PROSPECTING CELLULOLITICAL FUNGI DERIVATE FROM MANGROVE SEDIMENTS

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ABSTRACT

The mangrove ecosystem, characterized by high biodiversity and extreme conditions, harbors fungi that play a crucial role in organic matter decomposition and nutrient cycling. Prospecting these fungi involves exploring and identifying species, which is fundamental for understanding mangrove ecology and discovering new biotechnological resources. This study aimed to isolate and characterize fungi from mangrove sediment based on their morphology and potential to produce cellulases. Fungi were isolated from 10 g of sediment in 100 mL of 0.9% saline solution under constant agitation. Serial dilutions of the supernatant were plated on Sabouraud dextrose agar and incubated at 28°C for 14 days. Cellulolytic activity and enzymatic index were evaluated. The results show that six fungi (A, B, C, D, E, and F) were isolated, predominantly exhibiting rapid growth and white macroscopic characteristics. Microscopic characterization indicates that the isolates have similarities with the genus *Aspergillus* and degrade carboxymethyl cellulose, indicating the production of cellulases. Thus, this study contributes to the scientific valorization of mangroves and the exploration of new cellulases sources.

Keywords: Sediment. Isolation. Fungi.

1 INTRODUCTION

The mangrove ecosystem comprises coastal environments that serve as dynamic interfaces between marine and terrestrial realms. Mangroves are characterized by high biodiversity adapted to extreme conditions of salinity, tidal fluctuations, and anoxic soils. Among the organisms constituting the mangrove microbiota, fungi play a crucial role in ecological processes, including the decomposition of organic matter and nutrient cycling[1].

The prospecting of mangrove fungi involves exploring and identifying the diverse fungal species present in these habitats. This process is fundamental for understanding the ecology and biodiversity of mangroves and for discovering new biotechnological resources[2].

The biotechnological application of mangrove fungi is broad and diverse. These fungi exhibit potential to produce industrial enzymes, antimicrobial compounds, antioxidants, and other bioactive secondary metabolites[3]. Among industrial enzymes, cellulase stands out for its ability to catalyze the hydrolysis of cellulose into smaller sugars, such as glucose. The production of cellulase by mangrove fungi is particularly interesting due to the high efficiency and adaptability of these organisms in extreme conditions, which can enhance enzymatic yield and operational stability in industrial processes. These enzymes have important applications in the biofuel, food, animal feed and pulp industries[3].

Given the growing demand for new sources of bioactive compounds and sustainable technologies, the prospecting of mangrove fungi emerges as a highly relevant research area. This study aims to explore the fungal diversity of mangroves and assess the biotechnological potential of the identified species, thereby contributing to the expansion of scientific knowledge and the development of new technological applications.

2 MATERIAL & METHODS

Sediment from the mangrove region of Rio Real-SE was collected during the winter, in the morning, at low tide, from depths of 0-2 cm, 2-5 cm, and 5-10 cm. Sterile PVC tubes, measuring 15 cm in length and 5 cm in diameter, were used for the collection. The collected sediment was wrapped in cling film and transported in a cooler box to the Molecular Biology Laboratory (LBM) at the Institute of Technology and Research (ITP) in Aracaju-SE.

Fungi were isolated as described[4]. The plates were incubated at 28°C for 14 days, before isolating the fungi on Petri dishes containing malt extract agar (MEA) (30 g/L malt extract, 3 g/L peptone, 15 g/L agar)[5].

The selection of fungi with cellulolytic activity was carried out according to[6]. Isolates with a hydrolysis halo diameter per colony >1.50 were considered potential cellulase producers[7]. After isolation, fungi were morphologically identified based on their macroscopic characteristics (colony growth, colour, diameter, texture, colony topography, and edges) and microscopic features (microstructure). The fungi were maintained by subculturing on MEA and stored in a refrigerator at 22°C.

The experimental results, in triplicate, were statistically analyzed, expressing them as mean ±SD, using analysis of variance (One-way ANOVA) with GraphPad software version 5.0. Statistical significance was considered at $p<0.05$.

3 RESULTS & DISCUSSION

From the mangrove sediment samples, six fungi were isolated under the experimental isolation conditions used. Figure 1 illustrates the growth of these fungi on solid MEA medium

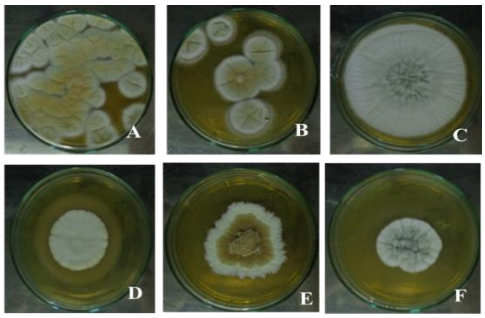

Figure 1 Macroscopic aspect of the growth of mangrove fungal isolates in MEA medium after 11 days in na oven at 28ºC. Images (A, B, C, D, E and F) of the macroculture of isolates from mangrove soil.

The macroscopic characteristics of fungi, including colony, growth, color, diameter, texture, and colony topography were evaluated. Isolates A and C exhibited rapid growth, while isolates B and E showed moderate growth, and fungi D and F displayed slow growth. Regarding colony coloration, colonies were orange, light green, greyish, brown, soft yellow, white, and grey. The diameter of the isolates after 11 days of cultivation also varied, ranging from 7.3 cm for isolate C to 2 cm for isolate A, which had the smallest diameter. In terms of texture, isolates A, B, C, D, and F displayed a cottony appearance, while isolate E exhibited a granular texture. Regarding topography, colonies A, B, C, D, E, and F showed a rough appearance with regular edges, while isolate E was warty with irregular edges.

The microscopic characteristics of the fungi were analyzed by comparison to images described by[8]. In Figure 2, conidiophores, vesicles, metulae, and phialides were observed in isolate A; and in isolate B: conidiophores, vesicles, metulae, phialides, and conidia; while septate hyphae were observed in isolates C and D. Additionally, isolate E presented conidiophores and conidia, whereas conidiophores, vesicles, and conidia were observed in isolate F.

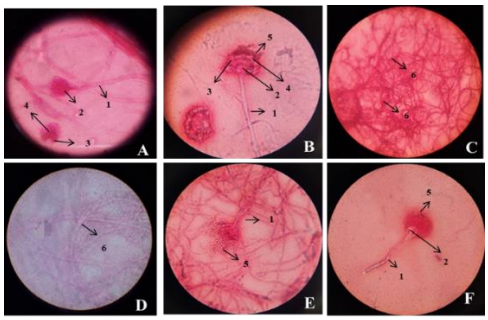

Figure 2 Microscopy of fungal isolates on slides stained with Fuchsin. Arrows and numbers indicate: 1. Conidiophore, 2. Vesicle, 3. Metulae, 4. Phialides, 5. Conidia, 6. Septate hyphae. (1000x).

These morphological structures are characteristic of fungi of the genus *Aspergillus* sp., suggesting that the isolates belong to this genus. Although morphological aspects are important for identifying microorganisms, molecular identification is required due to its high sensitivity and specificity at the genus and species levels[9].

In the quantitative analysis, the relation >1.50 obtained between hydrolysis halo diameter/colony halo diameter allowed the selection of fungi with the highest cellulase production (Table 1). Fungi B (3.0) and C (1.9) exhibited an enzymatic index >1.50, indicating high cellulolytic potential.

Table 1 Enzymatic index of fungal isolates indicating their cellulolytic capabilities. Average values of the 3 repetitions. Means with the same letter do not differ from each other, according to Tukey's test ($p<0.005$).

Isolated	Hydrolysis halo diameter (cm)	Colony halo diameter (cm)	Enzyme index (i.e) >1.50
Control	0.0^{c}	0.0^{c}	0.0
A	3.7^{a}	1.2^{b}	3.0
B	3.3^{a}	1.7^{ab}	1.9
C	2.9^{ab}	2.4^{a}	1.2
D	3.0^{a}	2.0^{a}	1.5
E	3.0^{ab}	1.6^{ab}	1.8
F	2.2^{b}	1.8^{ab}	1.2

These results are consistent with studies assessing fungal cellulolytic capacity, which have shown variations in the enzymatic index ranging from 1.2 to 3.0 cm. These values enable the screening of fungi with potential cellulolytic activity, allowing for the selection of fungi capable of degrading cellulose for use in biotechnological research and industrial applications[10].

Thus, the diversity of mangrove microbiota generates scientific interest in prospecting fungi that produce bioactive compounds, such as enzymes[11]. Fungi are important soil decomposers that symbiotically interact with plants, playing a crucial role in ecological and biogeochemical processes. Consequently, mangrove fungi significantly contribute to the degradation of organic matter, acting as primary mineralizers and a food source for benthic fauna[12]. During these processes, fungi produce metabolites that are exploitable for industrial applications.

4 CONCLUSION

From the mangrove sediment, six fungi with characteristics of the genus *Aspergillus* were isolated. Of these fungi, four presented enzymatic indices above 1.5, indicating high potential to produce cellulases. These results encourage the search for new microbial metabolites of industrial interest in mangrove environments and contribute to highlighting the potential of this biome contributing to its conservation.

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