Prospection of cellulolytic fungi from compost samples of saturated equine bedding

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Annually each adult horse can generate up too 10 tons of waste such as the bedding of plant materials installed in stalls. The direct and untreated application of this type of waste in pastures increases the emission of greenhouse gases, contaminates the soil and increases the incidence of pathogenic microorganism (Westendorf et al., 2020). The bioconversion of organic waste by employing the composting process is a functional and low-cost alternative for the safe recycling of these materials, giving rise to a stabilized final product (Busato et al., 2019). Through this practice, the circular bioeconomy is fostered (Khan e Ali, 2022) and negative environmental impacts on the environment are minimized and composting is a tool recognized by the United Nations Agenda 2030 (UN, 2015)

Due to the nature of the materials used in the making of the horse bedding, the process of composting this material can be long. The enrichment of the piles with specific microorganisms can optimize the composting process, which is a promising biotechnological tool capable of increasing the activity of enzymes that degrade lignocellulosic biomolecules (Awasthi et al., 2022). However, research involving the isolation of fungi with potential for degradation directly from equine bedding is scarce. Thus, the presented work aimed to isolate and identify fungi present in compost piles of saturated bedding of horses made with shavings and rice straw, aiming to select those with greater potential for cellulolytic activity.

Compost piles composed of horse manure combined with wood shavings or rice straw were assembled at the Large Animal Veterinary Hospital of the University of Brasilia. The pyramidal piles were monitored daily for temperature and humidity and When They reached the thermophilic phase (temperatures above 60°C) the material was sampled in five different points to form a composite sample. For fungal isolation the sample were serially diluted in 0.9% NaCl saline solution (up to 10^{-8}) and inoculated in cellulolytic selective medium, according to Parkinson et al. (1971), with adaptations. To replace the carboxymethylcellulase, compounds based on wood shavings and rice straw, the same used as basic components of horse bedding were used. Seven isolates were isolated in solid medium using the streak depletion technique (Ribeiro e Soares, 2002), and these were morphologically characterized using the slide culture technique, allowing the observation of their hyphae and sporulation pattern by optical microscopy. All isolates obtained were identified as belonging to the genus *Aspergillus* (Figure 1).

Molecular identification of the isolates was also performed by extraction of fungal DNA using the FavorPrepTM Soil DNA Isolation Mini Kit, followed by amplification of genetic material by the polymerase chain reaction (PCR) technique, using the primes ITS1 and ITS4 (White et al., 1990). In this case, all isolates were identified as belonging to the *Aspergillus fumigatus* species.

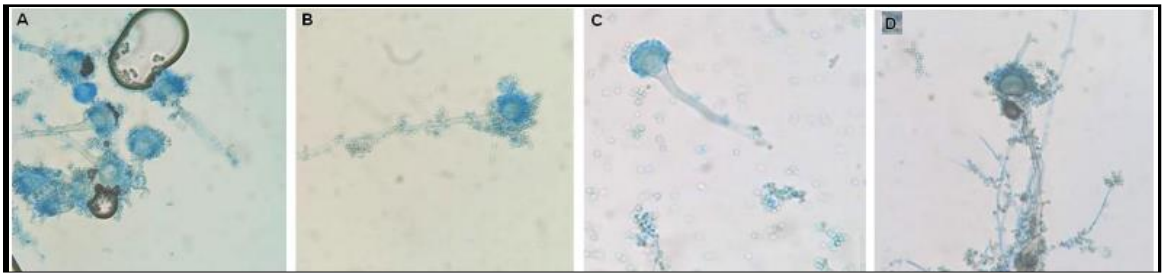

Figure 1. Micromorphological characteristics of isolated fungi. Photomicrographs performe using a microscope. 40 X magnification.

To evaluate the cellulase enzyme activity the isolates obtained were inoculated in liquid basal culture medium containing, as carbon source, the substrate from which they isolated (wood shavings or rice straw). After incubation, the total cellulase activity was evaluated using the 3,5 dinitrosalicylic acid (DNS) (Ghose, 1987). From the 7 isolates showing total cellulase activity, 4 were obtained from the wood shavings substrate and 3 from the rice straw substrate. Isolates PA-7 5 and PA-7 7, obtained from rice straw, showed the highest cellulolytic activities (0,376 e 0,358 UI mL^{-1}, respectively, Figure 2).

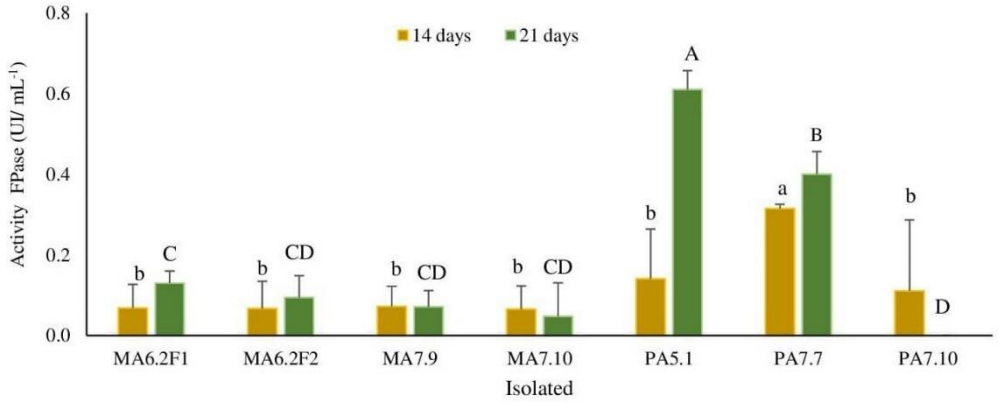

Figure 2. Graphic representation of the averages of the enzymatic activities of total cellulase (FPase; UI mL^{-1}). MA: fungi isolated from horse bed compost made with wood shavings; PA: fungi isolated from horse bed compost made with rice straw. Low case letters compare the enzymatic activity in the incubation period of 14 days. Capital letters compare the enzymatic activity of the isolates in the incubation period of 21 days. Different letters indicate difference by t (Student) at 5% de probability.

The high cellulolytic activity of *A. fumigatus* isolates was also recognized in research isolating fungi from organic composts of fruit waste (Danish et al., 2022) and municipal solid waste (Awasthi et al., 2022). This species was also recognized among the dominant species in the thermophilic phase of composting cow, goat and poultry manure (Noreen et al., 2019). Promising results were also observed in inoculation of a consortium of 3 species of fungi of the genus aspergillus (*A. fumigatus*, *A. flavus* e *A. terreus*) reduced the stabilization time of rice straw and poultry manure waste compost (Khyalia et al., 2022). In the present work, the isolate PA-7 5 and PA-7 7 showed the highest total cellulase activity, standing out as promising to be reapplied in equine saturated bedding compost piles, potentially allowing accelerating compost maturation.

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