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# ISOLATION AND IDENTIFICATION OF ENDOPHYTIC FUNGI FROM THE AMAZONIAN PALM *Oenocarpus bataua* MART

# ISOLAMENTO E IDENTIFICAÇÃO DE FUNGOS ENDOFÍTICOS DA PALMEIRA AMAZÔNICA *Oenocarpus bataua* MART

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**ABSTRACT**

The aim of this study was to characterize the community of endophytic fungi of the Amazonian palm patauá *Oenocarpus bataua* in the state of Acre, Brazil. Samples of leaf and stem of *O. bataua* were collected, washed and disinfected by immersion in 70% ethanol (1 min), 2% sodium hypochlorite (5 min), 70% alcohol (30 sec) and washing in sterile distilled water (1 min) three times. The disinfected samples were fragmented and inoculated in PDA and Oat culture media and incubated at 28 ºC for 30 days. For identification, morphological characteristics of the colonies were observed. 63 endophytic fungi were isolated from *O. bataua*, 50 (79.3%) of leaf and 13 (20.7%) of stem. Regarding the culture medium, 24 (38.1%) fungi were isolated in BDA medium and 39 (61.9%) in Oat. Six genera were identified, *Xylaria* (33.3%), *Phomopsis* (12.6%), *Phoma* (4.8%), *Geotrichum* (4.8%), *Penicillium* (3.2%), *Nigrospora* (1.6%) and unidentified fungi (39.7%). This is the first report from the endophytic fungi community of *O. bataua.*
**Keywords:** Biodiversity, Patauá, *Xylaria*, *Phomopsis*, *Phoma.*

## RESUMO

O objetivo deste estudo foi caracterizar a comunidade de fungos endofíticos da palmeira amazônica Patauá (*Oenocarpus bataua*) no estado do Acre, Brasil. Amostras de folha e caule de *O. bataua* foram coletadas, lavadas e desinfetadas por imersão em etanol a 70% (1 min), hipoclorito de sódio a 2% (5 min), álcool a 70% (30 seg) e lavagem em água destilada estéril (1 min) três vezes. As amostras desinfetadas foram fragmentadas e inoculadas em meio de cultura PDA e aveia e incubadas a 28 ºC por 30 dias. Para identificação, foram observadas as características morfológicas das colônias. 63 fungos endofíticos foram isolados de *O. bataua*, 50 (79,3%) de folhas e 13 (20,7%) de caule. Quanto ao meio de cultura, 24 (38,1%) fungos foram isolados no meio BDA e 39 (61,9%) na aveia. Foram identificados seis gêneros, *Xylaria* (33,3%), *Phomopsis* (12,6%), *Phoma* (4,8%), *Geotrichum* (4,8%), *Penicillium* (3,2%), *Nigrospora* (1,6%) e fungos não identificados (39,7%). Este é o primeiro relatório da comunidade de fungos endofíticos de *O. bataua*.
**Palavras-chaves:** Biodiversidade, Patauá, *Xylaria*, *Phomopsis*, *Phoma.*

## 1. INTRODUCTION

*Oenocarpus bataua* Mart. is popularly known as patauá, patoá e patuá [1]. It occurs throughout the Amazon, and it is also possible to find it in the southern part of the central region [1,2]. The propagation of *O. bataua* occurs through seeds, which germinate in periods ranging from 44 to 52 days after sowing [4,5], grow to 25 m in height and produce large clusters of dark purple fruits [2,3].

*O. bataua* has great cultural, medicinal and economic importance in the Amazon region. The name given to the genus *Oenocarpus* comes from the Greek oinos = wine + karpus = fruit, or "fruit of wine", related to the more traditional use of this palm, the manufacture of juices, regionally called wines, in addition to the use as a source of oil for medicinal, cosmetic or culinary purposes[3,4,5]. The leaves are used in woven crafts and the stipe is used in bridge construction [4].

As for medicinal properties, the nutraceutical characteristics of patauá deserve attention, due to the antioxidant effect present in oils and fruits [3,6]. These effects are related to polyphenols, such as stilbenes, phenolic acids and condensed tannins, especially for tannins with a very high quantity [7]. In addition, the plant is used in the composition of ointments, balms, ointments and keratos, used empirically in the treatment hair loss control, dandruff, bronchitis, tuberculosis and malaria [2,8].

Studies have also shown that the *O. bataua* fruit has antiproliferative and antioxidant effects in liver carcinoma cells (Hep-G2), showing antineoplastic potential [9]. However, these medicinal properties are still poorly studied in experimental tests, demonstrating that this plant has a rich source of bioactive compounds to be explored.

Despite the medicinal and economic importance of *O. bataua*, there is no planting technology for this species to supply the market, so that commercialization depends entirely on extraction [8]. Such activity can be considered a risk when the sustainable management of natural populations is not carried out, and the species may disappear from the natural ecosystem [8]. Thus, the exploration of the community of microorganisms that live in association with the plant is an interesting way to be explored, being able to perform functions similar to the plant, being a source of active compounds [10].

Endophytic microorganisms, especially fungi, produce secondary metabolites, with wide biotechnological use [11]. Inhabit the interior of plant tissues and organs without causing apparent damage to their host [12]. Studies report the efficiency of endophytic fungi in producing pharmacological substances that are also synthesized by the host plant, such as the endophyte *Colletotrichum gloeosporioides* that produces the active metabolite piperine, also isolated from the host plant *Piper nigrum* [13], with antimicrobial and antitumor properties [14,15]. Another classic example from the literature is Taxol, an anticancer substance isolated from plants of the genus *Taxus* and, later, from the endophyte *Taxomyces andreanea* [16,17].

In addition to the pharmaceutical importance of these substances, many can help in the development of *ex situ* technology for plant cultivation, allowing their use in a sustainable way. Endophytic fungi are commonly used in agriculture to promote plant growth and development and also to control pests and phytopathogens [18,19,20]. The knowledge of the endophytic fungi community of *O. bataua*, generates inputs for the exploration of its medicinal and agronomic potential. Thus, the objective of this study was to characterize the community of endophytic fungi of the Amazonian palm *Oenocarpus bataua* in the state of Acre, Brazil.

## 2. MATERIAL AND METHODS

### Vegetable Material Collection

The plant material was collected in the city of Rio Branco, state of Acre, Brazil. Three plants of *Oenocarpus bataua* were collected at two different points, two plants located on the Transacreana Highway AC - 90 (10°00'15.0"S 67°50'28.9"W) and one plant on BR 317 (Km 30) (10°40'05.6"S 68°16'23.0"W).

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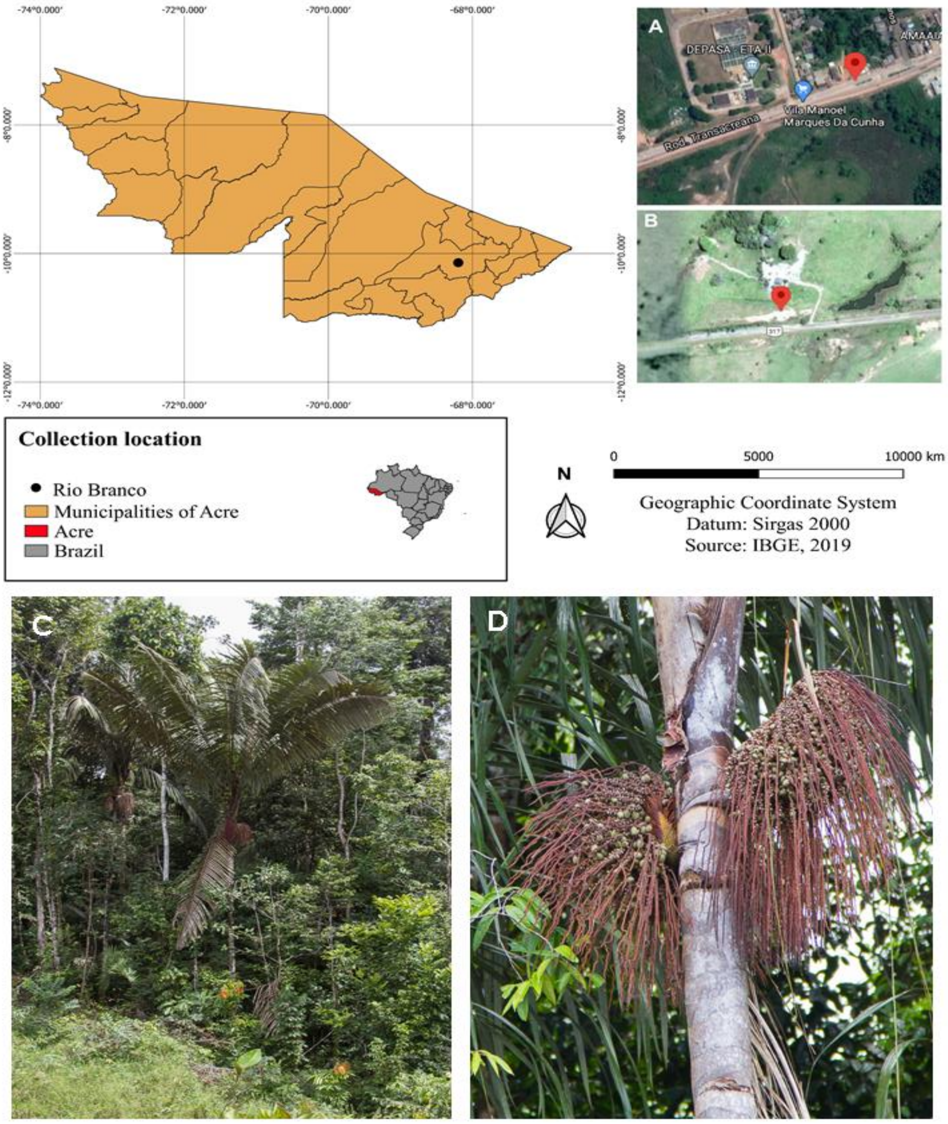


**Figure 1**. Location map of the collections of *Oenocarpus bataua*; A. Collection point 1 - Transacreana Highway AC - 90 (10 ° 00'15.0 "S 67 ° 50'28.9" W); B. Collection point 2 - BR 317 (Km 30) (10 ° 40'05.6 "S 68 ° 16'23.0" W); C. Palm *Oenocarpus bataua*; D. Fruit and seed of *Oenocarpus bataua*.

### Isolation of Endophytic Fungi

Samples of leaf and stem of *Oenocarpus bataua* were washed with sponge and detergent in running water to remove solid residues and epiphytic microorganisms. After washing, surface disinfection was performed by immersion in 70% ethanol (1 min), 2% sodium hypochlorite (5 min), 70% alcohol (30 sec) and washing in sterile distilled water (1 min) three times. The disinfected plant material was cut into fragments of 5 mm in diameter and inoculated in a Petri dish with Potato-Dextrose-Agar-PDA culture media and Oat medium supplemented with chloramphenicol antibiotic (100 mg.$L^{-1}$), and the plates incubated at 28 °C for 30 days [21].

Fungal colonies with distinct characteristics according to macroscopic observations (color and growth characteristics in culture medium) were purified using the streak technique by depletion in Petri dishes with PDA culture medium and incubated at room temperature for 48 h. After the purity of the colonies was confirmed, the isolates were inoculated in tubes containing inclined PDA medium[21], and fungi were preserved in distilled water [22] and mineral oil [23].

**Morphological Characterization**

For the macromorphological characterization, the fungi were grouped into morphospecies according to the characteristics of the colony, such as color, texture and pigment production. One fungi of each morphospecies was used for micromorphological identification. For that, microculture was done, the fungi were inoculated in 1 $cm^2$ cubes of BDA and covered with coverslips, inside Petri dishes. The plates were incubated at room temperature for 7 days for mycelial growth and the coverslips were stained with lactophenol blue to view reproductive structures under an optical microscope [24,25].

**Data analysis**

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

## 3. RESULTS AND DISCUSSION

A total of 63 endophytic fungi were isolated from *O. bataua*, 50 (79.3%) from leaf and 13 (20.7%) from stem. As for the culture medium used, 24 (38.1%) fungi were isolated in BDA medium and 39 (61.9%) in Oat medium (Tabela 1). 38 (60.3%) fungi have been identified, from six genera. *Xylaria* was the most frequent genus with 33.3%, followed by *Phomopsis* (12.6%), *Phoma* (4.8%) *Geotrichum* (4.8%), *Penicillium* (3.2%), *Nigrospora* (1.6 %) and unidentified fungi (39.6%) (Figura 1).

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**Table 1.** Endophytic fungi isolated from *Oenocarpus bataua* according to plant tissue and culture medium.

| **Genus** | **Plant tissue** | | **Culture medium** | | **Total** | **RF (%)** |
|---|---|---|---|---|---|---|
| | Leaf | Stem | PDA | Oat | | |
| *Xylaria* | 17 | 4 | 4 | 17 | 21 | 33.3 |
| *Phomopsis* | 8 | 0 | 3 | 5 | 8 | 12.7 |
| *Phoma* | 2 | 1 | 3 | 0 | 3 | 4.8 |
| *Geotrichum* | 2 | 1 | 0 | 3 | 3 | 4.8 |
| *Penicillium* | 2 | 0 | 0 | 2 | 2 | 3.2 |
| *Nigrospora* | 1 | 0 | 1 | 0 | 1 | 1.6 |
| Unknown | 18 | 7 | 13 | 12 | 25 | 39.6 |
| **Total** | **50** | **13** | **24** | **39** | **63** | **100** |
| **RF %** | **79.3** | **20.7** | **38.1** | **61.9** | **100** | |

RF = Relative Frequency

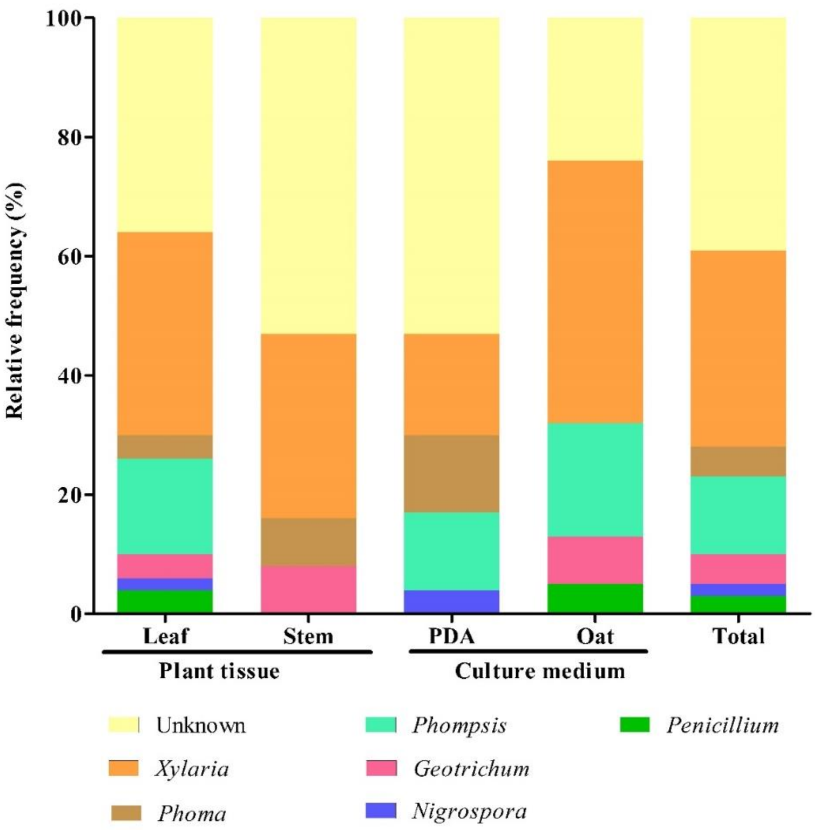


**Figure 1.** Relative frequency of isolated endophytic fungi in different culture media and plant tissue of *Oenocarpus bataua*.

Some fungal genera had specificity in relation to the conditions that were isolated. To culture media, *Nigrospora* was isolated only in PDA medium and *Penicillium* and *Geotrichum* isolated only in oat medium, demonstrating specificity for this nutritional condition. On plant tissue, *Nigrospora*, *Penicillium* and *Phomopsis* showed specificity for leaf. *Xylaria* was a generalist, isolated in all conditions analyzed (Figura 2).

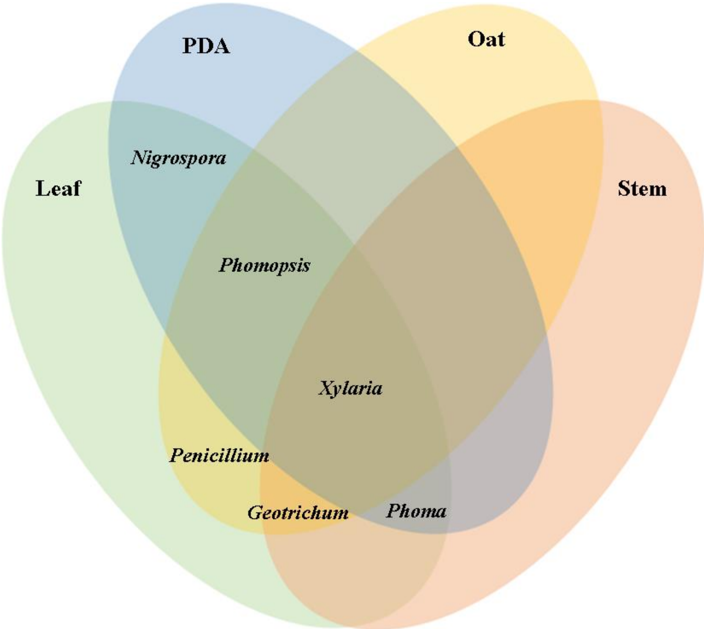


**Figure 2.** Venn diagram showing the intersection of the conditions for the isolation of endophytic fungi from *Oenocarpus bataua*.

The Amazonian palm *Oenocarpus bataua* demonstrated in this study to be associated with important genera of endophytic fungi, with potential for biotechnological applications. Among the insulation conditions used, leaf was the plant tissue with the highest frequency of isolation (79.3%). This fact may be related to the anatomy of the leaf, due to the presence of natural openings such as hydatodes and stomata that favor colonization by the entry of fungal spores [26].

Regarding culture media, differently most studies of isolations of endophytic fungi, the medium with the highest number of isolates was Oat with 61.9%. This result shows different nutritional conditions in the method used, favors the growth of morpho-species with different nutritional needs [27,28]. This fact was observed with *Penicillium* and *Geotrichum* that grow only in medium Oat, as well as *Nigrospora* that grew only in medium PDA. This experimental tool allows the observed morpho-species data not to be underestimated [29]. In addition, the community can be affected by other variables such as the substances present in the plant tissue, the genotype of the plant and the abiotic and biotic environmental conditions, including temperature and humidity [30].

Regarding genus, *Xylaria* was the most frequent genus (33.3%). *Xylaria* has also been isolated from other palms such as *Trachycarpus fortunei* [31], *Calamus kerrianus* and *Wallichia caryotoides* [32], *Livistona chinensis* [33], *Licuala ramsayi* and *Licuala* sp. [34] and *Cocos nucifera* [35]. Thus, it is a fungal genus commonly isolated from plants, considered generalist for growing under different conditions [28,36].

It is also a source of metabolites of pharmacological interest described in the literature, such as phthalide, an antimicrobial substance produced by the strain *Xylaria* sp. GDG-102, isolated from the medicinal plant *Sophora tonkinensis* [37]. Substances with cytotoxic activity have also been described in *Xylaria* isolates, and natural antioxidants [38,39]. It is also responsible for producing enzymes for therapeutic use in preclinical studies in thrombolytic therapy, such as xylinase [40]. In agriculture, this genus has also demonstrated benefits for the formulation of biopesticides, antifungals and phytotoxics for weed control [41].

*Phomopsis* was the second most isolated fungal genus in *O. bataua*, with 12.6%. Fungi of this genus have shown important biotechnological applications in the pharmaceutical industry, agriculture, where strains can produce plant hormones to promote plant growth [42], antimicrobial [43] and for biological control [44].

The endophytes *Phoma* and *Geotrichum* had 4.8% of colonization each. *Phoma* is an important fungal genus, due to the production of secondary metabolites with antifungal and cytotoxic activity in tumor lines, such as (3S) -3,6,7-trihydroxy-α-tetralone and cercosporamide metabolites [45]. In addition, *Phoma* has brought benefits in agriculture by promoting plant growth and inducing seed germination, as evidenced in corn (*Zea mays*) [46]. *Geotrichum* is a producer of secondary metabolites derived from dihydroisocoumarin (1-3) isolated from the endophyte *Geotrichum* isolated from *Crassocephalum crepidioides*, with antimalarial, antituberculosis and antifungal properties [47]. Nematicidal properties in metabolites produced by the *Geotrichum* sp. AL4, presenting an important sustainable tool for biological control [48].

*Penicillium* obtained a low colonization rate with 3.2%. *Penicillium* is a fungal genus considered cosmopolitan, explored in the pharmaceutical industry [49]. As an example, *Penicillium* has pancreatic lipase inhibitory activity with the potential for the development of a drug to control obesity, antimicrobial activity against human pathogens, antimalarial activity and antituberculosis [50,51,52]. An endophyte of this genus also has important results in agriculture, due to the production of plant hormones such as gibberellins and phosphate solubilization, in addition to providing the plant with greater resistance to the stress caused by the presence of heavy metals in the soil [53,54]

Finally, *Nigrospora* was the fungal genus with the lowest frequency of isolation, representing 1.6% of the total. This genus is reported in the literature as an endophyte of medicinal plants such as *Moringa oleifera* and *Emblica officinalis*, producing the substances griseofulvin, an important antimicrobial against human pathogens, and declorhydriseofulvin, 8-dihydroamulosin and mellein, with antifungal activity for plant pathogens [55,56]. It has also presented potential for biological control, as it was inoculated in cauliflower plants, it started to reproduce a cytotoxic characteristic for larval hemocytes of *Spodoptera litura* [57].

This is the first report about the endophytic fungi community of *O. bataua*, having a rich community of fungi, with biotechnological potential to be explored. Bioprospecting of substances present in extracts of these fungi, can serve as a basis for elucidating new compounds and subsequently developing new drugs. They can also provide a basis for *ex situ* technology of this species, which may have the potential to promote plant growth and development and to control pests and phytopathogens, allowing the sustainable management of *O. bataua*.

## 4. CONCLUSIONS

*Oenocarpus bataua* proved to be a rich source of endophytic fungi, leaf was the plant tissue with the highest frequency of colonization, and the oat medium the most conducive to isolation. Among the identified genera, *Xylaria* was most frequently isolated, demonstrating to be a generalist fungus. This is the first report of isolation of endophytic fungi from *Oenocarpus bataua*.

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