CUTINASE ACTIVITY OF FUNGAL STRAINS ON DIFFERENT PLASTICS: IMPLICATIONS FOR SUSTAINABLE DEGRADATION

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ABSTRACT

Plastic is widely used due to its durability, strength, and low cost, making it essential in various sectors. However, its extensive use has resulted in significant environmental challenges, affecting marine and terrestrial ecosystems. There are different types of plastics, each with unique properties and uses, such as polyurethane (PU), high-density polyethylene (HDPE), low-density polyethylene (LDPE), polypropylene (PP), polyvinyl chloride (PVC), polystyrene (PS), and polyethylene terephthalate (PET). This study aimed to evaluate the cutinase activity of fungal strains grown on polyester film and various disposable plastics (PET, HDPE, and PP) as sole carbon sources. Stationary cultures and cultures under agitation were used to grow fungi, and enzymatic activity was measured using p-nitrophenylbutyrate hydrolysis. Fungi from the Atlantic Forest and soybean plantation soil demonstrated cutinase activity, with *Cunningamella echinulata* showing the highest enzyme production after 10 days. *Aspergillus niger* exhibited greater cutinase activity with all three plastics after 5 days, while C. *echinulata* sustained its production for 5-20 days. The findings underscore the potential of these fungi, particularly *C. echinulata*, for developing effective and sustainable plastic degradation processes, highlighting their promising role in future environmental applications.

Keywords: Degradation. *Cunningamella echinulata*. environmental contamination. PET. plastic.

1 INTRODUCTION

Plastic is one of the most versatile and widely used materials in modern society because of its durability, strength, and low production cost, making it indispensable in sectors ranging from packaging and consumer products to electronics and medicine. However, despite its many benefits, plastic has also become one of the greatest environmental and social challenges of our time. The increasing production and rampant consumption of plastic have had serious negative impacts on our planet, with visible effects in oceans, rivers, and terrestrial ecosystems directly affecting marine and terrestrial life[1].

There are various types of plastics, each with distinct chemical properties, characteristics, and specific applications, such as polyurethane (PU), high-density polyethylene (HDPE), low-density polyethylene (LDPE), polypropylene (PP), polyvinyl chloride (PVC), polystyrene (PS), polyethylene terephthalate (PET), and polymethylmethacrylate (PMMA)[2]. Despite being made from thermoplastic polystyrene, Styrofoam is regarded as a distinct material due to its unique cellular structure and specific properties [3]. Styrofoam consists of closed cells filled with air, making it lightweight, insulating, shock-absorbing, and buoyant. These features set it apart from traditional plastics, which possess solid, three-dimensional polymer structures.

This study specifically aimed to evaluate the cutinase activity of selected fungal strains[4-6] when grown on polyester film and different disposable plastic packaging, including PET, HDP and PP, as the only carbon sources. Through detailed enzyme activity assays, this investigation sought to identify fungal species with significant plastic degradation capabilities, thus contributing to the development of effective and sustainable plastic degradation processes. The findings highlight the promising role of these fungi in future environmental applications for plastic waste management.

2 MATERIAL & METHODS

Polyester film assay:

Stationary cultures were maintained for 20 days at 28°C in 30 mL of Czapeck medium without glucose, using 1% (w/v) Polyester film (Applied Biosystems®) as the sole carbon source. This polyester film is typically used for sealing microplates in real-time PCR assays. Liquid cultures were grown under the same conditions with orbital shaking at 120 rpm. Each culture was inoculated with 2.2×10^4 spores/mL in 1.5 mL of sterile aqueous solution containing different fungal species. After 20 days, the cultures were filtered using a vacuum pump in a laminar flow cabinet, and the cutinase activity in the filtrate was measured.

Single-use packaging plastic assay:

Fungal growth was conducted in Czapec medium without glucose in both stationary mode (S) and under constant agitation (A) at 120 rpm and 28°C. Each 150 ml flask containing 30 ml of medium (w/v) was inoculated with 1.5 ml of sterile aqueous solution containing spores ($2.2x10^4$/mL) from each of the different tested strains. The various types of plastics obtained from different new

single-use packaging, including polyethylene terephthalate (PET), high-density polyethylene (HDP), and polypropylene (PP), were cut into 1x1 cm squares and sterilized using physicochemical methods. The cultures were maintained under different conditions for 5, 10, 15, or 20 days and then aseptically filtered in a laminar flow cabinet, after which the filtrate was used to determine the enzymatic cutinase activity.

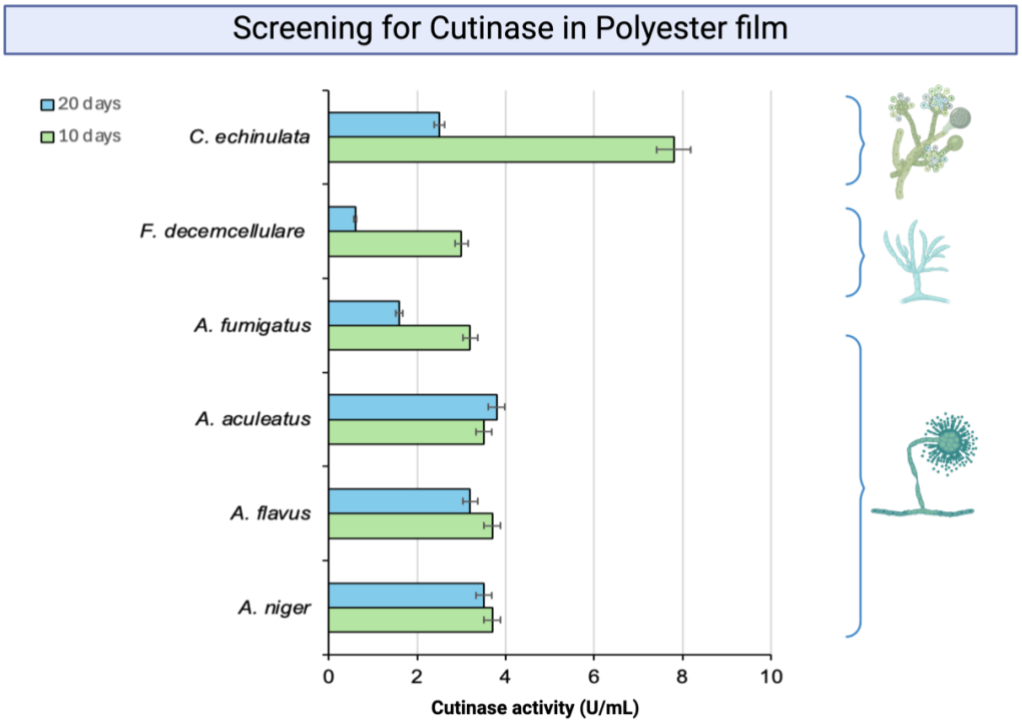
Cuttinase activity:

The enzymatic activity was measured by the hydrolysis of p-nitrophenylbutyrate (p-NPB) at a concentration of 1.12 mM using a reaction mixture containing 0.43 M tetrahydrofuran (THF) and 0.2% Triton X-100 in 50 mM phosphate buffer (pH 7.2). A 3.43 mL aliquot of the substrate was added to 70 µL of the fungal sample and incubated for 15 minutes at 30°C. After the incubation period, the absorbance of the reaction mixture was measured at 405 nm using a spectrophotometer. One enzyme unit (U) was defined as the amount of enzyme required to produce 1 µmol of p-nitrophenol per minute of reaction.

3 RESULTS & DISCUSSION

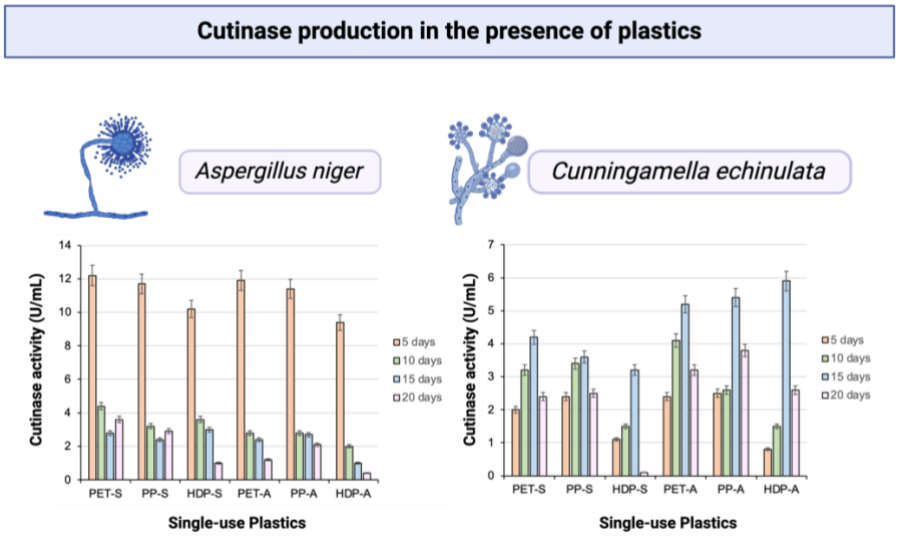
This study explored the potential for plastic degradation of cutinase-producing fungi isolated from the Atlantic Forest biome at the Bela Vista Biological Refuge in Foz do Iguaçu, Paraná, Brazil. Fungi capable of growing on polyester microfilms were selected for cutinase activity measurements. The analyses identified three Aspergillus species (*A. flavus* strain KJ470626, *A. aculeatus* strain KM382062, and *A. fumigatus* strain KM382061) (Corrêa et al. 2019) and one *Fusarium* species (*F. decemcellulare* strain KY523049)[4] from the Atlantic Forest, as well as *Cunninghamella echinulata* from soil in a soybean plantation in Nova Aurora, Paraná[5-6]. These five fungal strains demonstrated cutinase activity in the presence of plastic film as the sole carbon source (Figure 1), with *C. echinulata* showing the highest enzyme production (approximately 8 U/mL) after 10 days of stationary culture (Figure 1).

Figure 1. Cutinase activity in Polyester film.



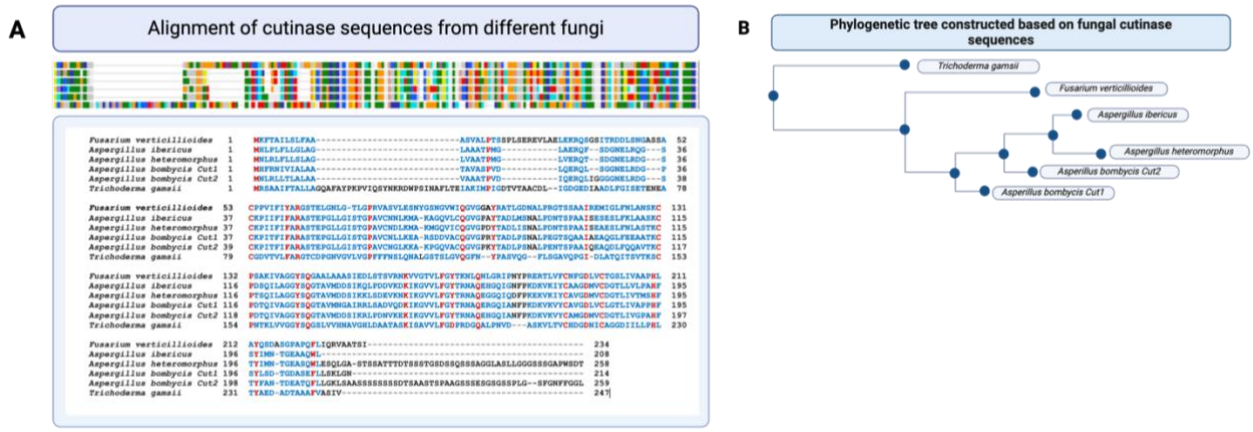
Additionally, the cutinase activities of *Aspergillus niger* (strain ATCC 16404) and *C. echinulata* (strain PA3S12MM) were compared using different single-use packaging plastics (PET, HDP, and PP) as the sole carbon source in stationary mode (S) and under constant agitation (A) (Figure 2). *A. niger* exhibited greater cutinase activity (>12 U/mL) after 5 days of cultivation with all three plastics, while *C. echinulata* maintained cutinase production (approximately 6 U/mL) over extended growth periods (5-20 days) (Figure 2). The ability of *C. echinulata* to sustain cutinase production over long periods with different types of petrochemical plastics underscores its potential for optimizing plastic degradation processes. However, all the fungal species studied are promising candidates for future experiments aimed at enhancing the activities of cutinase or other enzymes and developing controlled plastic degradation processes.

Figure 2. Cutinase activity in different plastic



There are few cutinase genes as well as their predicted proteins deposited in GeneBank. However, some fungal cutinase sequences were obtained from NCBI (*National Center for Biotechnological Information*) and used for a sequence alignment that showed a great structural conservation of these proteins (Figure 3A) except for the cutinase from a *Trichoderma* species that presents additional sequences absent in the other proteins. The phylogenetic tree obtained from this alignment of cutinase protein sequences also places the *Trichoderma* specie phylogenetically more distant from the *Aspergillus* and *Fusarium* species (Figure 3-B). The structural analysis of similar protein sequences is an important step in attempting to clone protein genes whose genome has not yet been sequenced, such as the fungal species in the present work.

Figure 3 Alignment of cutinase and phylogenetic analysis



4 CONCLUSION

In conclusion, the findings of this study highlight the potential of cutinase-producing fungi, particularly *C. echinulata*, for optimizing and developing effective plastic degradation processes, emphasizing the promising role of these fungal species in future environmental applications.

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ACKNOWLEDGEMENTS

P.M.C.R, J.P., M.T. and A.F. was supported by CAPES; J.T.K.M. was supported by CNPq; J.O. was supported by PIBIC-Unioeste and R.C.G.S. was supported by FUNDEP: Fundação de Apoio ao Ensino, Extensão, Pesquisa e Pós-Graduação and Unioeste, Cascavel, PR.