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SHORT COMMUNICATIONS

The downstream process choice interferes with the recovery of ß-glucans of mushrooms cultured in submerged fermentation

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Highlights

- Mushrooms are valuable sources of ß-glucans
- Submerged fermentations can provide considerable amounts of mycelial biomass
- Different mushroom species demand appropriate protocols for ß-glucan obtaining

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KEYWORDS
ß-glucans;
Trametes versicolor;
Schizophyllum commune;
Ethanol extraction;
Alkali extraction.

Abstract: Mushroom ß-glucans (ßG) have several health-benefiting properties, but the production and extraction of these polysaccharides need to be improved. This study aimed to identify species of edible/medicinal basidiomycetes that produce greater biomass in submerged fermentation and determine the most effective extraction method for those with the best yield. *Ganoderma lucidum*, *Schizophyllum commune*, *Trametes versicolor*, and *Pleurotus eryngii* were cultivated in nutrient broth for 120 h. The extraction of ßG was done by boiling in ethanol and sodium hydroxide. Only *S. commune* and *T. versicolor* presented polysaccharide levels, showing greater biomass. Extraction with NaOH was more effective for *T. versicolor*, while ethanol was better for *S. commune*. The study concluded that the extraction of ßG must be customized for each strain, and the yield of the conventional enzymatic extraction method was comparable to that obtained.

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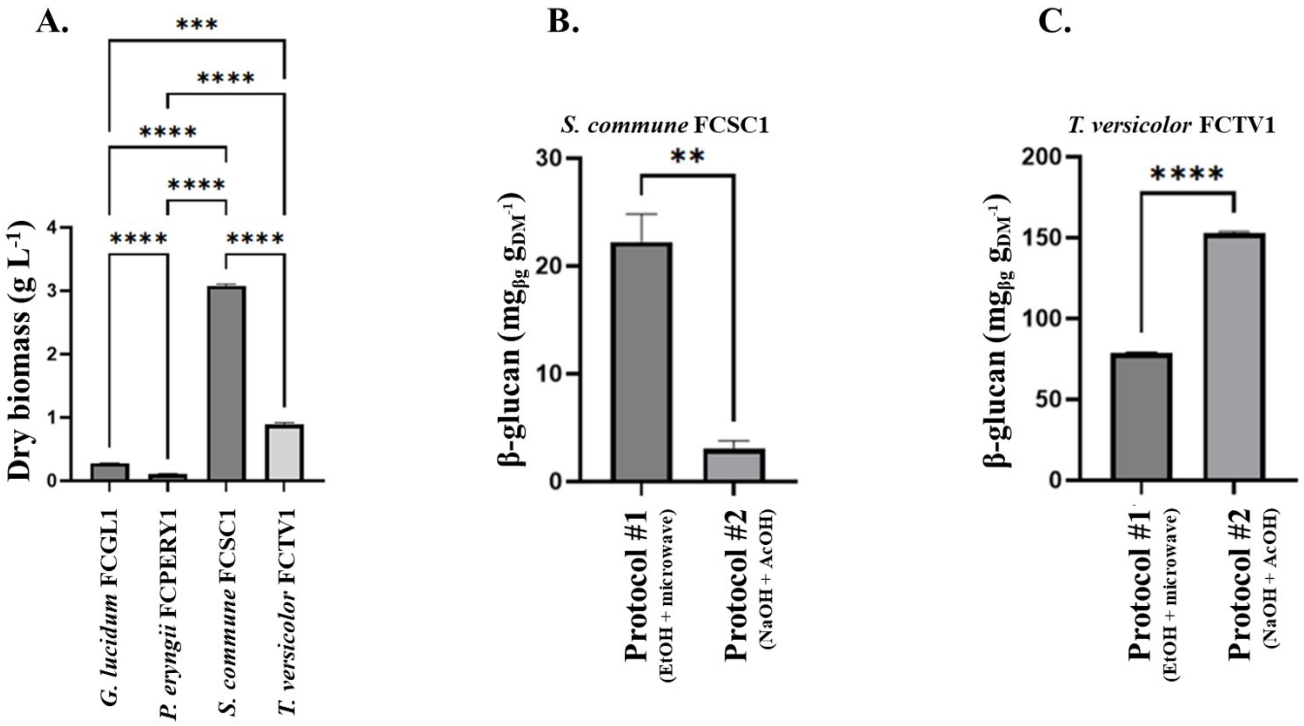
Graphical Abstract



Introduction

Despite their relatively easy upstream steps, the best extraction methodologies (downstream) for ßG from basidiomycetes still need to be determined to be used as fine chemicals for immunomodulatory, antitumor, and cholesterol-lowering medicines (Rop et al., 2009). As different producers extract these polysaccharides following different procedures, and no concordance seems to exist, we evaluated two popular protocols for extracting them from fungal biomasses obtained by submerged fermentation (SmF) and determined which one allows the highest yield.

Materials and methods

Ganoderma lucidum FCGL1, *Schizophyllum commune* FCSC1, *Trametes versicolor* FCTV1, and *Pleurotus eryngii* FCPERY1 were cultivated in 250 mL Erlenmeyer flasks (five repetitions each) with 100 mL of 2% Sabouraud dextrose broth (SDB). Each flask received one 5 mm Ø disc of mycelium and was kept at 28 °C, 110 rpm for 120 h. The biomasses were collected by filtration on quantitative filter paper under negative pressure (wet masses) and were dried at 60 °C until constant masses (dry masses; DM). The DM were powdered with mortars and pistils.

The first extraction protocol (Frioui et al., 2018) was carried out using a Brastemp® Crisp 38 microwave oven at 900 W, with a frequency of 2450 MHz. The biomasses' powders were boiled in 80% ethanol for 3 h under a reflux regimen. The water-soluble fractions were extracted by treatment in a microwave oven for 30 min. The aqueous extracts obtained were separated from the residues by filtration in quantitative Whatman #3 filters. They were concentrated and precipitated by adding five volumes of cold 96% ethanol, followed by incubation at 4 °C. The precipitates were filtered and dried at 70 °C under airflow and pulverised. The masses obtained were evaluated gravimetrically.

The second protocol (Mahmoud Amer et al., 2021) was followed by combining DM with 1 M NaOH in a 1:5 ratio and heating for 2 h at 80 °C under a reflux regimen. The biomasses were twice washed by centrifugation with distilled water ($_dH_2O$). Extractions were carried out with acetic acid in a 1:5 ratio. The precipitates were collected and dried at 60 °C. The masses obtained were evaluated gravimetrically.

The masses of both protocols were dissolved in $_dH_2O$ at 10% (m v^{-1}), and the ßG concentrations were evaluated as their total carbohydrate content using the phenol-sulfuric acid method with absorbance read at 490 nm. D(+)glucose was used as a standard for quantification.

Statistical analysis was done through descriptive data analysis to obtain mean and standard deviation values. Normality was analyzed using the Shapiro-Wilk test. Data comparison was performed using the One-Way ANOVA test with Tukey and T-test; the results were considered significant when $p \leq 0.05$ with parametric data. Analyses were performed using GraphPad® Prism 10.2.3 software.

Results and discussion

The biomass yield was *S. commune* FCSC1 (3.08 ± 0.02 g L^{-1}) > *T. versicolor* FCTV1 (0.88 ± 0.29 g L^{-1}) > *G. lucidum* FCGL1 (0.28 ± 0.00 g L^{-1}) > *P. eryngii* FCPERY1 (0.11 ± 0.00 g L^{-1}) as shown in Figure 1A. The discrepancies observed in biomass obtaining may be attributed to species- and strain-dependent characteristics. Indeed, it has been demonstrated that different strains of the same species present markable variations in mycelial growth when cultured in liquid media (Krupodorova et al., 2024).

Although *S. commune* and *T. versicolor* presented the highest biomass values, these can still be considered low compared to the literature, as there are reports of *S. commune* yields exceeding 10 g L^{-1}. The lower biomass yields observed for both fungi in this study may be related to the cultivation medium used, which may be the most indicated. Pilafidis et al. (2024) reported *T. versicolor* yields ranging from 0.9 to 2.6 g L^{-1}, with the highest yield achieved using wine distillery effluent, and *S. commune* yields ranging from 1.73 to 10.7 g L^{-1}, with the highest yield obtained using brewer's spent grain extract. In the present study, *S. commune* also demonstrated higher yields than *T. versicolor*. The authors further suggest that the biomass levels of these fungi could be increased by utilizing a medium derived from agro-industrial byproducts. Furthermore, the study highlights that the cultivation medium may influence ßG production, potentially increasing the percentage of polymers relative to fungal biomass, thereby making the process more efficient.

Due to their low biomass yields, the evaluation with *G. lucidum* FCGL1 and *P. eryngii* FCPERY1 was discontinued. Upstream SmF conditions for these species need to be better established; however, this was not the focus of this study.

As *S. commune* and *T. versicolor* showed higher yields, these two strains were used in subsequent experiments. Figure 1B and C shows the yield of ßG, expressed in milligrams of polysaccharide per gram of DM for the two basidiomycetes.

It is perceptible that there are differences in the amounts of ßG between species and that the yield varies according to the extraction technique adopted. *Trametes versicolor* FCTV1, although not the largest biomass producer, provided more significant amounts of the polysaccharide in both treatments. Also, extraction with NaOH provided more significant obtaining (152.80 ± 0.92 $mg_{ßG}\ g_{DM}^{-1}$) than with ethanol (78.37 ± 0.89 $mg_{ßG}\ g_{DM}^{-1}$). On the other hand, for *S. commune* FCSC1, the treatment with ethanol (22.24 ± 1.48 $mg_{ßG}\ g_{BM}^{-1}$) was more effective than with NaOH (3.00 ± 0.46 $mg_{ßG}\ g_{DM}^{-1}$). Therefore, it was demonstrated that tailored extraction approaches for different species/strains are needed.

We believe that the differences in the yields of the extraction methods are related to the specific structural and biochemical characteristics of each cell wall and how they interact with the chemical agents used. Chioru & Chirsanova (2023) state that mushroom strains have ß-(1→3) and ß-(1→6) linkages. However, because they are distinct strains, they may present differences in the locations of these linkages, in the degree of branching, and in the overall composition of the ßG. These variations directly affect the solubility of the polysaccharide, which explains the need for different methods for each strain. Based on the authors' findings, the higher yield of the alkaline

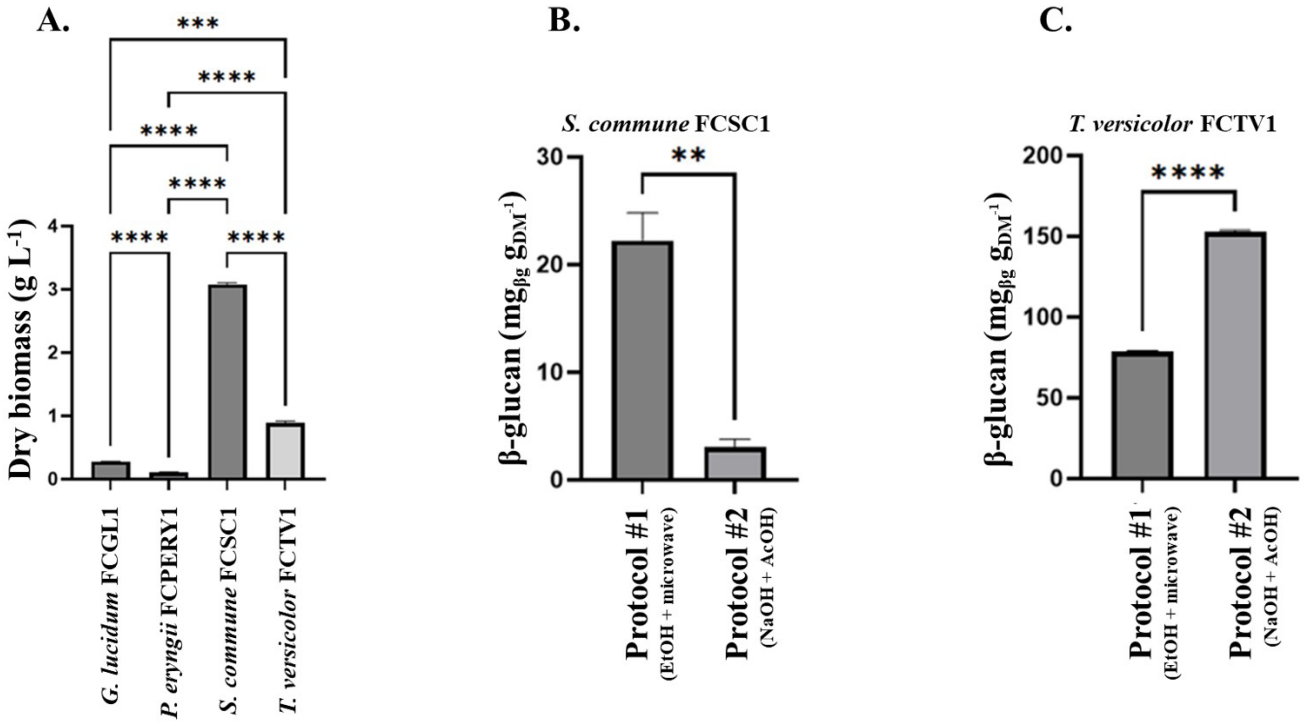


Figure 1. Biomass values of *G. lucidum* FCG1, *S. commune* FSC1, *T. versicolor* FCTV1, and *P. eryngii* FCPERY1 cultivated by SmF (A). Yield of obtaining ßG according to extraction method for *S. commune* FCSC1 (B) and *T. versicolor* FCTV1 (C). Legend: The number of asterisks refers to statistical significance, being ** indicates $p<0.05$, *** indicates $p<0.01$, and **** indicates $p<0.001$. EtOH = ethanol, NaOH = sodium hydroxide, AcOH = acetic acid.

extraction (NaOH) for *T. versicolor* can be explained by the greater complexity of the ß-glucan linkages, which are possibly associated with other molecules through covalent bonds, making the polymer less accessible to the ethanol-based method. In contrast, in *S. commune*, the ßG likely have lesser complex linkages and/or are located in an outer layer of the cell wall, which allows their extraction using ethanol alone. Other studies have evaluated the production of ßG in basidiomycetes; however, they focused on quantifications made on basidiocarps, the edible/medicinal parts of mushrooms (Sari et al., 2017; Pérez-Bassart et al., 2023). Even using mycelia, our results for *T. versicolor* FCTV1 were higher than the 82 $mg_{ßG}\ g_{DM}^{-1}$ obtained by Frioui et al. (2018) and that from 56.3 to 82 $mg_{ßG}\ g_{DM}^{-1}$ obtained by Pérez-Bassart et al. (2023), that used basidiocarps. Our results suggest that the ethanol-microwave and the NaOH-based methods can also efficiently extract this polymer. Manzi & Pizzoferrato (2000) extracted ßG from *Pleurotus* species using a treatment with 50% ($v\ v^{-1}$) ethanol. This was followed by hydrolysis with lichenase and degradation with ß-glucosidase. The authors reported ßG concentrations ranging from 2.2 to 5.3 $g_{ßG}\ g_{DM}^{-1}$, inferior to our results.

Based on the results, new possibilities arise for studies to scale up SmF to extract ßG from the mycelia of *S. commune* and *T. versicolor*. As an initial step, it is essential to increase the biomass production of these fungi to enable more significant quantities of the polymer to be obtained. Dudekula et al. (2020) suggest that to achieve this higher production, it is crucial to optimise cultivation parameters, identifying the best conditions for temperature, pH, aeration rate, incubation time, nutrient sources (mainly carbon and nitrogen), and agitation rate. On the other hand, to improve ßG yields, Venkatachalam et al. (2021) indicate that genetic engineering through the overexpression of target genes could be a promising alternative. This approach enables the production of more efficient polymers for specific applications (such as wound-healing or anti-inflammatory properties) and contributes to the economic viability of the process. The authors further emphasize that genetic engineering of the target strains, combined with cultivation optimization and reactor design, are critical steps toward obtaining high-quality polymers in a more economically feasible manner.

Finally, we would like to highlight that an experiment focused on analyzing the purity of ßG could enhance the results and aid in selecting the most appropriate extraction method. Furthermore, a detailed FT-IR, HPLC-MS/MS, or NMR analysis could confirm the structural differences between the extracted ßG and provide more precise insights into the biochemical phenomena occurring during the extraction process.

Conclusion

Results like ours demonstrate the feasibility of using mycelial structures cultivated in submerged fermentation to obtain ßG, considering that different extraction protocols for other species/strains must be evaluated.

Conflict of interests

The authors declare no conflict of interests.

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