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Lignocellulosic supplementation of the culture medium for mycelial development and production of phenoloxidase enzymes from Amazonian strains of gasteroid fungi (Basidiomycota)

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I N F O

Keywords
Mycelial cultures
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A B S T R A C T

Strains of gasteroid fungi are capable of oxidizing phenolic components and producing enzymes of great commercial value. However, the difficulty in producing biomass and, consequently, the low levels of expression of enzymes produced by these fungi hinder the advancement of biotechnological studies. A possible alternative may be supplementing the culture medium with lignocellulosic substrates, as observed in the cultivation of edible mushrooms, for example. Thus, the objective of this study was to analyze the effect of supplementing a potato-based culture medium with different concentrations of wheat and rice bran on mycelial development and the production of phenoloxidase enzymes of Amazonian strains of *Cyathus* and *Geastrum*. The basidiomas were collected in the Amazon of Pará, and the strains were obtained from mycelial isolation on Potato Dextrose Agar (PDA) medium. The strains were inoculated in semi-solid and liquid potato-based medium containing 10% and 20% rice bran and 10% and 20% wheat bran. For a period of 20 days, mycelial growth was observed in solid form, and for 40 days, the development of the strains in liquid medium was monitored, all incubated at 25°C in the dark. Growth and production of fresh and dry biomass were measured, as well as the production of phenoloxidase, arising from growth in liquid medium. Supplementation using 10% wheat bran was the most promising for the radial growth of four of the six species tested (*C. limbatus, G. hirsutum, G. echinulatum,* and *G. schweinitzii*), in addition to being the best treatment for the production of biomass and phenoloxidase enzymes for all species. Considering that wheat bran is easily accessible, this study stimulates basic science, especially in the Amazon, where Funga is still underestimated in terms of diversity and biotechnological potential.

Palavras-chaves
Culturas miceliais
farelos lignocelulósicos
fungos estrela-da-terra
fungos ninho-de-pássaros
produção enzimática

R E S U M O

Suplementação lignocelulósica do meio de cultura para desenvolvimento micelial e produção de enzimas fenoloxidase de cepas amazônicas de fungos gasteroides (Basidiomycota)

Cepas de fungos gasteroides são capazes de oxidar componentes fenólicos e produzir enzimas de grande valor comercial. Porém, a dificuldade na produção de biomassa e consequentemente os baixos níveis de expressão das enzimas produzidas por esses fungos, impedem o avanço dos estudos biotecnológicos. Uma auternativa possível pode ser o uso da suplementação do meio de cultura com substratos lignocelulógicos, como como observado no cultivo de cogumelos cultiváveis, por exemplo. Assim, o objetivo desse estudo foi analisar o efeito da suplementação do meio de cultura a base de batata com diferentes concentrações de farelo de trigo e arroz no desenvolvimento micelial e na produção de enzimas fenoloxidases de cepas amazônicas de *Cyathus* e *Geastrum*. Os basidiomas foram coletados na Amazônia paraense e as cepas obtidas a partir do isolamento micelial em meio Batata Dextrose Ágar (BDA). As cepas foram inoculadas em meio semisólido e líquido a base de batata contendo 10 e 20% de farelo de arroz e 10 e 20% de farelo de trigo. Por um período de 20 dias observou-se o crescimento micelial emisólido e por 40 dias o desenvolvimento das cepas em meio líquido, todos incubados 25°C no escuro. O crescimento e a produção de biomassa fresca e seca foram mensuradas, assim como a produção de fenoloxidase, oriunda do crescimento em meio líquido. A suplementação usando farelo de trigo a 10% foi a mais promissora para o crescimento radial de quatro, das seis espécies testadas (*C. limbatus, G. hirsutum, G. echinulatum* e *G. schweinitzii*), além de ter sido o melhor tratamento para a produção de biomassa e enzimas fenoloxidases para todas as espécies. Considerando que o farelo de trigo é de fácil acesso, este estudo estimula a ciência de base, principalmente na Amazônica, onde a Funga ainda é subestimada em diversidade e potencial biotecnológico.

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INTRODUCTION

Given the increasing global demand for bioproducts associated with sustainable production, studies on fungi and its enzymes, particularly those that have potential for biotechnological application, have increased (Troiano et al., 2020). In this respect, gasteroid fungi (Basidiomycota) are recognized as important (Wang et al., 2014; Badshah et al., 2015; Srisurichan et al., 2017) for being able to produce several substances such as phenoloxidaseenzymes (PO) (Santana et al., 2016). PO have wide application in pollutantdegradation, effluent discoloration, wood pulp bleaching, removal of phenolics from wines, dyes oxidation, enzymatic conversion of chemical intermediates, biofuels production, xenobiotics degradation and bioremediation (Valmaseda et al., 1990; Banerjee and Vohra, 1991; D'Souza et al., 1999).

For some groups of mushrooms, the detection of PO can be done by direct reaction (Parra, 2008) or by grinding the basidiome. However, for gasteroid fungi, e.g. *Geastrum* Pers., the former technique is not efficient (Zamora et al., 2013) and the latter requires large or numerous basidiomes, which is not always possible given the nature of these fungi, that occur in low abundance and limited by climatic seasonality like most Amazonian fungi (Braga-Neto et al., 2008). Hence, the cultivation of strains may present advantages over other methods, mainly due to the availability of material for enzyme extraction over time.

Despite the recognition of the significant biotechnological potential of gasteroid fungi (Liu and Zhang, 2004), studies involving this group are predominantly taxonomic in nature. One of the contributing factors to this trend may be the challenges associated with obtaining mycelial cultures of these fungi, especially in the case of *Geastrum* species. *Geastrum* is a promising genus for the production of intriguing substances (Dore et al., 2007; Kuhar et al., 2016); however, its mycelial growth in conventional culture media is slow, posing difficulties in maintaining in vitro cultures and precluding enzyme production tests due to the limited amount of biomass generated (Sunhede, 1989; Stoytchev et al., 2001; Zamora et al., 2014).

Santana et al. (2016) reported that Amazonian strains of *Geastrum* have the capacity to produce phenoloxidase (PO). Although they have made recent advancements in obtaining mycelial cultures, testing different media, and studying improved cultivation conditions to facilitate investigations within this group (Santana et al., 2020), there remains a need to enhance the yield of in vitro cultures. In contrast, for *Cyathus* Haller, another gasteroid genus, there are more studies focusing on the extraction of substances of biotechnological interest, even though they are concentrated in a few species and specific regions of the world (Vasdev et al., 1995; Dhawan and Kuhad, 2002; Mishra and Bisaria, 2006). Obtaining and growing mycelial cultures for these fungi does not appear to be a significant challenge when compared to *Geastrum* cultures (Dhawan and Kuhad, 2003; Sharvit et al., 2021). Nevertheless, there is room for improvement, especially when considering cultivation systems.

Consequently, it is expected that mycelial cultures of gasteroid fungi can be enhanced through the supplementation of the culture medium, as observed in other fungal groups (Rossi et al., 2001; Benassi et al., 2012; Pérez-Pacheco et al., 2014; Olguin-Maciel et al., 2017; Szambelan et al., 2018). Utilizing rice and wheat bran can be particularly advantageous for achieving greater biomass or increasing enzyme production, as evidenced in the cultivation of edible mushrooms for similar purposes (Donini et al., 2006; Minotto et al., 2008). In the Brazilian Amazon regions, where the biodiversity of these genera continues to expand each year, it becomes imperative to propose protocols that open up new avenues for scientific research, including basic science, involving these species (Santana et al., 2020).

In this context, the aim of this study was to examine the impact of supplementing a potato-based culture medium, widely regarded as one of the most crucial culture media for fungi today, primarily due to its accessibility and the favorable findings reported by Santana et al. (2020). We investigated the influence of varying concentrations of rice bran and wheat bran on the mycelial development and phenoloxidase (PO) enzyme production in Amazonian strains representing different species of *Cyathus* and *Geastrum*.

MATERIAL AND METHODS

Sampling and identification of gasteroid fungi

Fresh and mature specimens of *Cyathus* and *Geastrum* were collected manually (Lodge et al. 2004) in a rainforest fragment (Amazon dense rainforest) near the Sílvio Braga Hydroelectric Power Plant (02°48'44.45"S, 54°17'56.23"W), Santarém, western Pará, Brazil. The specimens were packed separately in labeled plastic boxes. Species identification was performed based on morphological characters according to descriptions in the literature (Sunhede, 1989; Calonge et al., 2005; Sousa et al., 2014; Gois et al., 2021). Part of the samples were destined to obtain strains and

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another part was assembled in voucher specimens and incorporated into the collection of fungi of the HSTM Herbarium of the Federal University of Western Pará (Table 1).

Table 1 - Species used to obtain Amazonian strains of gasteroid fungi

Family	Species	Voucher number
Agaricaceae	*Cyathus albinus* Accioly, R. Cruz & Baseia	14790
	C. limbatus Tul. & C. Tul.	14883
	C. setosus H.J. Brodie	14957
Geastraceae	*Geastrum echinulatum* T.S. Cabral, B.D.B. Silva & Baseia	14901
	G. hirsutum Baseia & Calonge	14865
	G. schweinitzii (Berk. & M.A. Curtis) Zeller	14896

Mycelial culture obtaining

The *Geastrum* strains were obtained from sections removed from the pseudoparenchymatous layer of fresh basidiome exoperidium, set in PDA (Difco®). The *Cyathus* strains were obtained from sections of the inoculated peridioles in PDA. For all strains, the presence of a clamp connection in the mycelium, after satisfactory growth, was used as confirmation of a dicariotic mycelium (Taylor et al., 2000; Taylor et al., 2006). From the confirmation, mycelium fragments (3 × 3 mm) from the obtained strains were removed and inoculated in the center of Petri dishes (ø 90 mm) containing 15 mL of PDA and incubated at 25°C in the dark (Santana et al., 2020) to obtain pure cultures.

Effect of culture medium supplementation on mycelial growth

For the evaluation of mycelial growth, pure culture fragments (3 ×3 mm) were inoculated in the center of Petri dishes (ø 90 mm) containing 15 mL of PDA supplemented with 0 (control), 10 or 20% wheat bran or rice bran, locally sourced, previously sterilized at 121°C for 15 minutes. Petri dishes were then incubated for 20 days at 25°C in the dark (Santana et al., 2020). After this period, the average radial growth of the cultures was measured with a millimeter ruler set in orthogonal directions.

After the growth measurements, the Petri dishes were taken to the microwave oven (800 W for 20 seconds) to fuse the culture medium and then separate the fungal mycelium by filtration (Vargas-Isla and Ishikawa, 2008). After filtered, the mycelium was transferred to aluminum foil crucibles (of known mass) to measure their fresh mass. Finally, the crucibles were dehydrated in an oven at 60°C for 24 hours, and the temperature increased to 105°C until the mycelium mass reached a constant dry weight.

Effect of supplementation on biomass production in submerged culture

Supplemented mycelial growth was also analyzed in submerged culture of Potato Dextrose (PD). For this, strain fragments (3 × 3 mm) were set in 250 mL Erlenmeyer bottles containing 50 mL of PD + supplementation of 10 and 20% wheat bran or rice bran, previously sterilized at 121°C for 15 minutes, for 40 days. After the incubation period, mycelium was separated from the culture medium by filtration, and the fresh and dry mycelial mass was determined as described in the previous experiment.

Phenoloxidase production

The filtrates resulting from the previous experiment were used to evaluatethe supplementation by wheat bran and rice bran in the production of phenoloxidaseenzymes. Petri dishes (ø 90 mm) containing 15 mL of tannic acid agar culture medium (5%) sterile, three cup plates of 9 mm diameter were dug, filled with 0.1 mL of the filtrate. After 24 hours of incubation, the distance between the edge of each cup plate and the edge of the brownish oxidation halo was measured in orthogonal directions, with a millimeter ruler.

Data analysis

The experiments were carried out in a completely randomized design. For the experiments in solid culture medium, the experimental unit consisted of a Petri dish with five replicates per treatment; for experiments in liquid culture medium, the experimental unit was an Erlenmeyer bottle, also with five replicates per treatment. To evaluate the production of phenoloxidaseenzymes, the average of the three cup plates per Petri dish was considered, and each Petri dish was considered a sampling unit, with five

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replicates.

In the end, the measurements of growth and mycelial biomass of the strains and the size of the PO oxidation halo of the five repetitions for each species were submitted to two-way analysis of variance (ANOVA) between the five treatments (no supplementation, 10 and 20% wheat bran, 10 and 20% rice bran), followed by Tukey test if necessary. For the analyses, a significance level of 0.05 was set and software BioEstat 5.0 was used.

RESULTS

Effect of supplementation on mycelial growth

The radial mycelial growth of the strains was significantly influenced by the species of gasteroid fungi and by the supplementation (wheat bran or rice bran at 10 or 20%). Table 2 shows the final mycelial growth, where it can be observed that the strains of *Cyathus* species showed higher growth in relation to the strains of *Geastrum*. Overall, the best results of mycelial growth were observed in the treatment with 10% wheat bran supplementation. Rice bran supplementation in both concentrations conferred the lowest results in mycelial growth for all tested strains. Different results were only observed for *C. limbatus*, with higher growth in not supplemented culture medium (control); and for *C. albinus* strains, in which the control treatment did not differ from the treatments supplemented with wheat.

Table 2 - Mycelial growth (mm) of Amazonian strains of gasteroid fungi grown in Potato Dextrose Agar (PDA) medium supplemented with different concentrations of wheat bran and rice bran after 20 days of incubation at 25°C in the dark

Species	Treatments				
	PDA	PDA + Wheat		PDA + Rice	
		10%	20%	10%	20%
Cyathus limbatus	80.2±1.1^{Ba}	61.4±0.9^{Cb}	48.6±1.1^{Cc}	37.6±1.6^{Cd}	32.0±3.4^{Bd}
C. albinus	90.0±0.0^{Aa}	90.0±0.0^{Aa}	90.0±0.0^{Aa}	80.0±0.0^{Ab}	52.6±0.0^{Ac}
C. setosus	64.0±1.9^{Cb}	74.0±1.4^{Ba}	60.0±0.9^{Bb}	45.0±5.5^{Bc}	31.0±0.7^{Bd}
Geastrum schweinitzii	16.2±1.1^{Dc}	24.6±0.5^{Da}	19.0±0.7^{Db}	12.8±1.3^{Dd}	6.4±0.1^{Ce}
G. echinulatum	12.2±1.6^{Eb}	15.6±1.1^{Fa}	7.4±0.5^{Fc}	11.6±1.1^{Db}	4.3±0.2^{Dd}
G. hirsutum	10.0±4.7^{Fb}	18.5±2.5^{Ea}	10.4±5.3^{Ec}	1.3±1.0^{Ed}	2.4±2.2^{Ed}

Mean ± standard deviation of five replicates; different lettersindicate significantly different values (ANOVA, $p < 0.05$); lowercase letters refer to the comparison between treatments and uppercase ones refer to the comparison between strains.

In addition to the results presented in Figure 1, the variation in mycelial growth of fungal strains is visually shown. The strains cultivated without supplementation (control) presented irregular margins, fine and dense hyphae. The strains grown in medium supplemented with wheat bran presented denser hyphae, slightly irregular edges, larger in length and numerous branches and clamp connections. Supplementation also provided the emergence of basidiome initials in *C. albinus* strains. The strains grown in the presence of rice bran presented less dense mycelium, shorter hyphae and few branches that were closer to the surface of the culture medium

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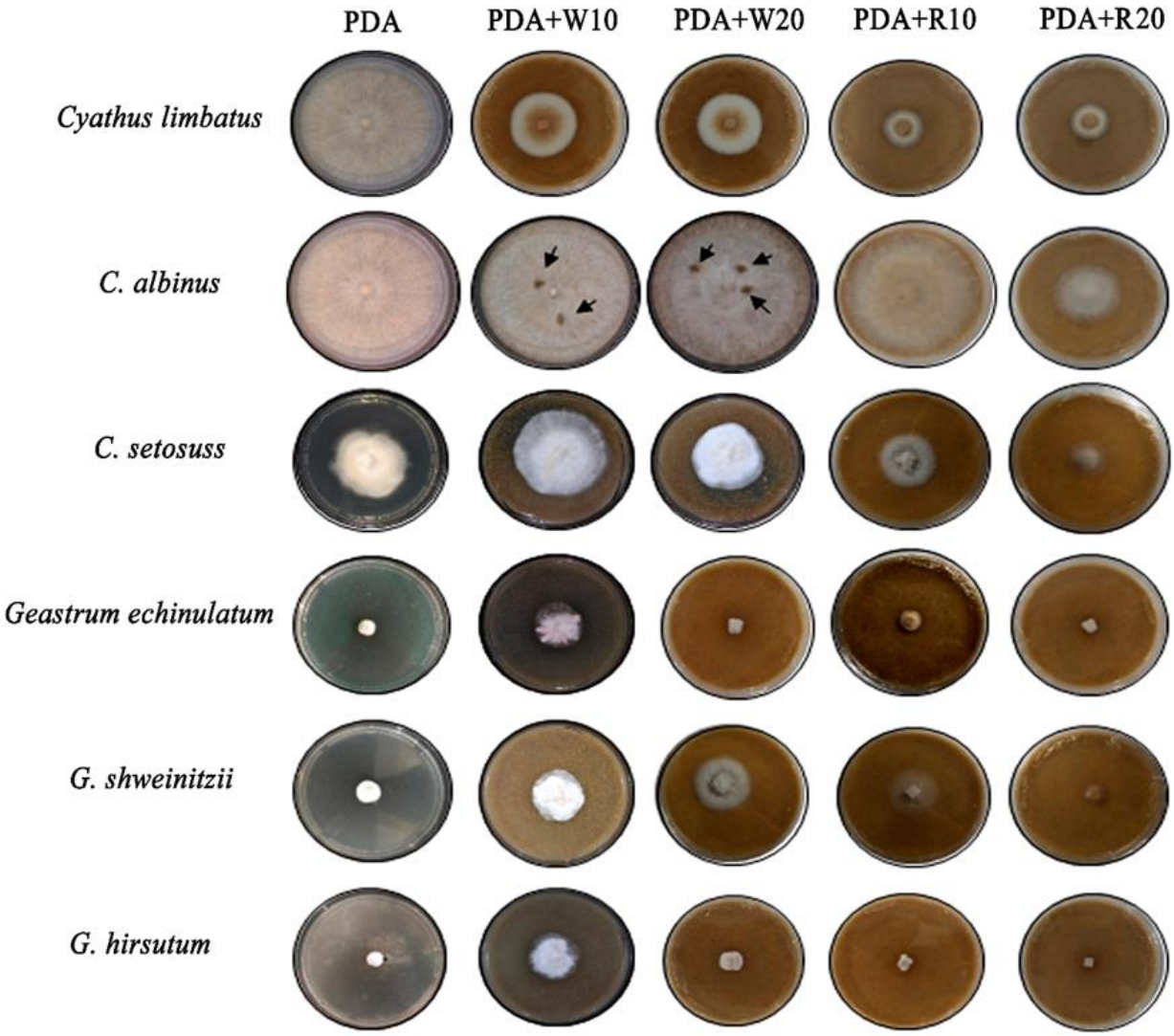

Figure 1 - Morphological aspects of Amazonian strains of gasteroid fungi grown in Potato Dextrose Agar (PDA) medium supplemented with wheat 10% (PDA + W10), wheat 20% (PDA + W20), rice 10% (PDA + R10) and rice 20% (PDA + R20) after 20 days in the dark. Arrows indicate the production of basidiome initials in culture medium

Similarly, biomass production was higher in the treatment supplemented with 10% wheat bran for both *Cyathus* and *Geastrum*; among the tested species, *Cyathus* strains were the most productive (Table3). Strains of *C. limbatus*, which had presented higher radial growth in non-supplemented medium, presented the highest biomass values in medium supplemented with 10% wheat bran, as well as the *C. albinus* strains, that reached the edge of the Petri dish in almost all treatments and showed the best results when supplemented with 10% wheat bran (Table 3). *G. echinulatum* produced the most biomass (compared to the control) among *Geastrum* strains, as well as the strains of *G. hirsutum*, which presented the lowest mycelial growth values, but obtained better biomass gains than the control (Table 3).

Effect of supplementation on biomass production in submerged culture

The biomass results in submerged culture medium reflected the same results as those observed in solid medium (Table 4). *Cyathus* strains presented the highest values of fresh and dry biomass production compared to *Geastrum* strains, especially when cultivated in the presence of 10% wheat bran, a treatment that conferred the best results in fresh and dry biomass production for both taxa. The treatment with 20% rice bran supplementation presented the lowest biomass gain (Table 4), and 10% rice bran had low values as well.

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Table 3 - Biomass produced by Amazonian strains of gasteroid fungi grown in Potato Dextrose Agar (PDA) medium supplemented with different concentrations of wheat bran and rice bran after 20 days of incubation at 25°C in the dark

Species	Treatments								
	Fresh mass (g)					Dry mass (g)			
	PD	PD + Wheat		PD + Rice		PD	PD + Wheat		PD + Rice	
		10%	20%	10%	20%		10%	20%	10%	20%
C. limbatus	5.7±0.4^{Ab}	11.3±0.6^{Aa}	4.0±1.1^{Ac}	3.0±0.4^{Bc}	1.4±0.4^{Ad}	0.2±0.1^{Bd}	0.9±0.1^{Aa}	0.4±0.2^{Bc}	0.6±0.1^{Ab}	0.4±01^{Ac}
C. albinus	4.7±0.6^{Bb}	7.3±2.4^{Ba}	2.4±0.8^{Bc}	4.0±0.8^{Ab}	1.3±0.2^{Ad}	0.3±0.1^{Ac}	0.8±0.0^{Aa}	0.6±0.1^{Ab}	0.6±0.1^{Ab}	0.3±0.1^{Bc}
C. setosus	3.9±0.3^{Cb}	6.3±0.5^{Ca}	2.7±0.7^{Bc}	1.2±0.2^{Cd}	0.7±0.3^{Bd}	0.2±0.1^{Bc}	0.8±0.1^{Ba}	0.4±0.1^{Bb}	0.2±0.1^{Bc}	0.1±0.0^{Cd}
G. schweinitzii	1.6±0.3^{Db}	2.9±0.2^{Da}	1.2±0.2^{Cc}	0.4±0.3^{Dd}	0.2±0.1^{Cd}	0.4±0.0B^{Cb}	0.7±0.1^{Ca}	0.1±0.0^{Cc}	0.01±0.0^{Cd}	0.01±0.0^{Cd}
G. echinulatum	0.8±0.1^{Eb}	2.0±0.2^{Ea}	1.0±0.3^{Cb}	0.7±0.1^{Dc}	0.3±0.1^{Cc}	0.3±0.1^{Db}	0.7±0.1^{Da}	0.1±0.0^{Dc}	0.02±0.0^{Cd}	0.01±0.0^{Cd}
G. hirsutum	0.5±0.1^{Fb}	0.8±0.1^{Fa}	0.3±0.1^{Dc}	0.2±0.1^{Ec}	0.2±0.1^{Cc}	0.02±0.0^{Ebc}	0.15±0.1^{Da}	0.05±0.0^{Db}	0.01±0.0^{Cc}	0.01±0.0^{Cc}

Mean ± standard deviation of five replicates; different letters indicate significantly different values (ANOVA, p <0.05); lowercase letters refer to the comparison between treatments and uppercase ones refer to the comparison between strains.

Table 4 - Biomass produced by Amazonian strains of gasteroid fungi grown in of Potato Dextrose (BD) submerged medium supplemented with different concentrations of wheat bran and rice bran after 40 days of incubation at 25°C in the dark

Species	Treatments								
	Fresh mass (g)					Dry mass (g)			
	PD	PD + Wheat		PD + Rice		PD	PD + Wheat		PD + Rice	
		10%	20%	10%	20%		10%	20%	10%	20%
C. limbatus	12.2±0.3^{Bb}	15.4±0.4^{Ba}	8.5±0.6^{Bc}	4.3±0.4^{Cd}	2.8±0.4^{Ce}	0.4±0.0^{Cb}	1.5±0.1^{Ba}	0.3±0.1^{Cc}	0.2±0.0^{Cc}	0.1±0.0^{Bd}
C. albinus	17.2±0.3^{Ab}	19.4±0.4^{Aa}	8.5±0.5^{Bd}	13.4±0.4^{Ac}	7.9±0.9^{Bd}	0.7±0.0^{Bb}	2.3±0.1^{Aa}	0.5±0.1^{Ac}	0.8±0.2^{Ab}	0.3±0.1^{Ad}
C. setosus	11.1±0.5^{Bb}	14.2±0.3^{Ba}	11.9±0.9^{Ab}	11.1±0.3^{Bb}	9.4±0.3^{Ac}	0.9±0.1^{Ab}	1.3±0.0^{Ca}	0.5±0.0^{Bc}	0.3±0.0^{Bd}	0.1±0.0^{Be}
G. schweinitzii	2.5±0.3^{Db}	3.5±0.3^{Ea}	1.2±0.2^{Dc}	2.1±0.^{2Db}	1.4±0.3^{Dc}	0.3±0.1^{Db}	1.1±0.0^{Da}	0.1±0.0^{Dc}	0.3±0.0^{Bb}	0.01±0.0^{Bc}
G. echinulatum	2.9±0.1^{Db}	6.6±0.4^{Da}	2.1±0.1^{Cc}	1.3±0.2^{Ed}	0.8±0.1^{Ee}	0.1±0.0^{Eb}	0.9±0.1^{Da}	0.1±0.0^{Dcb}	0.1±0.0^{Cb}	0.01±0.0^{Bb}
G. hirsutum	3.7±0.1^{Cb}	9.1±0.2^{Ca}	1.1±0.1^{Dc}	1.2±0.1^{Ec}	0.3±0.1^{Ec}	0.8±0.0^{Ab}	1.1±0.0^{Ea}	0.04±0.0^{Ed}	0.2±0.0^{Cc}	0.02±0.0^{Cd}

Mean ± standard deviation of five replicates; different letters indicate significantly different values (ANOVA, p <0.05); lowercase letters refer to the comparison between treatments and uppercase ones refer to the comparison between strains.

Phenoloxidase production

Only the strains of *Cyathus* species expressed the production of phenoloxidaseenzymes without supplementation. However, with supplementation, particularly wheat bran, there was an intensification in the production of enzymes for *Cyathus* strains. Also, the production of the enzyme by *Geastrum* strains was then visible (Figure 2; Table 5).

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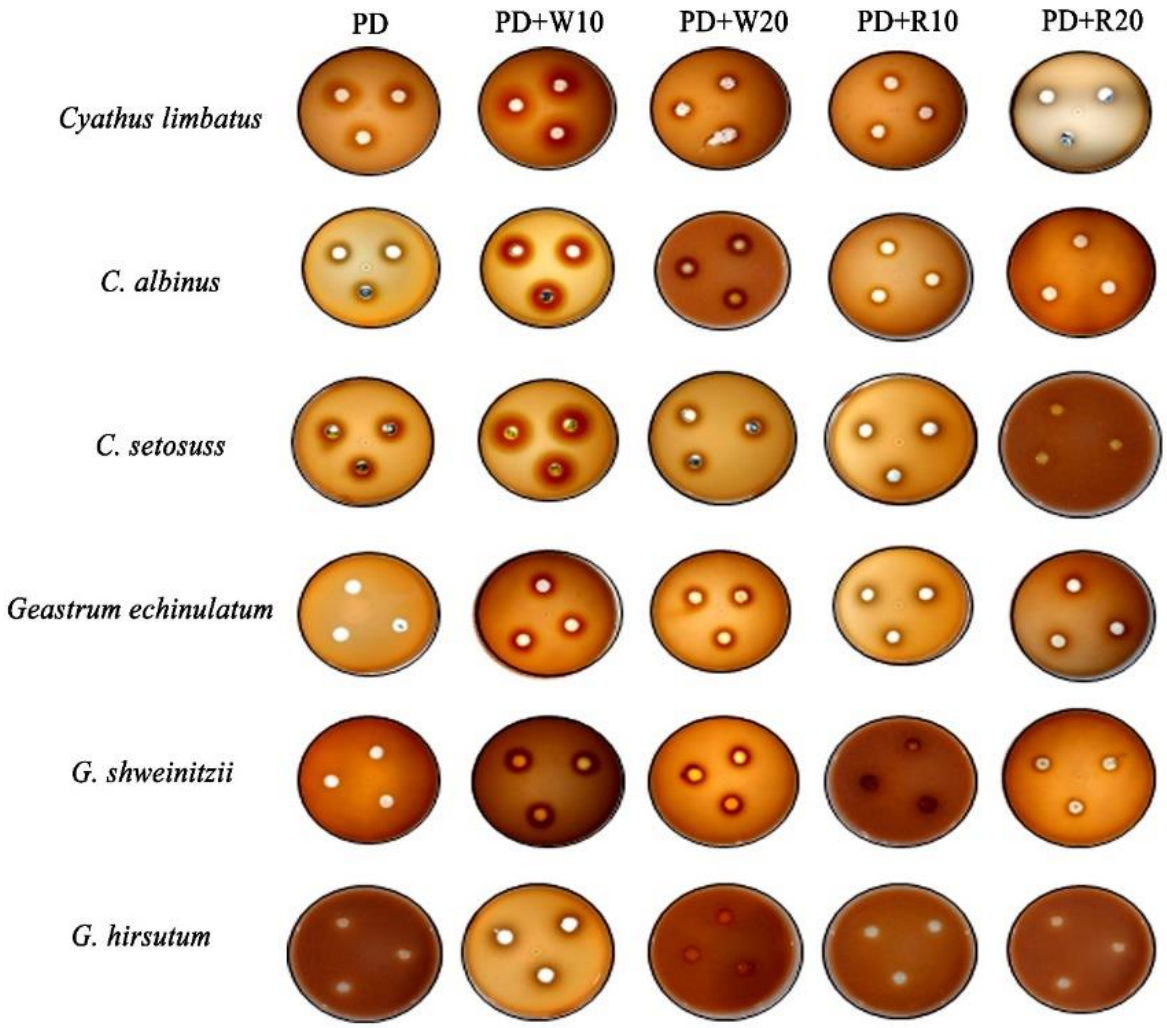

Figure 2 - Production of PO by Amazonian strains of gasteroid fungi grown in Potato Dextrose (PD) liquid medium supplemented with wheat 10% (PD + W10), wheat 20% (PD + W20), rice 10% (PD + R10) and rice 20% (PD + R20) after 20 days of cultivation in the dark

Supplementation with 10% wheat bran intensified the production of PO with larger oxidation halos for all strains of the gasteroid fungi tested, although the best result was measured for *Cyathus* species. Supplementation with rice bran contributed to the smallest halos observed (Table 5).

Table 5 - Oxidation halo (mm) indicated by the production of phenoloxidaseenzymes by Amazonian strains of gasteroid fungi grown in different concentrations of wheat and rice bran

Species	Treatments				
	PD	PD + Wheat		PD + Rice	
		10%	20%	10%	20%
Cyathus limbatus	13.8 ± 1.3^{Ab}	27.6 ± 1.1^{Aa}	11.6 ± 0.9^{Bc}	0.5 ± 0.1^{Bd}	0.2 ± 0.0^{Ae}
C. albinus	14.0 ± 1.0^{Ab}	25.8 ± 0.8^{Ba}	11.6 ± 1.1^{Bc}	0.5 ± 0.5^{Bd}	0.2 ± 0.1^{Ae}
C. setosus	13.8 ± 1.1^{Ab}	27.6 ± 1.1^{Aa}	14.0 ± 1.0^{Ab}	0.7 ± 0.4^{Ac}	0.2 ± 0.0^{Ad}
Geastrum schweinitzii	0 ± 0^{Bd}	11.6 ± 0.9^{Ca}	4.2 ± 0.8^{Cb}	0.3 ± 0.0^{Cc}	0.2 ± 0.1^{Ac}
G. echinulatum	0 ± 0^{Bd}	19.4 ± 1.1^{Ba}	3.8 ± 0.8^{Cb}	0.2 ± 0.1^{Cc}	0.2 ± 1^{Ac}
G. hirsutum	0 ± 0^{Bc}	1.3 ± 0.1^{Da}	0.3 ± 0.0^{Db}	0 ± 0^{Dc}	0 ± 0^{Bc}

Mean ± standard deviation of five replicates; different letters indicate significantly different values (ANOVA, $p < 0.05$); lowercase letters refer to the comparison between treatments and uppercase ones refer to the comparison between strains.

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DISCUSSION

The difference in development between the strains of the two genera may be attributed to the ecophysiological differences among species. These differences, coupled with substrate preferences, can influence mycelial growth and the production of biotechnologically significant enzymes (Wicklow et al., 1984). It is essential to understand that, under laboratory conditions, the growth and maintenance capacity of mycelial cultures depend on the adequate supply of nutrients provided by the culture medium (Prescott et al., 2002). Supplementation of the culture medium with lignocellulosic bran can be a valuable and advantageous technique for accelerating mycelial growth and biomass production in fungal strains (Donini et al., 2006; Minotto et al., 2008).

The *Geastrum* strains in this study were isolated from basidiomas that grew on leaf litter, where the need for enzyme production to degrade lignin is reduced (Wesenberg et al., 2003). This reduction may explain the absence of phenoloxidase enzyme production in a potato-based medium, for example. On the other hand, *Cyathus* strains were obtained from basidiomas that decompose trunks and branches, which stimulates a higher production of lignocellulosic enzymes (Sen et al., 2016). This difference in ecological niches likely contributed to the observed variation. When the culture medium was supplemented with 10% wheat bran, enzyme production was observed in *Geastrum* strains and increased in *Cyathus* strains.

For certain edible mushroom species, such as *Pleurotus ostreatus* (Jacq.) P. Kumm., wheat bran serves as a favorable substrate for mycelial development (Wang et al., 2001; Locci et al., 2008), as it did for the species in this study. Additionally, wheat-based substrates have been reported as effective inducers of enzyme activity (Mikiashvili et al., 2004; Papinutti and Lechner, 2008; Zhang et al., 2019; Cai et al., 2021). However, this enhancement comes at the expense of nutritional and cultural conditions, particularly oxygen levels and the availability of nitrogen (Silva, 2004). This relationship may help explain the differences in results observed with each type of bran used in the formulation of the culture media in this study. The low nitrogen content in rice bran may have had a similar inhibitory effect on gasteroid fungal strains, akin to what Rossi et al. (2003) observed in the cultivation of *Lentinula edodes* (Berk.) Pegler when they utilized the same type of bran.

These characteristics are likely associated with the ability to trigger the physiological mechanisms necessary for nutrient utilization from the culture medium (Mata et al., 2001). For instance, nitrogen, which is present in larger quantities in wheat bran, can be used more effectively, and its addition can also stimulate mycelial enzyme activity (Donini et al., 2006). However, it's worth noting that at high concentrations, nutrients can inhibit the development of certain fungal strains (Naraian et al., 2009). This observation might help explain the results obtained in this study, particularly in the case of the treatment with 20% wheat bran.

While the correlation between mycelial growth and phenoloxidase (PO) enzyme production hasn't been tested, the association between supplementation and enzyme production is widely documented for white rot fungi (Mikiashvili et al., 2005; Souza et al., 2006; Songulashvili et al., 2006, 2007; Osma et al., 2007), and this study may provide further corroboration. In the case of gasteroid fungi strains, the observed differences in mycelial growth, biomass production, and PO enzyme production, particularly in *Cyathus*, were also noted by Bernardi et al. (2008) in strains of *Agaricus brasiliensis* Fr. These findings can serve as motivation for advancing basic science with new research, highlighting the importance of obtaining fungal enzymes with significant biotechnological potential, especially in regions like the Amazon that have been underestimated in the scientific community.

CONCLUSION

This study deals with the first report, which presents promising results, regarding mycelial development and the production of enzymes of biotechnological interest using lignocellulosic products as a supplement to the culture medium for strains of gasteroid fungi in the Brazilian Amazon. Supplementing the potato-based culture medium with 10% wheat bran is recommended to enhance the mycelial development of the strains targeted in this study. It also serves as a significant inducer in the production of phenoloxidase enzymes, primarily by stimulating their synthesis in *Geastrum* strains and amplifying it in *Cyathus* strains. Furthermore, considering that culture media based on potatoes and wheat bran are readily available, this study broadens research horizons in regions with limited financial and human resources and little focus on basic science, particularly in the Amazon, where the diversity and biotechnological potential of fungi are still underestimated.

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