FUNGI FROM THE DIGESTIVE TRACT OF *PHYLLOICUS*: BIOTECHNOLOGICAL POTENTIAL IN CELLULASE PRODUCTION

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ABSTRACT

Microbial enzymes are crucial in optimizing industrial processes, driving enzymatic technology, and fueling a growing market. Cellulases, which degrade cellulose, the most abundant biopolymer on Earth, are particularly relevant. Filamentous fungi, such as those found in the digestive tract of insects, possess diverse metabolisms due to their adaptation to different habitats. *Phylloicus* larvae, aquatic insects, feed on leaves and participate in nutrient recycling along with fungi. This study aimed to evaluate the production of cellulases by fungi isolated from the digestive tract of *Phylloicus* larvae and identify the species of these insects in streams of the Serra do Tepequém, Amajarí-RR. The fungi were isolated and tested in a medium containing cellulose as a carbon source. Of the 71 fungi tested, 60% produced cellulases, with 13% being considered good cellulase producers. Molecular identification revealed that the best producers belonged to the genera Devriesia, Microascus, Clonostachys, and Aspergillus.These results highlight aquatic shredding insects as an important source for isolating new fungi with biotechnological potential, especially in cellulase production.

Keywords: Cellulases. Filamentous fungi. *Phylloicus*.

1 INTRODUCTION

Microbial enzymes have become a crucial alternative for optimizing various industrial processes, driving the development of enzymatic technology and meeting the growing demand for more stable, specific biocatalysts capable of operating under industrial conditions. With the increase in industrial production, there is also a rise in waste generation, which can be reused as carbon sources, reducing pollution and increasing efficiency. There is a significant search for harmless, stable, and specific enzymes, especially in sectors such as food, beverages, paper, textiles, and biofuels. Fungi, due to their ability to inhabit diverse environments and assimilate a variety of substrates, are particularly important in enzyme production. They play a crucial role in the production of second-generation biofuels, utilizing agricultural waste as raw material. Studies have shown that insects, especially shredders, are important for isolating new fungal species, with insect guts standing out as a conducive environment for discovering new enzymes. However, studies of this nature are still scarce in the Amazon region, highlighting the importance of bioprospecting to find solutions in areas such as food, health, and the environment. An example is the creation of the thematic network "Ecology and bioprospecting of mutualistic fungi and aquatic insects in Amazonian ecosystems," funded by BIONORTE, which aims to develop and apply these biotechnological resources. The study in question focuses on the isolation, identification, and selection of cellulase-producing fungi in the digestive tract of *Phylloicus*, as well as the identification of insect species in streams in the Serra do Tepequém, Roraima.

2 MATERIAL & METHODS

2.1 Collection, Rearing, and Identification of *Phylloicus*: In the Serra do Tepequém, *Phylloicus* were collected from ten streams, with five individuals per stream. The larvae were disinfected and transported to the laboratory, where their digestive tracts were dissected and fungi isolated. Additional *Phylloicus* larvae were collected for rearing in the laboratory to facilitate the specific identification of the adults. The larvae were reared in containers with water and leaves, being monitored and fed periodically. The adults were preserved and prepared for taxonomic identification, following specific methods.

2.2 Fungal Isolation: Three of the five larvae collected from each stream were chosen for fungal isolation, based on the most advanced developmental stage and the integrity of the digestive tract. The digestive tracts were placed in microtubes with sterile water for plating on potato dextrose agar (PDA) medium. The colonies were observed daily and grouped into morphotypes. The morphotypes were purified, subcultured, and characterized for both macromorphology and micromorphology.

2.3 Selection of Cellulase-Producing Strains and Semiquantitative Evaluation: A qualitative enzymatic test was conducted on a selective solid medium containing carboxymethyl cellulose (CMC) to select cellulase-producing strains. Colonies with positive enzymatic activity were identified by the formation of a hydrolysis halo around the CMC. The enzymatic index (EI) was established for the semiquantitative evaluation of enzymatic production, with colonies having an EI ≥ 2 considered potential for biotechnological application.

2.4 Molecular Identification: The ITS1-5.8S-ITS2 regions of ribosomal DNA (rDNA) were analyzed for the identification of fungal isolates. DNA fragments were amplified by PCR, visualized on agarose gel, purified, and sequenced. The sequences were compared with the NCBI GenBank using BLASTn, with identification criteria based on similarity.

3 RESULTS & DISCUSSION

3.1 Collection, Rearing, and Identification of *Phylloicus*

Phylloicus were collected from two streams, with 15 larvae reaching the adult stage, comprising three *Phylloicus fenestratus* and twelve *Phylloicus passulatus*, each species being recorded exclusively in one stream. *P. passulatus* was previously restricted to Venezuela, making this the first record of the species in Brazil [1]. This work highlights the importance of Roraima for the study of Trichoptera. Besides its taxonomic value, the information about *Phylloicus* species in each stream helps to understand the observed differences in fungal density.

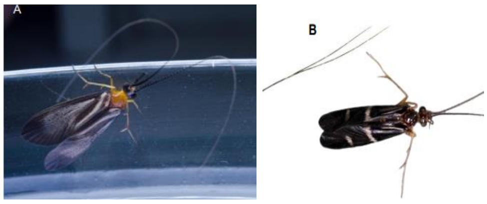

Figure 1 – (A): Adult male Phylloicus passulatus obtained through laboratory rearing of larvae, dorsal view of the insect; (B) Adult female Phylloicus fenestratus obtained through laboratory rearing of larvae, dorsal view of the insect.

3.2 Fungal Isolation

Overall, filamentous fungi were isolated from all dissected *Phylloicus*, indicating interactions between fungi and decomposer insects. [2] observe that fungi are common in the gut of insects that feed on wood or plant debris, aiding in the digestion of lignocellulosic materials. Therefore, studying the fungi associated with the digestive tract of *Phylloicus* is crucial for the discovery of new fungi that produce hydrolytic enzymes relevant for industrial applications.

3.3 Selection of Cellulase-Producing Strains and Qualitative Test

Of the morphotypes qualitatively tested for cellulase production, 42 isolates showed positive enzymatic production. These fungi were evaluated semi-quantitatively through the enzymatic index, which ranged from 0.3 to 5.5. The highest enzymatic index (5.5) was from a representative of the genus Devriesia. Saprobic, phytopathogenic, and thermotolerant forms of this genus are known [3] and [4] and are of particular interest for investigating enzymatic production, given their adaptation to high temperatures.

Table 1 - Screening for cellulase production by filamentous fungi isolated from Phylloicus in streams of Serra do Tepequém.

Cellulase Screening	**Total fungi**	**Enzymatic index (min. – máx.)**
Negatives	29	-
Positive	37	0.3 – 1.6
Biotechnologically Potential Positives	5	2.1 – 5.5
Total	71	-

3.4 Molecular Identification

The fungi that demonstrated an enzymatic index with potential for biotechnological application (IE ≥ 2) were subjected to molecular identification. Although the ITS region is recognized as an efficient barcode for Dikarya, there is greater success in identifying Basidiomycota than Ascomycota [5]. Most of the fungi isolated in the present work belong to the Phylum Ascomycota.

Table 2 - Molecular identification of filamentous fungi isolates that demonstrated an enzymatic index with potential for biotechnological application (IE ≥ 2).

Isolated	**Fragment Size in base pairs**	**Identification**	**E-Value**	**Identity**	**GenBank Accession Number**

R10PAF1	426 pb	*Devriesia* sp.	0.0	99%	KT329205
R10PBF8	476 pb	*Microascus* sp.	0.0	92%	MG595648
		Scopulariopsis sp.	0.0	92%	KP764411
R10PAF3	503 pb	*Clonostachys phyllophila*	0.0	95%	JF311937
R7PEF25	950 pb	*Aspergillus ellipticus*	0.0	99%	FJ629328
R10PCF24	461 pb	*Aspergillus flavus*	0.0	100%	MH469496
		Aspergillus fumigatus	0.0	100%	MF196886
		Aspergillus parasiticus	0.0	100%	KX270347
		Aspergillus sydowii	0.0	100%	MH634483
		Aspergillus versicolor	0.0	100%	MH333219

4 CONCLUSION

The study of the streams in the Serra do Tepequém revealed abiotic characteristics typical of pristine Amazonian environments. Although the physicochemical factors did not show a direct relationship with the distribution of *Phylloicus*, several species were identified, including a new record for Brazil. The presence of larvae was observed in areas with high habitat integrity. The combination of abiotic and biotic metrics, such as the habitat integrity index, proved crucial for understanding the distribution of the insect community. There were differences in the density of fungi isolated from different *Phylloicus* larvae, and their diversity varied among collection sites, with notable enzymatic production by certain isolates. Many of the fungi tested exhibited cellulase activity, with biotechnological potential for various applications, including plastic polymer bioremediation. Molecular identification confirmed the morphologically identified genera, and the study expanded knowledge about *Phylloicus* and filamentous fungi in Roraima, highlighting their relevance as a source of fungi with biotechnological potential, especially in cellulase production.

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