Artigo científico

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Cultivable endophytic fungi associated with the murumuru Amazon palm (*Astrocaryum ulei* Burret)

Fungos endofíticos cultiváveis associados a palmeira amazônica Murumuru (*Astrocaryum ulei* Burret)

Abstract: Murumuru (*Astrocaryum ulei* Burret) is an Amazon palm tree of great value in Brazil, based in the cosmetic industry and an important source of active compounds with biotechnological applications. It is known that plants share numerous bioactive substances with the endophytic fungi they harbor. However, there is no knowledge of these fungal communities in *A. ulei*. Therefore, the aim of the study was to characterize the endophytic fungi community of the Amazon palm *Astrocaryum ulei* in the state of Acre, Brazil. First, samples of *A. ulei* leaves were collected, washed and disinfected. Afterwards, they were fragmented and inoculated in PDA and PDA culture media + leaf extract and incubated at two different temperatures 18 ºC and 28 ºC. The isolated fungi were characterized regarding their macromorphology and micromorphology. A total of 88 fungi of *A. ulei* were isolated, grouped into five genera, of which *Xylaria* was the most frequent genus, with 48.8% of relative frequency, followed by *Penicillium* (19.4%), *Phomopsis* (8%), *Fusarium* (2.2%) and *Aspergillus* (2.2%) and unidentified with 17%. These genera are commonly reported in the literature as a source of bioactive molecules, with applications in the agricultural, pharmaceutical and industrial areas. This is the first study on the community of endophytic fungi of *A. ulei*, which can be explored in future studies for the development of new biotechnologies. **Keywords:** Biodiversity, Amazon, Endophytes, Xylaria.

Resumo: Murumuru (*Astrocaryum ulei* Burret) é uma palmeira amazônica de grande valorização no Brasil, base na indústria cosmética e importante fonte de compostos ativos com aplicações biotecnológicas. Sabe-se que as plantas compartilham inúmeras substâncias bioativas com fungos endofíticos que elas albergam. Entretanto não há conhecimento destas comunidades fúngicas em *A. ulei*. Logo o objetivo do estudo foi caracterizar a comunidade de fungos endofíticos da palmeira amazônica Astrocaryum ulei no estado do Acre, Brasil. Primeiramente, amostras de folhas *A. ulei* foram coletadas, lavadas e desinfetadas. Posteriormente foram fragmentadas e inoculadas em meios de cultura BDA e BDA + extrato da folha e incubadas em duas temperaturas distintas 18 ºC e 28 ºC. Os fungos isolados foram caracterizados quanto sua macromorfologia e micromorfologia. Foram isolados um total de 88 fungos de *A. ule*i, agrupados em cinco gêneros dos quais *Xylaria* foi o gênero mais frequente, com 48,8% de frequência relativa, seguido por *Penicillium* (19,4%), *Phomopsis* (8%), *Fusarium* (2,2%) e *Aspergillus* (2,2%) e não identificados com 17%. Estes gêneros são comumente relatados na literatura como fonte de moléculas bioativas, com aplicações na área agrícola, farmacêutica e industrial. Trata-se do primeiro estudo acerca da comunidade de fungos endofíticos de *A. ulei*, aos quais podem ser explorados em estudos futuros para o desenvolvimento de novas biotecnologias. **Palavras-chave:** Biodiversidad, Amazônia, Endófitos, Xylaria.

Introduction

Astrocaryum ulei Burret is an Amazonian species known as murumuru, which can be found in Brazil (Acre, Amazonas and Rondônia), Bolivia (Pando) and Peru (Madre de Dios) (KAHN, 2008). The Amazonian palm of the genus *Astrocaryum* is highly valued in Brazil, as it is a base in the cosmetics industry of renowned companies. These products mostly come from *A. ulei* almonds, in the production of lotions, creams, facial masks and skin moisturizer (DE ARAÚJO et al., 2007).

In addition, the oil extracted from the fruit has provided biotechnological applications, mainly in the context of sustainable agriculture. Studies for the control of phytopathogens such as *Colletotrichum gloeosporioides* (causal agent of anthracnose in passion fruit) and

Mycosphaerella fijiensis (causal agent of black Sigatoka in bananas), had in vitro, significant inhibition of mycelial growth of the pathogen (ABREU et al., 2014; NASCIMENTO et al., 2014). Despite this, there are no studies in the literature on the phytochemistry of *A. ulei*, much less on the relationships that the community of endophytic microorganisms plays in the production of these substances.

Endophytic microorganisms live in association with plant tissues, and act in a beneficial way, protecting against pests and pathogens, and thus sharing numerous bioactive substances with the host plant (SANTOS; VARALLO, 2011; DE SILVA et al., 2019). Thus, studying the endophytic community of Amazonian plants generates subsidies for biotechnological studies, mainly in the bioprospecting of substances of pharmacological and agricultural interest (SEGARAN; SATHIAVELU, 2019).

Within this community, endophytic fungi stand out for producing secondary metabolites and, thus, the possibility of discovering new molecules with antifungal activity (endophytes from *Phoenix dactylifera*) (ABDENNABI et al., 2017), against phytopathogens (endophytes of *Euterpe precatoria*) (PETERS et al., 2020), enzymatic (endophytes of *Phoenix dactylifera* and *Oenocarpus bacaba*) (BEN MEFTEH et al., 2019; DINIZ et al., 2020) and antibacterial (endophytic from *Uncaria tomentosa*) (RODRIGUES et al., 2018). Thus, the objective of this work was to characterize the community of endophytic fungi associated with the murumuru Amazon palm (*Astrocaryum ulei*), as the first report, in order to contribute to the knowledge of the Amazonian biodiversity.

Material and Methods

Vegetable Material Collection

Plant material was collected in the city of Rio Branco, Acre, Brazil. Two adult *A. ulei* plants were collected and isolated from two different points of the Federal University of Acre campus (UFAC) (Figure 1). According to the Köppen system, Acre is classified into type A subdivisions (Af 3, Am 3 and Am 4), that is, it has high temperatures, dry season and rain in the Amazon winter (KÖPPEN, 1918). The average annual temperature in Rio Branco is 25.5 °C and the average annual rainfall is 1806 mm. In Rio Branco, the average relative humidity for the months of December to April is 87.4%, due to higher precipitation, the opposite is also observed for the months of July to September, when the humidity decreases to 80.7% (DE SOUSA, 2020).

Isolation of Endophytic Fungi

The experiment was carried out in a completely randomized design (DIC) with two replicates per treatment for each individual. Leaf samples of *A. ulei* were washed with a sponge and detergent in running water to remove solid residues and epiphytic microorganisms. The material was handled in a laminar flow chamber for surface disinfection with immersion in 70% ethanol (1 min), 2% sodium hypochlorite (4 min), 70% alcohol (30 s) and washing in sterile distilled water (1 min) 3 times. The disinfected plant material was cut into fragments of 5 mm in diameter and inoculated in a Petri dish containing culture medium (previously sterilized in an autoclave for 20 min). The treatments consisted of two culture media and two incubation temperatures: Potato-Dextrose-Agar (PDA), PDA + 10% extract, 18 °C and 28 °C. All media were

supplemented with chloramphenicol antibiotic (100 mg.L^{-1}) to suppress bacterial growth. In addition, 200 µl of the last wash water was seeded on the culture media evaluated to confirm the effectiveness of the disinfection process, through the absence of fungal colonies. To produce the medium with plant extract, 100 g of leaf were ground in 500 mL of distilled water, filtered and 500 mL of an infusion of 200 g of potato were added to the extract, in which the reagents were solubilized (ARAÚJO et al., 2010).

Fungal colonies with distinct characteristics according to macroscopic observations (color and growth characteristics in culture medium) were purified by the streak technique by depletion in Petri dishes with PDA culture medium, and incubated for 48 h. After the purity of the colonies was confirmed, the fungi were inoculated in tubes with PDA medium (ARAÚJO et al., 2010), and preserved in distilled water (CASTELLANI, 1963) and mineral oil (BUELL; WESTON, 1947).

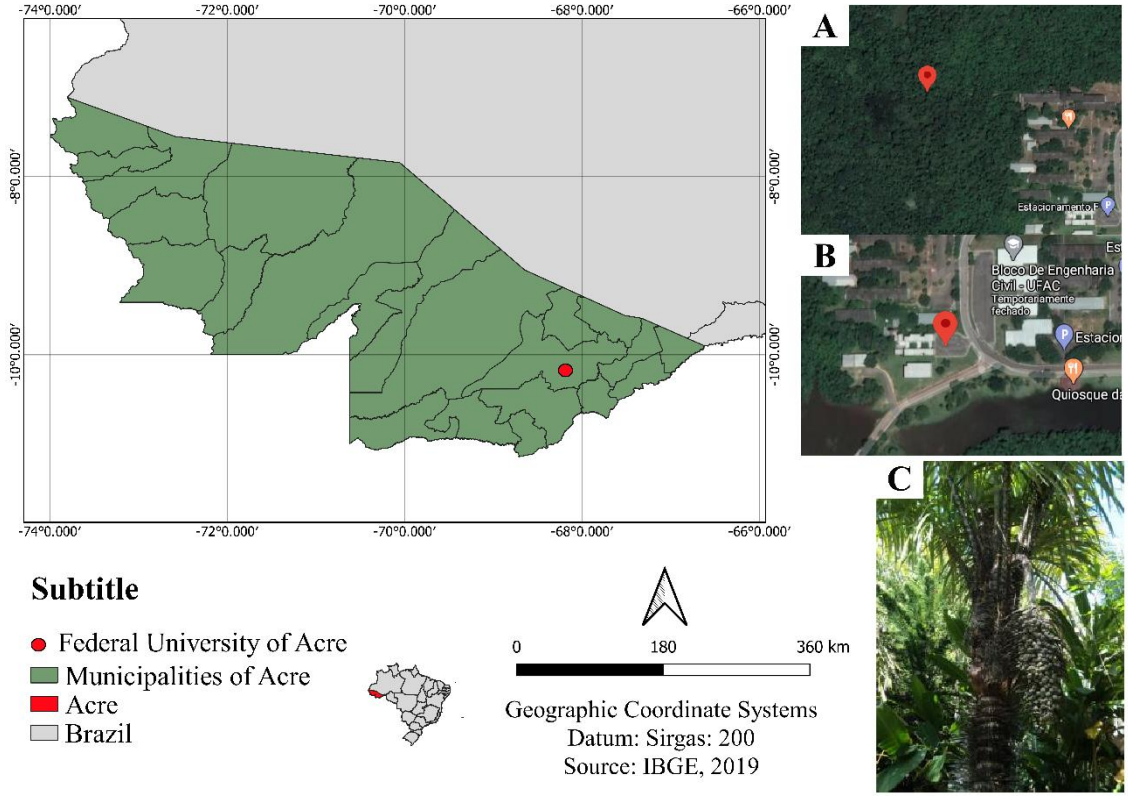

Figure 1 - Location map of *Astrocaryum ulei* collection points. A. Point 1: S09º 57.344 'W067º 52.288'; B. Point 2: S 09º 57,457 'W 067º 52,127'; C. *Astrocaryum ulei* (Murumuru). Fonte: Produzido pelos autores (2021).

Morphological Characterization

The fungi were organized into morphospecies according to the colony's macromorphological characteristics, such as color, texture and pigment production. One fungi of each morphospecies was used for micromorphological identification. For this, it were cultivated microcultures in Petri dishes, with the fungi inoculated in cubes of 1 cm2 of PDA medium and covered with coverslips. The plates were incubated at 28 ºC for 7 days, for mycelial growth, and

the coverslips stained with lactophenol blue for visualization of reproductive structures under an optical microscope (ARAÚJO et al., 2010; BARNETT; HUNTER, 1999).

Data analysis

For the calculation of the colonization frequency, the total number of isolates by genus, temperature or culture medium was divided by the total number of isolates and multiplied by one hundred, in the Excel program. GraphPad Prism 5.0 was used to make figures and QGIS for making the map.

Results

A total of 88 fungi were isolated from Astrocaryum ulei. The distribution of fungi by cultivation and insulation temperature is shown in Table 1.

Table 1 - Endophytic fungi recovered from *Astrocaryum ulei* according to culture medium and temperature.

Genus	Culture medium		Temperature		Total	RF%
	PDA	PDA+extract	18 °C	28 °C		
Xylaria	**21**	**22**	**19**	**24**	**43**	**48.8**
Penicillium	**5**	**12**	**7**	**10**	**17**	**19.4**
Phomopsis	**2**	**5**	**7**	**-**	**7**	**8.0**
Fusarium	**-**	**2**	**2**	**-**	**2**	**2.2**
Aspergillus	**-**	**2**	**2**	**-**	**2**	**2.2**
Unknow	**10**	**7**	**9**	**8**	**17**	**19.4**
Total	**38**	**50**	**46**	**42**	**88**	**100**
RF%	**43.2**	**56.8**	**52.3**	**47.7**		

Fonte: Produzido pelos autores (2021).

It was possible to identify 71 fungi (80.6%), distributed in five genera. Xylaria was the most frequent genus, with 48.8%, followed by *Penicillium* (19.4%), *Phomopsis* (8%), *Fusarium* (2.2%) and *Aspergillus* (2.2%). Fungi without reproductive structure for identification were considered unknow with 17% (Figure 2).

Some genera of fungi were generalists or specialists for isolation conditions. For culture medium, only *Xylaria* and *Penicillium* were generalists, being recovered in all media and temperatures used. Fusarium and *Aspergillus* proved to be specialists, growing only in one type of medium (PDA+extract) and a single temperature (18 °C) (Figure 3).

Dicussion

The Amazonian palm *Astrocaryum ulei* hosts important endophytic fungi, commonly reported in the literature. The study demonstrated a greater recovery of endophytic fungi in PDA medium with leaf extract, and the temperature most favorable to growth was 18 °C, in addition, *Fusarium* and *Aspergillus* were specific for these conditions. These results allow us to infer that different isolation conditions favor the growth of different endophytic fungi, providing a greater

representation of this community, avoiding underestimated data (CARNAÚBA et al., 2007; SIA, 2012; DA SILVA et al., 2020).

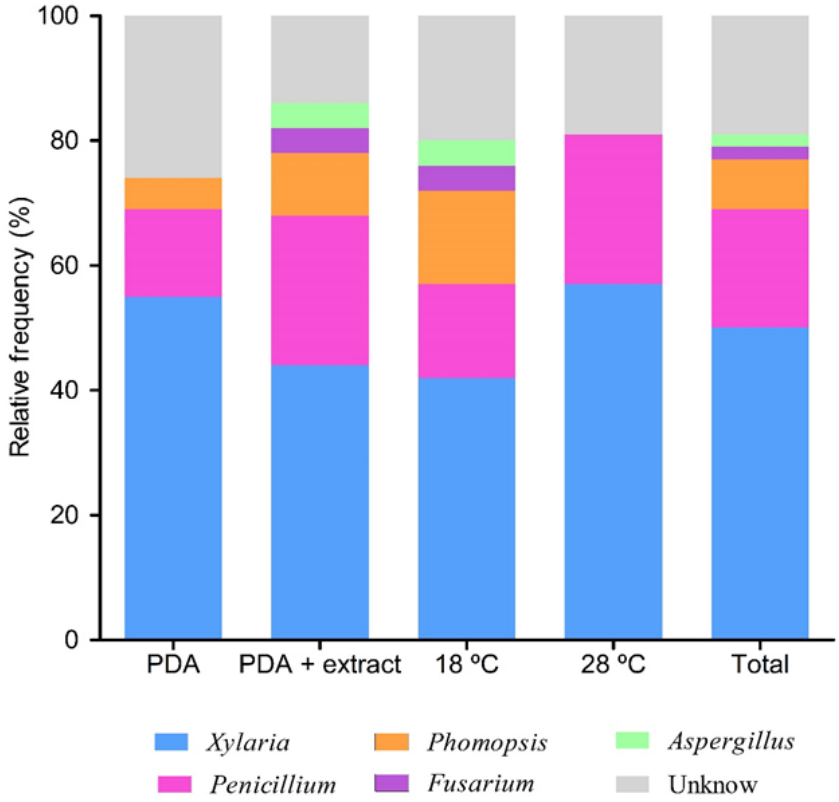

Figure 2 - Relative frequency of *Astrocaryum ulei* endophytic fungi according to culture medium and isolation temperature. Fonte: Produzido pelos autores (2021).

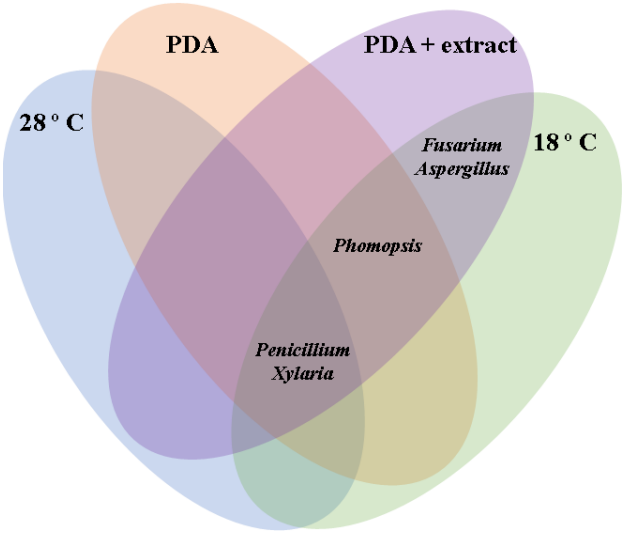

Figure 3 - Venn diagram showing the intersection of the conditions for isolation of endophytic fungi from *Astrocaryum ulei*. Fonte: Produzido pelos autores (2021).

Another interesting justification for the offer of different nutritional sources is that some species may be in latency and, therefore, take advantage of the conditions of the culture medium, such as supply of nutrients and pH, to start the growth (PEREIRA et al., 2003). Lower temperatures tend to favor the growth of demanding fungi, which need more time to start their initial growth. Thus, offering different temperatures, it prevents fast-growing fungi from overlapping the demanding ones, enabling their isolation (GUIMARÃES, 1998; DIX, 2012).

Macromorphological and micromorphological analysis of the 88 isolated fungi identified 71 (80.6%) endophytes from *A. ulei*. The genus *Xylaria* was the most frequent, with 48.8% relative frequency, followed by *Penicillium* (19.4%), *Phomopsis* (8%), *Fusarium* (2.2%) and *Aspergillus* (2.2%). *Xylaria* is commonly described in the literature as a wood decay fungus with high frequency of isolates in tropical plants when compared to subtropical (PHOTITA et al., 2001). Studies report the production of important substances by this genus, as the compounds griseofulfine and declorhydriseofulfina with antibacterial and antifungal activity (CAFÊU et al., 2005). In addition, it was also isolated as an endophyte in other palm trees, *Calamus kerrianus*, *Wallichia caryotoides* (LUMYONG et al., 2009) and *Livistona chinensis* (GUO et al., 2000).

Penicillium was the second most isolated genus from *A. ulei*. This fungus is considered cosmopolitan and has provided numerous biotechnological applications in agriculture over the years, as promoting plant growth, antibacterial activity and control of phytopathogens causing fusariosis (DEVI et al., 2012; TING et al., 2012; WAQAS et al., 2015). It also contributed to the emergence of new substances in the treatment of diseases such as malaria and tuberculosis (INTARAUDOM et al., 2013).

Phomopsis had 8% relative frequency in *A. ulei*. This genus is also reported as an endophyte of other Amazonian palms as Phoenix dactylifera, with the species *Phomopsis lagerstroemiae* and *Phomopsis asparagi* (MAHMOUD et al., 2017). In addition, *Phomopsis* isolates have demonstrated antibacterial and antioxidant potential (JAYANTHI et al., 2011). In agriculture, *Phomopsis liquidambari* stimulated organic mineralization and produced the release of $N\text{-}NH^{+4}$, making a process of nitrification in the soil (CHEN et al., 2013). Other strains of this fungus can also product plant hormones, stimulating plant growth and also causing insect infections contributing to biological pest control (CHITHRA et al., 2017; XIE et al., 2017).

Fusarium (2.2%) and *Aspergillus* (2.2%) presented the same relative frequency. They are fungi frequently isolated and have demonstrated important biotechnological applications for health and agriculture (KHAN et al., 2011; TAYUNG et al., 2011; RATNAWEERA et al., 2015). Fusarium is a genus widely reported as a phytopathogen, but also isolated as an endophyte and with beneficial activities to the plant, as production of growth hormones and pest control, as the excavator nematode *Radopholus similis* (NIERE et al., 2006; KAVROULAKIS et al., 2007). Studies with endophytes of the *Aspergillus genus* demonstrate the efficiency in producing plant hormones such as gibberellins and isoflavonoids, which also favors resistance against abiotic stresses (KHAN et al., 2011). It also acts in the biological control of *Spodoptera litura* larvae (ELANGO et al., 2020).

Thus, it was possible to observe that the Amazon palm tree *A. ulei* is colonized by important fungal endophytes that can be sources of active molecules for the development of new technologies in the pharmaceutical, industrial and, especially, in sustainable agriculture. Therefore, future studies are essential to detect molecules potentially useful to society and the environment.

Conclusion

The Amazonian palm *Astrocaryum ulei* has important endophytic fungi and this work contributes as the first report for the knowledge of the fungal symbiotic biodiversity of this plant. The culture medium BDA + extract and temperature of 18 °C obtained the highest amount of isolated fungi, and fungi of the genus *Xylaria* were the most frequent. The microorganisms isolated from *A. ulei* can contribute to future studies of possible bioactive products with economic and scientific potential.

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