K. J.; SUGUI, J. A. Aspergillus fumigatus—what makes the species a ubiquitous human fungal pathogen?. *PLoS pathogens*, **2013**, v.*9*(12), 1003743. doi:10.1371/journal.ppat.1003743

[40] BILAL, L.; ASAF, S.; HAMAYUN, M.; GUL, H.; IQBAL, A.; ULLAH, I.; LEE, I-J.; HUSSAIN, A. Plant growth promoting endophytic fungi Asprgillus fumiga-tus TS1 and Fusarium proliferatum BRL1 produce gibberellins andregulates plant endogenous hormones. Symbiosis, **2018,** v.76, p.117–127. doi: 10.1007/s13199-018-0545-4

[42] LIU, D.; LI, J.; ZHAO, S.; ZHANG R.; WANG, M.; MIAO, Y.; SHEN, Y.; SHEN, Q. Secrtome diversity and quantitative analysis of cellulolytic Aspergillus fumigatus Z5 in the presence of diferente carbon sources. Biotechnol Biofuels, **2013,** v.6, p.149 (2013). doi: 10.1186/1754-6834-6-149

[42] OTHMAN, A. R.; ISMAIL N. S.; SHEIKH S.R. Potential of indigenous biosurfactant-producing fungi from real crude oil sludge in total petroleum hydrocarbon degradation and its future research prospects. Journal of Environmental Chemical Engineering, **2022**, v. 10, n. 3, p. 107-621. doi: 10.1016/j.jece.2022.107621

[43] KHYALIA, P.; DANGI, J.; BARAPATRE, S.; DHANIA, G.; LAURA, J.; NANDAL, M. Comparative Analysis of Compost Quality Produced from Fungal Consortia and Rice Straw by Varying C/N Ratio and its Effect on Germination of Vigna radiata, **2022**, v.21, p.1235-1243. doi.org/10.46488/NEPT.2022.v21i03.029

[44] GREFF, B., SZIGETI, J., NAGY, Á., LAKATOS, E., & VARGA, L. Influence of microbial inoculants on co-composting of lignocellulosic crop residues with farm animal manure: A review. Journal of environmental management, **2022**, 302, 114088. doi.:10.1016/j.jenvman.2021.114088

[45] DE GOUVÊA, P.F;, BERNARDI, A.V.; GEROLAMO, L.E.; DE SOUZA SANTOS, E.; RIAÑO-PACCHÓN, D.M.; UYMURA, S.A.; DINAMARCO, T.M.; Transcriptome and secretome analysis of *Aspergillus fumigatus* in the presence of sugarcane bagasse. *BMC Genomics*, **2018**, v.19, p. 232.doi.:10.1186/s12864-018-4627-8

[46] VELASCO, J.; SEPULCHRO, A. G. V.; HIGASI, P. M.; PELLEGRINI, V. O.; CANNELLA, D.; DE OLIVEIRA, L. C.; SEGATO, F. Light Boosts the Activity of Novel LPMO from Aspergillus fumigatus Leading to Oxidative Cleavage of Cellulose and Hemicellulose. *ACS Sustainable Chem. Eng.* **2022**, 10, 50, 16969–16984. doi.:/10.1021/acssuschemeng.2c06281.

[47] BERNARDI, A. V. Identificação das principais enzimas hidrolíticas de Aspergillus fumigatus quando crescido em bagaço de cana-de-açúcar. Dissertação (Mestrado em Química). Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, **2017.** doi:10.11606/D.59.2017