EVALUATING THE PROTEIN POTENTIAL OF FUNGAL MYCELIUM USING DIFFERENT NITROGEN SOURCES

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ABSTRACT

With the increasing concern for environmental, nutritional, and sustainability issues, there has been a growing interest in alternative protein sources. One of these promising alternatives is the use of proteins derived from fungal mycelium. The mycelium, the vegetative part of fungi, has stood out in the production of alternative proteins due to its nutritional and functional value. In this study, we evaluated the mycelial growth of the fungi *Pleurotus albidus, Ganoderma australe*, and *Hericium erinaceus* in potato dextrose agar (PDA) medium with two nitrogen sources: yeast extract (YE) and ammonium sulfate (AS). We used standard medium cultivation (PDA), without nitrogen source as a control sample (CS). The results showed significant differences in protein content between the tested media and the control. *P. albidus* reached 45.82% protein with YE and 44.12% with AS, compared to 25.91% in the control. *G. australe* had 39.91% protein with YE, 32.47% with AS, and 29.91% in the control sample. There was no statistical difference in the protein content of *H. erinaceus* among different nitrogen sources and control. Yields per plate also showed no statistical difference. These results highlight the potential of fungal mycelial proteins from *P. albidus, G. australe*, and *H. erinaceus* as a sustainable alternative to protein sources.

Keywords: Mycelium. Protein. Nitrogen

1 INTRODUCTION

In recent years, the search for alternative protein sources has gained prominence due to environmental, nutritional and sustainability concerns. One of these promising alternatives is the use of proteins derived from fungal mycelium. Mycelium, the vegetative part of fungi, has demonstrated potential as a rich and sustainable source of proteins, with interesting nutritional and functional properties, representing a viable and sustainable alternative to traditional protein sources.

Fungi are a promising source of animal protein replacement, and the strains selected for fungal protein production are generally non-pathogenic, non-toxic, and fast-growing, with low nutritional requirements, simple processing systems and good biological value [1]. According to The Good Food institute, over the years, the number of people adopting a vegan lifestyle has increased, reducing the consumption of animal products [2].

As a result, research into the production of mycelium to be used as an alternative protein became necessary. Fungi need a source of nitrogen for the synthesis of proteins, amino acids, enzymes, and other compounds essential for their growth and metabolism. Nitrogen is a fundamental element in the composition of fungal biomolecules and plays a crucial role in vital metabolic processes. Therefore, the presence of an adequate nitrogen source is essential for the healthy development and biomass production of fungi during cultivation [4].

This study aims to evaluate the production of fungal mycelium from three different strains of *Pleurotus albidus*, *Ganoderma australe* and *Hericium erinaceus*, testing yeast extract (YE) and ammonium sulfate (AS) as nitrogen sources, to increase the protein content and yield of mycelial biomass.

2 MATERIAL & METHODS

The fungi *P. albidus, G. australe,* and *H. erinaceus* were provided by the Enzyme and Biomass Laboratory at the Institute of Biotechnology, University of Caxias do Sul.

The strains were stored at a temperature of 4 °C on Petri dishes containing Potato Dextrose Agar (PDA) medium. For strain maintenance, the cultivation procedure involved placing a 5 mm diameter disc of each fungus on Petri dishes with PDA medium and incubating at 28 °C in the absence of light.

Solid-state mycelium production was conducted using PDA medium enriched with different nitrogen sources, including yeast extract (YE) containing 11.8% nitrogen and ammonium sulfate (AS) containing 21% nitrogen in its composition. PDA medium was prepared without the addition of nitrogen source, PDA+YE, and PDA+AS in a concentration of 20 g/L of supplement. The cultivation using PDA without the addition of supplement was used as a control sample (CS). After preparation, the media were sterilized in an autoclave for 15 min at 121 °C and distributed into Petri dishes. Cultivation was conducted in triplicate for the three

fungi under investigation. In each Petri dish, a 5 mm diameter mycelium disc was placed at the center, and the dishes were incubated at 28 °C.

The radial growth of the fungi was measured every 24 h until complete colonization of the plate surface (10.5 cm in diameter), utilizing the ImageJ program for measurement evaluation. The biomass resulting from the cultivation was removed by heating the plate until the medium became liquid, facilitating the separation of medium and biomass. The separated medium portion was washed three times with distilled water and lyophilized for protein analysis using the micro Kjeldahl method and yield calculation. The results were submitted to analysis of variance (ANOVA) and the means were compared using the Tukey test ($p \leq 0.05$), using the software Statistic 8.0. These analyses were used to evaluate the significant differences between the PDA+YE and PDA+AS cultivations compared to the control sample (PDA) in terms of mycelium yield and protein content, considering the different nitrogen sources tested.

3 RESULTS & DISCUSSION

The growth of fungal mycelium is a fundamental process in many areas of biology and biotechnology. In this study, we investigated the effect of different nitrogen sources on the mycelial growth of the fungi *P. albidus, G. australe and H. erinaceus* in relation to productivity and protein content. The results obtained in cultures with two different supplements, YE and AS, were compared with the CS control sample (Figure 1). The development of the fungi was transmitted to the surface of the plate, without exceeding the time limit of 15 days. After 15 days the cultivation was stopped. For *P. albidus*, after the first three days of cultivation, it was possible to observe the growth of the fungus on the plate. After 6 days, half of the plate surface with the PDA and PDA+YE media had been taken, while growth with the PDA+AS medium was slower. Cultivation for *P. albidus* on PDA medium lasted 10 days, on PDA+YE 9 days, and on PDA+AS 11 days to take up the entire plate surface. *G. australe*, after 5 days of cultivation, showed growth on the plate. After 10 days, less than half of the plate surface was filled in with the PDA, PDA+YE, and PDA+AS media. Cultivation was terminated on the 15th day without the plate surface being fully taken over by the fungus. *H. erinaceus*, after the first 8 days of cultivation, exhibited growth on the plate surface. With 10 days of cultivation, less than half of the plate surface had been taken by the fungus. Upon reaching the 15th day, cultivation was terminated without the entire plate surface being taken.

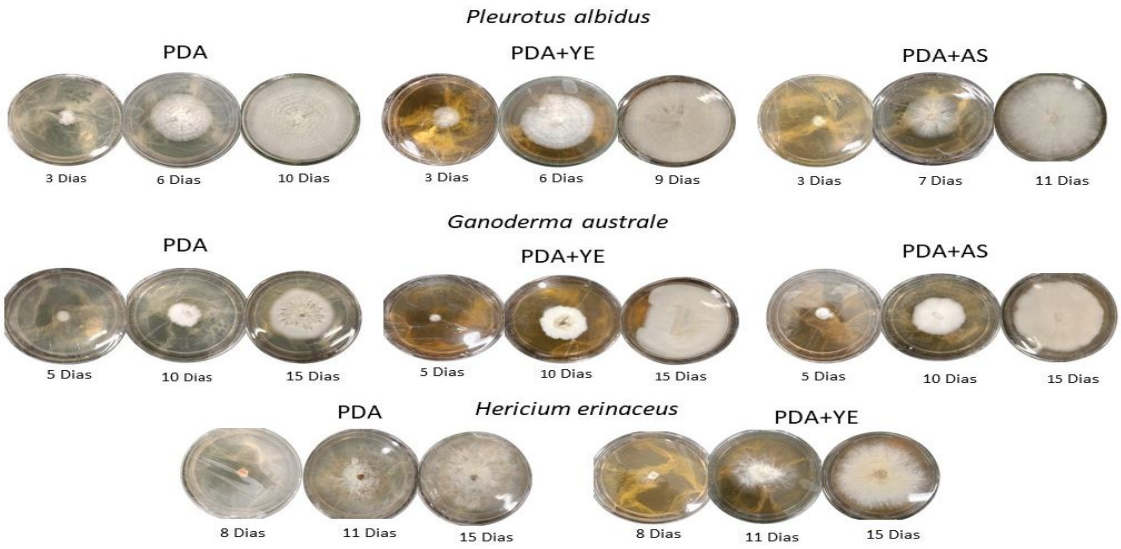

Figure 1 Radial growth of fungi during 15 days of cultivation at 28°C

The results of the statistical analysis indicate that *P. albidus*, when compared to the control sample, presented significant differences in protein content (Table 2). Cultivation with CS resulted in a protein content of 25.91%, while the medium supplemented with YE presented 45.82%, a value higher than that found in other studies [4], and AS presented 44.12% protein, indicating an increase in mycelial protein content with the addition of nitrogen source.

The growth of *G. australe* and *H. erinaceus* was slower compared to the cultivation time of *P. albidus*. Therefore, a cultivation time of 15 days was stipulated, even if the plate surface was not filled. CS from *G. australe* obtained 29.21% protein, while samples with YE obtained 39.91% and those with AS 32.42% protein, showing a significant difference in relation to the control sample. It was observed that *H. erinaceus* requires longer cultivation time to fill the entire surface of the plate. CS from H. erinaceus obtained

31.82% protein, while samples with YE supplement obtained 30.65% protein, showing no significant difference in relation to CS. The medium with AS at a concentration of 20 g/L apparently inhibited the growth of the mycelium.

Regarding biomass yield on a dry basis, no significant differences were observed when comparing the biomass concentrations of the CS sample between cultivation media with different nitrogen sources (YE and AS) and type of fungus. For *P. albidus*, yields were 0.084 g/plate for CS, 0.076 g/plate for YE, and 0.089 g/plate for AS. In the case of *H. erinaceus*, the yields were 0.076 g/plate for CS and 0.124 g/plate for YE. Among the cultivated strains, *G. australe* showed significantly higher productivity when cultivated with the nitrogen source YE (0.495 g/plate), compared to CS (0.208 g/plate) and AS (0.164 g/plate).

Table 2 Results of cultivation time, yield and protein content for each strain grown without nitrogen source (CS) and enriched with nitrogen source YE and AS.

Growth Medium	Pleurotus albidus			Ganoderma australe			Hericium erinaceus		
	*Time (days)	Yield (g/plate)	Protein (%)	*Time (days)	Yield (g/plate)	Protein (%)	*Time (days)	Yield (g/plate)	Protein (%)
PDA (CS)	10	0.08^{a}	25.9 ± 2.1^{a}	15	0.20^{a}	29.9 ± 2.5^{a}	15	0.07^{a}	31.8 ± 2^{a}
PDA + YE	9	0.07^{a}	45.8 ± 2.3^{b}	15	0.49^{a}	39.9 ± 0.7^{b}	15	0.12^{a}	30.6 ± 0.1^{a}
PDA + AS	12	0.08^{a}	44.1 ± 1.6^{bc}	15	0.16^{a}	32.4 ± 1.7^{c}	15	-	

*The time corresponds to the duration of growth. Times less than 15 correspond to the sampling time because the growth has reached the maximum diameter of the plate.; PDA - potato dextrose agar medium; YE - yeast extract; AS - ammonium sulfate

4 CONCLUSION

In conclusion, the results highlight the potential of proteins derived from fungal mycelium as a viable and sustainable alternative to traditional protein sources. When evaluating the growth of *P. albidus, G. australe* and *H. erinaceus* mycelium under different nitrogen sources, significant differences in the performance of each fungus were observed. Both *P. albidus* and *G. australe* showed high protein content when grown in medium enriched with YE when compared to CS. However, the yield of P. albidus is lower than that of *G. australe*, although the cultivation time of *G. australe* is longer than that of *P. albidus*. Therefore, further studies on cultivation parameters are needed to reduce the cultivation time of *G. australe* and increase the productivity of *P. albidus*. On the other hand, *H. erinaceus* showed lower productivity and protein content than *P. albidus* and *G. australe*, even when cultivated in a medium enriched with a nitrogen source.

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