Exploring Enzymatic Potential in the Atlantic Forest: Isolation of Pectinase-Producing Fungi

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ABSTRACT

The Atlantic Forest is a reservoir of biodiversity, and despite extensive knowledge about its fauna and flora, little is known about the diversity of filamentous fungi in this biome and, consequently, their potential to produce biologically active molecules. Therefore, this study aimed to collect, isolate, and select filamentous fungi producers of pectinases. The first step involved collecting water and soil samples from a forest fragment in the Paraná Atlantic Forest. The strains were isolated on BDA (Potato Dextrose Agar) supplemented with Ampicillin 50 mg L^{-1} to inhibit bacterial growth. After isolation, 78 strains of filamentous fungi and 3 yeast-like fungi were obtained. Of the 81 isolated fungi, 57 % grew at 28 °C and 43 % at 40 °C, considered mesophiles and thermophiles, respectively. According to the screening, the mesophilic filamentous fungus PA3S12MM stood out for its production of pectinases compared to the other evaluated fungi, with 15.9 U mL^{-1} of enzymatic activity under non-optimized conditions. Therefore, it can be inferred that new fungal strains can be collected from Atlantic Forest fragments, and that the PA3S12MM strain proved to be a good producer of pectinases.

Keywords: Bioprospecting. Pectinolytic complex. Fungal enzymes.

1 INTRODUCTION

The Atlantic Forest is a reservoir of biodiversity, boasting one of the highest species richness rates in the world, making it a conducive environment for microbial bioprospecting with biotechnological potential.[1]. Bioprospecting can be defined as the search for new active compounds in nature. These compounds represent valuable genetic and biochemical resources in various industries and are exploited to achieve commercial and conservation objectives[2]. Locations with high biodiversity, such as the Atlantic Forest, are conducive environments for this practice.

Fungi exhibit a remarkable capacity for enzymatic production, serving as the primary source of an enzymatic cocktail utilized across various industries. This cocktail includes cellulases, phytases, lipases, catalases, laccases, and pectinases[3]. Pectinases constitute a group of hydrolytic enzymes that catalyze the degradation of pectin, acting synergistically in the middle lamella and the primary cell wall of plants. These enzymes possess significant biotechnological potential, particularly in the food and beverage industry, paper manufacturing, and as additives in animal feed[4]. Therefore, the discovery of new fungal strains with high pectinase production is of paramount importance.

2 MATERIAL & METHODS

Microorganisms collection

For the experiments, microorganisms were collected in the municipality of Nova Aurora, located in the state of Paraná, between 24° 32' 00'' South and 53° 15' 10'' West, at an altitude of 520 meters above sea level, covering an area of 474.011 km². Seven sampling points were selected, and water and soil samples were collected from each location. Water samples were collected using sterile bottles, while soil samples were collected with the aid of a spatula. The samples were labeled with pertinent information, including geographic coordinates, temperature, and humidity.

Isolation of filamentous fungi

Water samples were incubated in sterile Petri dishes without culture medium, containing small fragments of Jararaca snake (*Bothrops sp.)* molt sterilized under ultraviolet light and apple pieces sterilized in an autoclave[5]. The material was incubated at room temperature in the dark for 4 days. After this period, only the molt or apple pieces (baits) were inoculated on plates containing BDA (Potato Dextrose Agar) supplemented with Ampicillin 50 mg L^{-1}. Soil samples were inoculated on plates containing BDA medium supplemented with Ampicillin 50 mg L^{-1} to inhibit the growth of bacteria and yeasts. Both samples were incubated at controlled temperatures (28 °C for mesophiles and 40 °C for thermophiles).

Screening of pectinolytic microorganisms and obtaining crude enzyme extract

For the selection of fungi producing pectinases, only mesophilic microorganisms were tested. The isolates were inoculated in Adams[6] liquid medium to verify the production of enzymes from the pectinolytic complex. The cultures were incubated at 28 °C for 72 hours under orbital agitation at 100 rpm. After the incubation period, the cultures were vacuum-filtered, obtaining a cell-free filtrate and mycelium. The filtrate was used for the determination of pectinolytic activity and protein quantification.

Determination of pectinolytic activity and protein quantification

Enzymatic activity was determined by the reaction with 3,5-dinitrosalicylic acid (DNS), as described by Miller[7], using 1% citrus pectin dissolved in 100 mM sodium acetate buffer pH 5.0 as the substrate. One enzymatic unit was defined as the amount of enzyme required to release 1 µmol of galacturonic acid per minute under the assay conditions. Protein quantification was performed according to the method described by Bradford[8], using bovine serum albumin (BSA) as a standard.

3 RESULTS & DISCUSSION

Collection and Isolation of filamentous fungi

The collection resulted in the isolation of 78 strains of filamentous fungi and 3 yeast-like fungi, the majority of which originated from soil samples. Of these 81 isolated fungi, 57 % grew at 28 °C, considered mesophiles, and 43 % grew at 40 °C, considered thermophiles. Similarly, a study aimed at expanding knowledge about representatives of *Ascomycota* associated with the *Arecaceae* family in Atlantic Forest areas isolated 81 species from 24 samples, of which 20 comprise new species for science[9].

Screening to select pectinolytic microorganisms

The screening was conducted with the 46 mesophilic isolates. Table 1 shows the values of enzymatic activity and proteins content. The strain PA3S12MM stood out for its production of pectinases compared to the other evaluated fungi, with 15.9 U mL^{-1} of enzymatic activity under non-optimized conditions. In addition to exhibiting high production of pectinases under non-optimized conditions, it has distinct morphological characteristics, such as colonies with aerial hyphae of cottony texture and brown color.

Table 1 Screening of mesophilic filamentous fungi producers of pectinases.

Fungi	Enzymatic Activity (U mL^{-1})		Protein (mg mL^{-1})		Fungi	Enzymatic Activity (U mL^{-1})		Protein (mg mL^{-1})	
PA2A1MB	1,0	±0,0	1,3	±0,1	PA3S12MM	15,9	±0,0	0,7	±0,1
PA3A1MV	0,0	±1,1	0,8	±0,0	PA3S13ML	1,2	±0,1	0,5	±0,2
PA1S1MY	0,2	±0,1	4,9	±0,1	PA3S14MB	2,2	±0,1	0,6	±0,0
PA1S2MV	0,0	±0,0	4,0	±0,1	PA3S15MB	0,4	±0,0	0,7	±0,2
PA1S3MM	0,6	±0,1	1,1	±0,2	PA3S16MC	1,6	±0,1	1,5	±0,1
PA2S1MY	0,0	±0,0	1,4	±0,1	PA3S17MC	1,3	±0,3	5,0	±0,4
PA2S2MC	5,2	±0,1	1,6	±0,0	PA3S18MV	7,7	±0,3	1,9	±0,0
PA2S3MC	5,8	±0,0	0,8	±0,1	PA3S19MV	0,7	±0,0	1,4	±0,0
PA2S4MY	0,0	±0,0	3,5	±0,3	PA3S20MB	1,4	±0,1	0,7	±0,1
PA2S5MC	3,6	±0,3	1,0	±0,0	PA4S1MR	0,6	±0,1	0,5	±0,1
PA2S6MM	0,0	±0,0	3,8	±0,1	PA4S2ML	0,9	±0,1	1,1	±0,0
PA2S7MM	0,4	±0,1	4,9	±0,1	PA4S3MB	8,8	±0,4	0,7	±0,0
PA3S1MM	0,6	±0,0	3,6	±0,1	PA4S4MB	1,9	±0,1	1,8	±0,0
PA3S2MV	8,5	±0,2	1,9	±0,1	PA4S5MC	5,9	±0,1	3,1	±0,2
PA3S3MB	0,5	±0,0	0,6	±0,1	PA4S6MV	8,7	±0,2	2,6	±0,0
PA3S4MV	0,7	±0,1	1,3	±0,0	PA4S7MM	1,2	±0,0	2,1	±0,1
PA3S5MB	1,1	±0,1	1,2	±0,3	PA4S8MM	1,6	±0,3	2,6	±0,1
PA3S6MV	1,0	±0,1	1,1	±0,1	PA4S9MM	0,9	±0,0	1,0	±0,0
PA3S7MM	1,1	±0,1	1,0	±0,2	PA4S10MM	0,4	±0,1	0,8	±0,0
PA3S8MM	1,0	±0,0	1,1	±0,0	PA4S11MV	2,7	±0,2	0,8	±0,0
PA3S9MV	3,8	±0,1	1,4	±0,1	PA4S12MB	0,8	±0,1	1,3	±0,1
PA3S10MB	0,6	±0,1	1,3	±0,1	PA4S13MB	3,5	±0,1	0,9	±0,0
PA3S11MR	1,1	±0,0	2,8	±0,1	PA4S14MV	3,0	±0,0	1,4	±0,1

4 CONCLUSION

Based on the results obtained, it can be inferred that new fungal strains can be collected from fragments of the Atlantic Forest, which is extremely promising, as they are excellent enzyme producers and can be applied in various industrial sectors, representing a valuable biochemical resource. Additionally, the strain PA3S12MM showed promising results as a producer of pectinases, indicating its potential for optimizing enzyme production.

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