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Biological activity and production of metabolites from Amazon endophytic fungi

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Obtaining bioactive metabolites from endophytic microorganisms has become increasingly more interesting in the last few decades, since endophytes are known for their biotechnological potential. However, studies involving endophytic microbiota from tropical hosts are still scarce. In this study, the production of bioactive metabolites from endophytic fungi isolated from Amazonian plants were evaluated. Two fungi (*Talaromyces* sp. F15 and *Aspergillus* sp. F18) isolated from *Myrcia guianensis* (Myrtaceae) and one (*Penicillium* sp. F3) isolated from *Euterpe precatoria* (Arecaceae) were analysed. The fungi were cultivated in liquid medium and their metabolites were tested for antimicrobial, antioxidant and cytotoxic activity. Amylase, cellulase and lipase production, as well as biosurfactant production, were also evaluated. The metabolites of *Aspergillus* sp. F18 showed 69.4% antioxidant activity against DPPH free-radical molecules and cytotoxic activity against *A. salina*. *Penicillium* sp. F3 showed cytotoxic activity and stood out as the best amylase producer (31 U/ml). *Talaromyces* sp. F15 was the best lipase producer (4.5 U/ml) and the best biosurfactant source, with 33.3% emulsification index. These Amazonian host-associated fungi showed biotechnological potential, which are believed should be further investigated in order to elucidate the chemical structure of the metabolites responsible for the activities described here, as well as optimize their production.

Key words: Enzymes, lipase, cellulase, amylase, biosurfactant, antioxidant, antimicrobial, cytotoxic.

INTRODUCTION

Brazil is a country with vast biodiversity, and much of this wealth is concentrated in the Amazon region. As such, the Amazon region has numerous plant species from which many benefits have been discovered to date and,

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in turn, made use of, especially in the food and pharmaceutical industry (Banhos et al., 2014). People have been using natural compounds for the preparation of herbal teas and remedies for the prevention and treatment of various diseases for a long time and many of the current medicines available on the market have come from plants and microorganisms isolated from various traditional sources (Amedei and D'Elios, 2012; Fokou et al., 2016; Huang and Lin, 2017).

Among the microorganisms which are used as sources of biologically-active biomolecules are endophytes, and these are characterized by the fact that they inhabit plant tissues without causing apparent damage to their hosts. Nevertheless, endophytic microorganisms can be pathogens, commensals, or mutualists, depending on plant-endophyte interactions. Secondary metabolites produced by endophytic fungi have a broad spectrum of bioactivity, such as for antimicrobial, antioxidant, antitumor, enzymatic and surfactant compounds (Canuto et al., 2012; Pamphile et al., 2017; Silva et al., 2019; Toghueo, 2019).

Endophytes have the ability to interact with the plant at complex levels and, in some cases, maintain symbiosis relationships (Yan et al., 2019). Endophytic micro-organisms can even produce the same metabolites as their hosts (Strobel at al., 2004; Facundo et al., 2008), and thus stand out as an alternative for the preservation of several plant species, whose extracts are used in the production of some medicines (Mussi-Dias et al., 2012). However, endophytic fungi isolated from tropical plants are still little studied (Oliveira, 2010; Specian et al., 2014; Silva et al., 2019).

Metabolites produced by endophytic fungi have already been identified as being effective against tumors in human cervical cancer cell cultures (Wijesekara et al., 2013). These metabolites have shown antimicrobial activity against multi-drug resistant strains of bacteria, and demonstrate effective and promising action in this application (Arivudainambi et al., 2014). Also, these molecules present antioxidant activity, an important characteristic, since the production of free radicals and other reactive oxygen species (ROS) can damage biomolecules such as RNA and DNA, and result in several physiological disorders, such as cancer and premature aging (Huang et al., 2007; Morais et al., 2014; Caicedo et al., 2019).

Other important bioactive metabolites that are produced by fungi are enzymes. Hydrolytic enzymes catalyze hydrolysis reactions of different substrates and have a wide range of industrial uses. Lipases are responsible for catalyzing the hydrolysis of triacylglycerides formed by long chain fatty acids, and have widespread application in biocatalysis. Cellulases are involved in the degradation of cellulose, which are utilized for the hydrolysis of biomass for ethanol production, and the amylases act by breaking down starch molecules, for which there is a high demand from the food industry (Roveda et al., 2010; Gopinath et al., 2017; Bentil et al., 2018).

Biosurfactants are also metabolites of industrial interest, which are amphiphilic molecules that are capable of reducing the interfacial and surface tension of liquids and have properties and applications that involve actions such as, emulsification, lubrication, foaming, wetting, solubilization, detergent actions and phase dispersion (Nitschke and Pastore, 2002; Varjani and Upasani, 2017).

Since little is known about the biotechnological potential of metabolites produced by fungi isolated from Amazonian species, this study aimed to primarily evaluate the antioxidant, cytotoxic and antimicrobial activities, as well as the production of hydrolases and biosurfactants in metabolites obtained from submerged cultivation of endophytic fungi which had been isolated from the tropical species *Myrcia guianensis* and *Euterpe precatoria*.

MATERIALS AND METHODS

Microorganisms

The endophytic fungi used in this study were previously selected as producers of bioactive metabolites (Batista, 2018; Matias, 2018) and are held in the Chemistry Applied to Technology (QAT) research group's work collection at the Superior School of Technology (EST) at the Amazonas State University (UEA).

The fungi *Aspergillus* sp. F18 (Figure 1A) and *Talaromyces* sp. F15 (Figure 1B) were isolated from *Myrcia guianensis* stem and root, respectively. The vegetable material from *M. guianensis* were collected in Santarém, Pará State. Their identifications were carried out at the INPA Herbarium (National Institute of Amazon Research), and a voucher specimen was deposited under the registration number 181913 (Banhos et al., 2014).

The isolate *Penicillium* sp. F3 (Figure 1C) was obtained from the leaves of *Euterpe precatoria.* The leaves were collected in Manaus, Amazonas State. The plant was identified at the IFAM Herbarium (Amazonas Federal Institute of Education, Science and Technology), and a voucher specimen was deposited under the registration number 16782 (Batista et al., 2018).

The endophytic fungi were identified by their macroscopic characteristics (color, texture, topography, diffuse pigmentation, color, and topography of the back of the colony), and well as by their microscopic reproductive structures, via the microculture technique and comparison of the obtained results with taxonomic keys (Barnett and Hunter, 1972; Hanlin, 1996) (Figure 1).

The three isolates were stored in sterile distilled water (Castellani, 1939). Reactivation of the fungi occurred by inoculation from a stock culture onto potato dextrose agar (PDA) with subsequent incubation in a microbiological chamber (BOD) at 28ºC for approximately 5 days.

Production of fungal metabolites for biological assays

Under sterile conditions, three mycelial plugs of approximately 5x5 mm were inoculated into 125 ml Erlenmeyer flasks with 80 ml potato-dextrose liquid medium (PD) supplemented with 0.2% yeast extract. Cultures were produced in duplicate. The flasks were incubated in a shaker at 28°C and shaken at 120 rpm for 8 days. After this period, the metabolic fluid was filtered through a 0.45 µm

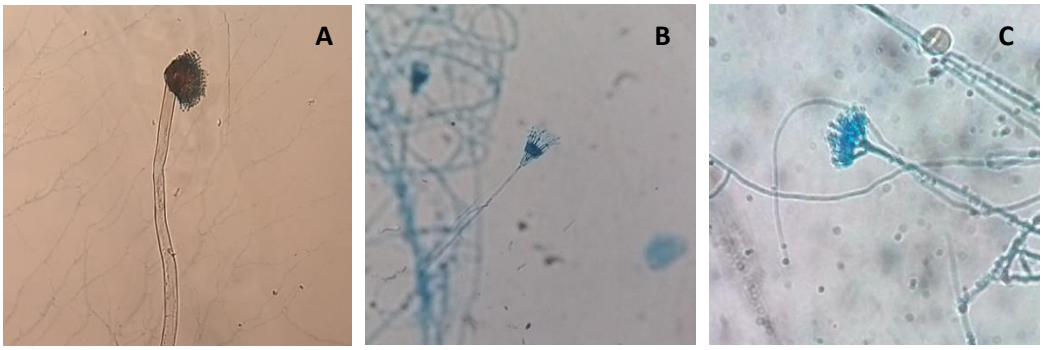

Figure 1. Microscopic images from Amazonian endophytic fungi. A: *Aspergillus* sp. F18 isolated from *Myrcia guianensis* stem. B: *Talaromyces* sp. F15 isolated from *Myrcia guianensis* root. C: *Penicillium* sp. F3 isolated from *Euterpe precatoria* leaves.

pore membrane filter (Souza et al., 2004). The filtered medium was stored at -18ºC for later use in biological activity assays.

Evaluation of antimicrobial activity

The microplate dilution technique was used (NCCLS, 2003) to verify the antimicrobial activity. The reducing of resazurin was used for antibacterial testing, and reducing of 2,3,5-triphenyltetrazoic chloride (TTC) for antifungal testing. The fungal metabolites were tested against strains of *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 35218), *Pseudomonas aeruginosa* (ATCC 9027) and *Candida albicans* (ATCC 12031).

The assay was performed using sterile 96-well microplates for each microorganism. 100 µl of the inoculum was pipetted at a concentration of 1.5 x 10^4 CFU/ml into the plate wells in triplicate. For sterility control and negative test control, we used the culture medium (Mueller Hinton broth for bacteria and potato dextrose broth for fungi). The positive control was Levofloxacin (15 mg/ml) for bacteria and Itraconazole (20 mg/ml) for fungi. Subsequently, the plates were incubated at 37ºC for 24 h. After this period, 20 µl of resazurin was added for the antibacterial activity assay and 20 µl of TTC for the antifungal activity assay. The plates were incubated again at 37 °C for approximately 2 h for reaction testing. Wells that remained colorless were considered as showing antimicrobial activity (Duarte, 2006).

Determination of antioxidant activity

Antioxidant activity was determined by the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH·) scavenging method. This assay is based on free radical reduction in the presence of an antioxidant (Molyneux, 2004). The DPPH solution was prepared at 0.06 mmol/l with methanol P.A., and protected from direct light exposure (Silva, 2012). The assay was performed by microplate spectrophotometry with 40 µl of the metabolic medium and the addition of 250 µl of the DPPH solution. For the negative control, 40 µl of methanol and 250 µl of DPPH solution were added (Duarte-Almeida et al., 2006). The microplate was protected from direct light exposure and after 10 min the absorbance readings were taken on a microplate spectrophotometer, at 517 nm. The flavonoid quercetin was used as standard. The percentage of DPPH radical scavenging was measured by the equation below using the absorbance decay values of the sample (Abs_{sample}) and of the control ($Abs_{control}$):

$$\boldsymbol{AA}\ (\%) = \frac{\left(\boldsymbol{Abs_{control} - Abs_{sample}}\right)}{\boldsymbol{Abs_{control}}}\ \boldsymbol{x\ 100}$$

Determination of cytotoxic activity

To evaluate the cytotoxic effect of the samples, the lethality test was performed with *Artemia salina* (Meyer et al., 1982; Mclaughlin, 1991). As a growth medium, a saline solution containing 20 g of synthetic sea salt in distilled water was used to prepare 1000 ml of 2% solution. For hatching, 10 mg of cysts of *A. salina* (Maramar) were used. Growth occurred at room temperature, with constant aeration under fluorescent lighting during 48 h. After hatching, the nauplii were transferred to 24-well plates, 10 nauplii distributed to each well and 1 ml of the test solutions added in duplicate. In the control, the test solution was replaced with the culture medium used for the fungal culture and, in another well, just the saline solution was used. 100 µl of Levofloxacin (15 mg/ml) was added to inhibit the proliferation of bacteria in the medium. After 24 h, the number of surviving larvae was analyzed in both control wells and treatments.

Preparation of spore suspension for the production of hydrolytic enzymes and biosurfactants

To produce the spore suspension, the fungi were cultivated in PDA in inclined test tubes (Gomes and Pena, 2016). 4 ml of sterile distilled water were added to each test tube. The tubes were shaken to float the spores present in the mycelium. An aliquot of 1000 µl was taken from each tube and from this tube the spore count was performed in a Neubauer chamber to adjust the inoculum to a concentration of 10^6 spores/ml for enzyme production and 10^8 spores/ml for biosurfactant production.

Amylase production

The fungi were grown in a liquid medium as described by Hegde et al. (2011), and composed of $NaNO_3$ (3.0 g/l), $MgSO_4.7H_2O$ (0.5 g/l), KCl (5.0 g/l), KH_2PO_4 (1.0 g/l), $FeSO_4.7H_2O$ (0.01 g/l), $CaCl_2$ (0.1 g/l) and starch (15 g/l), pH 7.0. 100 µl of spore suspension were added (10^6 spores/ml) and incubated in a shaker at 30°C, under constant stirring 120 rpm, for 7 days. Every 24 h, 1 ml aliquots were taken and filtered for subsequent measurement of enzymatic activity. Cultivations were performed in triplicate. Commercial amylase (Novozymes) was used as a standard for purposes of

comparison.

Determination of amylolytic activity

To measure amylase activity, a standard glucose curve was constructed according to the methodology of Vasconcelos et al. (2013). The measurement of enzymatic activity was carried out as described by Miller (1959), with some modifications, to determine the amount of reducing sugars formed during the incubation of the enzyme extract with the substrate using 3,5-dinitrosalicylic acid (DNS).

The reaction mixture was composed of 50 µl substrate (1% starch) diluted in 1 M sodium acetate buffer, pH 6 (m/v) and 50 µl enzyme extract. After incubation at 50°C for 30 min, 100 µl of DNS was added and the mixture was placed in a water bath for 5 min. 800 µl of distilled water were added and then the absorbance was read in a spectrophotometer at 540 nm. One unit of enzymatic activity (U) was defined as the amount of enzyme capable of releasing 1 µmol of reducing sugar per minute under the assay conditions.

Cellulase production

The fungi were grown in a liquid medium as described by Zanchetta (2012), composed of KH_2PO_4 (2.0 g/l), $(NH_4)_2SO_4$ (1.4 g/l), urea (0.3 g/l), $MgSO_4.7H_2O$ (0.3 g/l), $CaCl_2$ (0.1 g/l), $FeSO_4.7H_2O$ (5.0 mg/l), $MnSO_4.H_2O$ (1.6 mg/l), $ZnSO_4.7H_2O$ (1.4 mg/l), $CoCl_2$. H_2O (1.6 mg/l) and carboxymethylcellulose - CMC (10 g/l), pH 5.0. 100 µl of spore suspension was added (10^6 spores/ml) and incubated in a shaker at 28°C, 120 rpm during 7 days. Every 24 h, 1 ml aliquots were taken and filtered for subsequent measurement of enzyme activity. Cultivations were performed in triplicate. The commercial enzyme produced by *Aspergillus niger* (Sigma Aldrich) was used as a standard for comparative purposes.

Determination of cellulolytic activity

The standard curve was constructed with glucose. Enzyme activity was measured according to the methodology described by Miller (1959) with modifications. The reaction mixture was composed of 50 µl of enzyme extract and 50 µl of 1% CMC solution in sodium citrate buffer, 0.05 M pH 5, incubated at 50°C for 30 min. After incubation, 100 µl of DNS was added and then the mixture was placed in a water bath for 5 min at 100°C, followed by the addition of 800 µl of distilled water. The absorbances were read in a spectrophotometer at 540 nm against the blank which was composed of all the reaction components collected at time point zero of the experiment. One unit of enzymatic activity (U) was defined as the amount of enzyme capable of releasing 1 µmol of reducing sugar per minute under the assay conditions.

Lipase production

The fungi were grown in Erlenmeyer flasks containing 100 ml of liquid medium, as described by Nascimento et al. (2014). The medium was composed of NH_2NO_3 (1.0 g/l), $MgSO_4.7H_2O$ (0.6 g/l), KH_2PO_4 (1.0 g/l), peptone (20 g/l) and olive oil (1% and 0.065%), pH 6. 100 µl of spore suspension was inoculated (10^6 spores/ml) under sterile conditions and incubated in a shaker at 28°C, 160 rpm for 7 days. Every 24 h, 1 ml aliquots were taken and filtered for subsequent measurement of enzyme activity. Cultivations were performed in triplicate. The commercial enzyme produced by *Candida rugosa* (Sigma Aldrich) was used as a standard for comparative purposes.

Determination of lipolytic activity

The quantification of lipolytic activity was performed according to Winkler and Stuckmann (1979). An emulsion of *p*-nitrophenyl palmitate (pNPP) was prepared by the dropwise addition of 1 ml of solution A (30 mg of pNPP dissolved in 10 ml of isopropanol) in 9 ml of solution B (0.4 g of Triton X-100; 0.1 g of gum arabic and 90 ml of Tris HCl 50 mM pH 7.0) under intense agitation. The emulsion obtained and the aliquots taken from the fungal culture medium were stabilized for 5 min at 37°C. 0.1 ml of the supernatant was added to 0.9 ml of the substrate emulsion and the mixture was incubated for 15 min at 40°C. The absorbance of the mixtures was measured by spectrophotometer at 410 nm. One unit (U) of enzymatic activity was defined as the amount of enzyme required to release 1.0 µmol of *p*-nitrophenol per minute under these conditions (Pereira et al., 2015; Tombini, 2015). Enzyme activity was obtained by the relationship between the absorbance of the sample and the molar extinction coefficient of *p*-nitrophenol (ε = 12276).

Biosurfactant production

The liquid medium described by Jacobucci (2000) composed of $MgSO_4$ (0.5 g/l), Na_2HPO_4 (3.0 g/l), KH_2PO_4 (1.0 g/l) and yeast extract (1.3 g/l) was used. After autoclaving, 0.5 g/l of soybean oil was added to the medium and then homogenized. Afterwards, 1.0 ml of the spore suspension (10^8 spores/ml) of endophytic fungi (*Penicillium* sp. F3, *Talaromyces* sp. F15 and *Aspergillus* sp. F18) was inoculated into the medium. The fungi were grown in a shaker type incubator at 28 °C under constant stirring at 170 rpm for 8 days. At the end of the experiment, the metabolic medium was filtered to separate the mycelium with 0.45 µm filtering membrane and assisted by a vacuum pump. Afterwards, the cell free cultivation broth was used for the determination of the emulsification index E_{24} (%) and surface tension measurements. Cultures were performed in triplicate.

Determination of the Emulsification index

A mixture of 6 ml of the solvent (kerosene) and 4 ml of solution of the synthetic surfactant (control) or culture medium containing biosurfactant was mixed in a vortex-type stirrer for 2 min. The emulsifying activity was investigated after 24 h and the emulsification index E_{24} (%) was calculated by dividing the height measurement of the emulsion layer by the total height of the mixture, multiplying by 100 (Pornsunthorntawee et al., 2008). The assays were performed in triplicate. For positive control, 1% SDS solution was used and for negative control the culture medium without microorganism was used.

Surface tension measurements

Surface tension was measured by the ring method using a ring tensiometer (Krüss) at room temperature (25°C) (Du Nouy, 1925).

RESULTS AND DISCUSSION

Biological activities

The metabolites produced by the fungus *Aspergillus* sp. F18 isolated from *M. guianensis* showed antioxidant activity of 69.4%, while quercetin showed 90%. The other fungi tested showed no ability to sequester the DPPH free radical. Zhao et al. (2014) tested extracts obtained

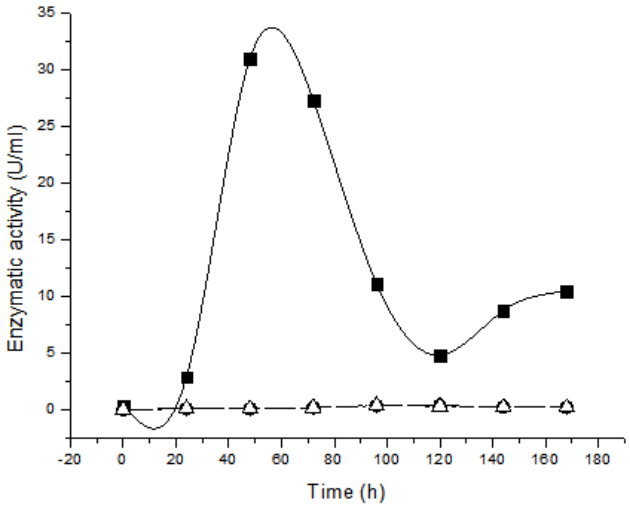

Figure 2. Amylase enzyme activity for the endophytic fungi *Penicillium* sp. F3 (■), *Talaromyces* sp. F15 (●) and *Aspergillus* sp. F18 (△), during the cultivation period.

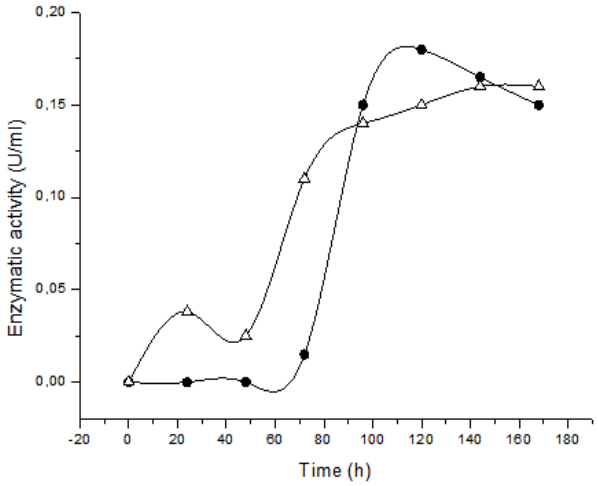

Figure 3. Cellulase enzyme activity for the endophytic fungi *Talaromyces* sp. F15 (●) and *Aspergillus* sp. F18 (△), during the cultivation period.

from the metabolites of the endophytic fungus *Aspergillus fumigatus* and observed that the ethyl acetoacetate extract was the most promising one, with considerable antioxidant activity (AA = 95%; CE_{50} = 38.64 µg/ml), which indicates the potential of endophytes as sources of antioxidant compounds.

Antimicrobial activity was determined by the microdilution technique. The fungal metabolites tested here showed no activity against the evaluated microorganisms. According to Furmanek et al. (2019), the microdilution method assesses the effectiveness of concentrations of used substances . In this study, the crude aqueous extract to access the antimicrobial activity was used, and the substances that could present the inhibition of microbial growth were probably not in an effective concentration (not detected by this method), thus it is necessary to concentrate the metabolites in order to access this biological activity. Proper interpretation of the results is also hampered by the dosages employed since, according to the Clinical and Laboratory Standards Institute's (CLSI) recommendations, a very major mistake in the interpretation of compound activity can be made in demonstrating the resistance according to this method (false sensitivity) (CLSI, 2015).

The metabolites of the fungi *Aspergillus* sp. F18 (isolated from *M. guianensis*) and *Penicillium* sp. F3 (from *E. precatoria*) were cytotoxic in the tests performed against *A. salina*, and lead to the death of all nauplii within 24 h. Miao et al. (2012) found that the substance 6.8-di-O-methylaverufin, produced by an endophytic fungus, showed significant toxicity for *A. salina*, with CL_{50} from 0.5 µg/mL. The lethality of simple organisms such as *A. salina* has been used for rapid and relatively simple monitoring of biological response (Meyer et al., 1982). The lethality assay allows the evaluation of general toxicity and is therefore considered essential as a preliminary bioassay in the study of compounds with potential biological activity (Colegate and Molyneux, 1993). According to Uzma et al. (2018), endophytic fungi represent a rich source of bioactive metabolites that can be manipulated to produce novel analogs for chemotherapy, such as taxol, podophyllotoxin, camptothecin, and vinca alkaloids. Therefore, the fungal metabolites tested here should be evaluated in further studies for their cytotoxic activity using cell assays.

It is worth mentioning that the secondary metabolites produced by endophytic fungi can suffer alterations when grown in the laboratory. Temperature, composition of the culture medium and aeration can interfere with the quantity and type of compounds that are produced (Strobel et al., 2004). Thus, it is important to optimize the cultivation conditions in order to increase the metabolite yields.

Enzyme production

Maximum amylase production (31 U/ml) was observed after 72 h of cultivation of the fungus *Penicillium* sp. F3, isolated from the *E. precatoria* leaf (Figure 2). The fungus *Talaromyces* sp. F15, isolated from the *M. guianensis* stem, however, showed low enzymatic activity (0.37 U/ml), as did the fungus *Aspergillus* sp. F18, isolated from the *M. guianensis* root (0.34 U/ml). The obtained result was compared with the commercial enzyme, which obtained activity of 38.5 U/ml, indicating the potential of *Penicillium* sp. F3 as a source of amylolytic enzymes.

The fungi *Talaromyces* sp. F15 and *Aspergillus* sp. F18 showed low cellulolytic activities after 120 h of cultivation of 0.18 and 0.16 U/ml, respectively, as shown in Figure 3.

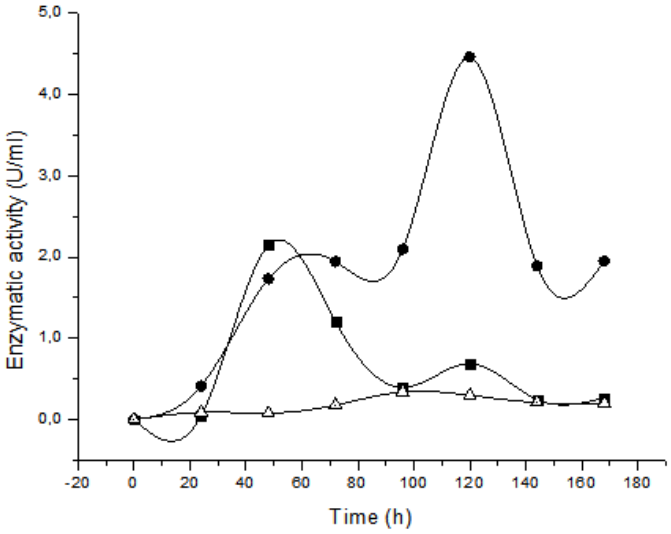

Figure 4. Lipase enzyme activity for the endophytic fungi *Penicillium* sp. F3 (■), *Talaromyces* sp. F15 (●) and *Aspergillus* sp. F18 (△), during the cultivation period.

However, the fungus *Penicillium* sp. F3 did not produce cellulase under the evaluated cultivation conditions. Reddy et al. (2015) obtained, under similar cultivation conditions used in this study, a cellulolytic activity of 2.48 U/ml for *A. niger*.

The evaluated commercial enzyme presented activity of 30.9 U/ml. Therefore, it is worth noting that the endophytic fungi evaluated in this study must be subjected to new culture conditions in order to increase cellulase production.

The maximum lipase production was observed after 120 h of cultivation of the fungus *Talaromyces* sp. F15 (Figure 4), and obtained an activity level of 4.45 U/ml. The fungus *Penicillium* sp. F3 showed a maximum activity level of 2.14 U/ml in just 48 h.

However, the fungus *Aspergillus* sp. F18 showed an activity level of 1.04 U/ml. Romdhane et al. (2013) obtained enzymatic activity of 9.8 U/ml for the purified lipase produced by the fungus *T. thermophilus*. Thus, the endophytic fungus *Talaromyces* sp. F15 isolated from *M. guianensis* is shown to be a promising lipase producer. The evaluated commercial enzyme presented activity of 24.4 U/ml.

Biosurfactant production

The 3 endophytic fungi were evaluated for emulsification index levels and reduction of surface tension for biosurfactant production. Table 1 shows the results obtained for emulsification index tests (E_{24}) in the presence of kerosene.

It is noted that the fungal culture media *Penicillium* sp. F3 and *Aspergillus* sp. F18 showed no emulsion formation in the presence of organic solvent. For the fungus *Talaromyces* sp. F15 emulsion formation was obtained, with E_{24} of 33.3%. SDS synthetic surfactant has higher emulsification capacity than fungal metabolites, which was expected since SDS is a pure substance.

Table 2 shows the reduction in surface tension of the cell-free culture medium for the 3 evaluated fungi. Note that for the fungus *Talaromyces* sp. F15, surface tension reduction was 33%, from 56.5 mN/m to 40.5 mN/m.

According to Haba et al. (2000), microorganisms that are good biosurfactant producers are able to reduce the surface tension of the medium to 40 mN/m or less, which indicates the potential of the endophytic isolate as a source of surfactant molecules. According to Ron and Rosenberg (2002), Bach et al. (2003) and Hamme et al. (2006), low molecular weight biosurfactants reduce surface tension more efficiently, while those with high molecular weight are characterized by the formation of more stable oil/water emulsions. Therefore, it can be inferred that the biosurfactant produced by the Amazonian endophytic fungus *Talaromyces* sp. F15 is a low molecular weight molecule.

Conclusion

The results showed that the endophytic fungi isolated from tropical species *M. guianensis* and *E. precatoria* are promising in the search for new bioactive compounds of industrial interest, mainly in the production of amylase and biosurfactants. The activities tested indicated significant results, which should be improved by further

Table 1. Results of the emulsification index tests (E_{24}) of the endophytic fungi cultivation broth and the 1% Sodium Dodecyl Sulfate (SDS) surfactant.

Endophytic fungi	E_{24} (%)
Penicillium sp. F3	*
Talaromyces sp. F15	33.3
Aspergillus sp. F18	*
SDS 1%	88.0

*Not detected.

Table 2. Reduction in the surface tension (ST) of the metabolic media after 8 days of cultivation of the endophytic fungi.

Endophytic fungi	Initial ST (mN/m)	Final ST (mN/m)	Reduction in ST (%)
Penicillium sp. F3	56.5	52.53 ± 1.5	5
Talaromyces sp. F15	56.5	40.5 ± 0.5	33
Aspergillus sp. F18	56.5	49.97 ± 2.5	10

studies of optimization of cultivation conditions.

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CONFLICT OF INTERESTS

The authors declare no conflict of interests.

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