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Development the Exopolysaccharides Production from *Ganoderma sichuanense* by Submerged Fermentation

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The genus *Ganoderma* is widely recognized for its therapeutic properties, mainly attributed to bioactive polysaccharides. This study investigated the production and characterization of exopolysaccharides (EPS) from *Ganoderma sichuanense* (CC22) cultivated by submerged fermentation, evaluating the effects of different carbon sources (glucose and sucrose) at concentrations of 20, 30, and 40 g/L. Glucose at 30 g/L resulted in the highest mycelial growth (8.27 ± 0.01 g/L), while sucrose at 20 g/L showed the lowest performance (2.32 ± 0.01 g/L). The highest EPS yield (4.53 ± 0.01 g/L) was obtained by the freeze-thaw method in a medium with glucose at 40 g/L. Structural analyses of the exopolysaccharide (SEM, FTIR, XRD, UV-Vis, and NMR) confirmed crystalline-amorphous characteristics and the presence of â-glycosidic linkages. The results demonstrate that the choice of carbon source and precipitation method directly influences the quantity and structure of EPS, reinforcing its potential for pharmaceutical and food applications.

Keywords: Basidiomycetes, Bioactive Compounds, Biopolymers, Polysaccharide Production, White-rot Fungi.

The *Ganoderma* genus has been widely used in traditional Chinese medicine for over 2000 years.[1,2] It is considered by many to be the most extensively studied and applied medicinal mushroom genus, renowned for promoting longevity and valued at approximately 2.5 billion

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USD in the global Market.[3] Among its species, *Ganoderma sichuanense* has gained increasing attention for its health-promoting properties and has been cultivated in China for over 100 years.[4,5]

The beneficial effects of macromycetes (*Ganoderma lucidum* and distinct species) are associated with the presence of bioactive molecules found in their mycelia and spores, such as triterpenoids, lactones, polysaccharides, alkaloids, proteins, fatty acids, amino acids, enzymes, lectins, and steroids.[6-9] Among these compounds, polysaccharides are considered some of the main biomolecules responsible for the biological activities of these fungi. They exhibit various health-promoting effects, including anti-inflammatory,[10] antioxidant,[11] immunomodulatory,[12] hypoglycemic,[13] antitumor,[14] gut-microbiota regulation,[15] and hypolipidemic activities,[16] among others.

The carbon source is one of the main factors influencing fungal growth, cellular metabolism, and the biosynthesis of metabolites, including extracellular polysaccharides. Among the different substrates available, glucose stands out for being widely used by fungi due to its easy assimilation and its central role in primary metabolic pathways, acting as a precursor of metabolic intermediates such as glucose-6-phosphate and UDP-glucose, which are fundamental for the synthesis of structural and extracellular polysaccharides.[17]

Recent studies have shown that glucose availability in the culture medium can increase mycelial biomass production and exopolysaccharide yield in *Ganoderma* species, as well as modulate the expression of enzymes involved in the biosynthetic pathway of activated sugars, which are crucial for the formation of these bioactive macromolecules.[18,19] Therefore, the selection and concentration of glucose as a carbon source represent determining variables in the optimization of submerged cultivation and in the production of compounds of biotechnological interest.

Current research on *Ganoderma* polysaccharides, particularly those from *G. lucidum*, has focused primarily on optimizing extraction methods, improving yields, analyzing structural characteristics, and investigating physiological activities. Therefore, this study aims to evaluate the submerged cultivation of *G. sichuanense*, investigating the influence of carbon sources (glucose and sucrose) at different concentrations (20 g/L, 30 g/L, and 40 g/L) to assess their effects on mycelial biomass production and exopolysaccharide synthesis.

In this context, the novelty of the present study lies in the evaluation of *Ganoderma sichuanense* under submerged cultivation conditions with controlled variations in carbon source type and concentration, focusing simultaneously on mycelial biomass formation and exopolysaccharide production. While most studies have centered on *G. lucidum* and extraction optimization, investigations addressing culture medium composition as a strategy to enhance metabolite biosynthesis in *G. sichuanense* remain limited. Thus, this work contributes to expanding the biotechnological knowledge of this species and provides experimental insights that may support the development of more efficient cultivation strategies for the production of fungal polysaccharides with potential industrial and pharmaceutical applications.

MATERIALS AND METHODS

Microorganism and maintenance

A commercial strain of *Ganoderma sichuanense* CC22 was provided by the company Funghi Flora and maintained in the Edible Fungus Cultivation Laboratory, belonging to the Coordination of Technology and Innovation, of the National Institute of Amazonian Research (COTEI-INPA), Manaus, Amazonas, Brazil. The fungus was reactivated in a Petri dish containing PDA (Potato Dextrose Agar) medium and maintained at 25°C in a BOD incubator until complete colonization and subsequently stored at 4°C until use.

This research was conducted in accordance with Brazilian regulations for access to genetic heritage and was registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) under registration number A7C9A6A.

Liquid medium composition for fermentations and cultivation conditions

The cultivation of *G. sichuanense* was carried out using Potato-Oligopeptone-Liquid (POL) medium, formulated with, composed of 5.0 g/L $(NH_4)_2SO_4$, 0.2 g/L $MgSO_4.7H_2O$, 1.0 g/L

K_2HPO_4, 2.0 g/L yeast extract, 1.0 g/L soy peptone, and 1.0 g/L $CaCO_3$, with the pH adjusted to 6.0.[18] The inoculum consisted of 7 mycelial discs (Ø = 7 mm) grown on plates containing PDA medium, which were inoculated into 250 mL Erlenmeyer flasks containing 100 mL of liquid medium and incubated in a shaker (Thermo SCIENTIFIC) at 120 rpm, at 28°C for 10 days in the absence of light. The cultures were carried out using two carbon and energy sources, glucose and sucrose, at concentrations of 20, 30, and 40 g/L for each source.[20]

After the fermentation period, the cell concentration was determined by gravimetry. The fermented broths were centrifuged at 5000 rpm (Thermo SCIENTIFIC, SORVALL LYNX 4000) for 20 minutes at 4°C, and the mycelial biomass was filtered through Whatman No. 1 filter paper. The biomass retained on the filter paper was dried in an oven at 60°C for 24 h.

Determination of pH of fermented broth

The fermented broths, in triplicate, were subjected to pH determination after the fermentations were complete, using a pH meter (PHtek – model PHS – 3B).

Separation and precipitation of EPS

Following separation of the mycelial biomass, aliquots of the post-culture fermentation broth were subjected to different exopolysaccharide (EPS) fractionation methods to compare recovery yields. For EPS precipitation, each broth aliquot was mixed with 96° GL ethanol at a 1:4 (v/v) ratio and incubated at 7°C for 24 h, allowing complete polysaccharide precipitation.

Freeze–thaw fractionation was another technique applied for the extraction of EPS from the fermented broth. The material was thawed at room temperature (± 22°C). The recovered EPS were dialyzed against distilled water under refrigeration (7°C) for 5 days. The samples were lyophilized using a bench-top freeze dryer (Liotop K108).

Analysis by scanning electron microscopy (SEM)

To obtain EPS micrographs, lyophilized samples were adhered to metal stubs on carbon strips and subjected to gold metallization (approximately 15 nm) followed by analysis using a Tescan scanning electron microscope (VEA3), in secondary electron mode, at magnifications of 50, 100, and 200 times.

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

The FTIR spectrum was obtained using a Frontier spectrophotometer (Agilent Technologies) in the 4000 to 400 cm{ ¹ range, with a resolution of 8 cm{ ¹, using 128 scans. A Total Attenuated Reflectance (TAR) device with a germanium crystal accessory was used.

X-ray diffraction (XRD)

The X-ray diffractogram of the lyophilized EPS was obtained on the XRD-7000 diffractometer (Shimadzu) using a copper radiation source (CuKá), 30 mA current, 40 kV voltage, scanning range of 2è = 10° to 60°, step of 0.0001, and 5 s/step.

Color analysis and ultraviolet-visible (UV-Vis) spectral analysis

The color analysis of the EPS was performed using a colorimeter (Delta Vista 450G from Delta Color) with the CIELAB color scale (L*, a*, and b*). L* is defined as lightness (ranging from 0, black, to 100, white), a*, which assumes positive values for reddish colors and negative values for greenish colors, and b*, which assumes positive values for yellowish colors and negative values for bluish colors. Color-related parameters, such as chroma and hue angle 'è', were calculated.

Ultraviolet-visible (UV-Vis) spectral analysis was performed to analyze the UV-Vis absorption of the EPS using a Thermo Scientific spectrophotometer (Evolution 220) between wavelengths of 190 and 500 nm.

Monosaccharide composition

The neutral monosaccharide composition of the fraction was determined by gas chromatography (GC) in the form of alditol acetate derivatives, according to the methodology described by Wang et al.[16] Briefly, 100 ìL of sample (1 mg/mL) were mixed with 100 ìL of 5 M trifluoroacetic acid (TFA) and kept for 2 h and 30 min at 100°C in an oven, followed by reduction and acetylation of the hydrolysis products. GC analyses were performed on a gas chromatography platform (TRACE ULTRA, Thermo Fisher Scientific Inc, Waltham, Massachusetts, USA), using a DB-225 capillary column (30 mm x 0.25 mm i.d.), heated to 100°C during injection and then programmed

at 60°C/min up to 230°C (constant). The relative percentages of monosaccharides were calculated by comparison with the retention times of the standard alditol acetates rhamnose (Rha), arabinose (Ara), xylose (Xyl), galactose (Gal), mannose (Man), and glucose (Glc).

Nuclear magnetic resonance (NMR) analysis

The samples (30 mg) were dissolved in 500 µL of D_2O and subjected to an ultrasonic bath at 50°C for 30 min. The solution was then filtered and transferred to an NMR tube containing 50 µL of D_2O with trimethylsilylpropanoic acid (TMSP). The analysis was performed on a Bruker AVANCE III HD NMR spectrometer operating at 11.75 T, equipped with a probe (BBFO Plus SmartProbe™) at 25°C. For structural confirmation, two-dimensional Heteronuclear Single Quantum Coherence (HSQC) and Heteronuclear Multiple Bond Correlation (HMBC) experiments were conducted, and the spectra were processed using TopSpin™ 3.5 software.

Statistical Analysis

The values for mycelial biomass, pH, and yields of precipitated exopolysaccharides were calculated in triplicate and presented as means, with significance $p < 0.05$ after analysis of variance (ANOVA).

RESULTS

pH of the fermented broth after the fermentation process

The fermentation process was completed after 10 days of cultivation, and the pH of the media was analyzed to evaluate the variations that occurred throughout the fermentation. Initially, the initial growth of the fungus occurred in a medium with a pH of 6.0, and at the end of the cultivation, a reduction in this value was observed, with a final average of 5.35 ± 0.01, indicating the production of acidic metabolites during the process due to the consumption of sugars (Figure 1).

Influence of carbon source at different concentrations on mycelial biomass production

Mycelial growth varied significantly depending on the carbon source and concentration used in the fermentation medium. The highest mycelial biomass value was recorded using glucose as a carbon source at 30 g/L, reaching 8.27 ± 0.01 g/L, showing that this concentration was the most favorable for fungal development. Conversely, the lowest biomass production was observed under the condition with sucrose at 20 g/L, resulting in only 2.32 ± 0.01 g/L (Figure 2).

Yield of exopolysaccharides

The freeze-thaw method applied to the culture broth containing 40 g/L glucose resulted in the highest recovery of exopolysaccharides (EPS),

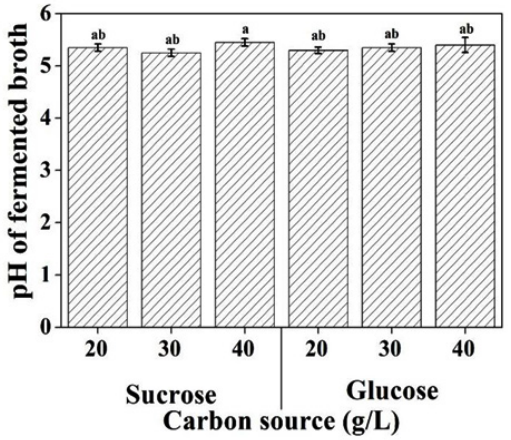


Fig. 1. pH of the culture broths after the end of the fermentation process using sucrose and glucose as carbon sources at different concentrations (20, 30, and 40 g/L). Values represent the mean ± standard deviation (n = 3). Different letters above the bars indicate statistically significant differences ($p < 0.05$).

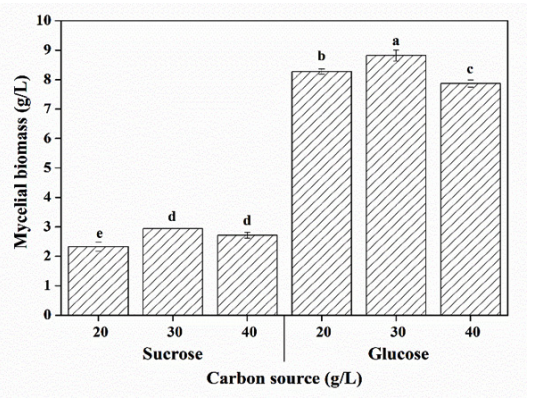


Fig. 2. Types of sugars (sucrose and glucose) in different concentrations (20, 30, and 40 g/L) used as a carbon source for the production of mycelial biomass. Values represent the mean ± standard deviation (n = 3). Different letters above the bars indicate statistically significant differences ($p < 0.05$).

reaching 4.53 ± 0.01 g/L (Figure 3a). In contrast, the exopolysaccharide precipitation technique with 95% ethanol (EPS) showed a maximum yield of 2.72 ± 0.01 g/L (Figure 3b). These results demonstrated that precipitation conditions significantly affect the yield of exopolysaccharides, and it is possible that there may be structural differences within a single polysaccharide or between different polysaccharides.

Characterization of exopolysaccharides

SEM do EPS

In SEM analysis, the EPS structures exhibited a crystalline structure, with irregularly shaped blocks and a large, smooth surface area (Figure 4). Xu et al.[37] extracted polysaccharides from *G. lucidum* with microstructures similar to those demonstrated in this work, with a square shape and flat surface.

Fourier Transform Infrared Spectroscopy (FT-IR) of EPS

The FT-IR spectrum for EPS is shown in Figure 5. The strong signals at 3234 cm{ ¹ corresponded to the single-bond OH stretching vibration of carbohydrates.[41] The next five bands in the 1404–1209 cm{ ¹ region were close to each other, and most of them were of low intensity. The signal at 1404 cm{ ¹ showed asymmetric and symmetric stretching vibrations of the carboxylate.[42] The band around 1329 cm{ ¹ corresponded to the bending of the CH single bond into CH*f* , while the bands at 1209 cm{ ¹ belong to the attenuated lipid.[42] A band at 849 cm^{-1} was characteristic of â-type glycosidic linkages.[43,44]

X-ray diffraction is a technique capable of analyzing the crystallinity of materials, being of great importance for verifying the structure of

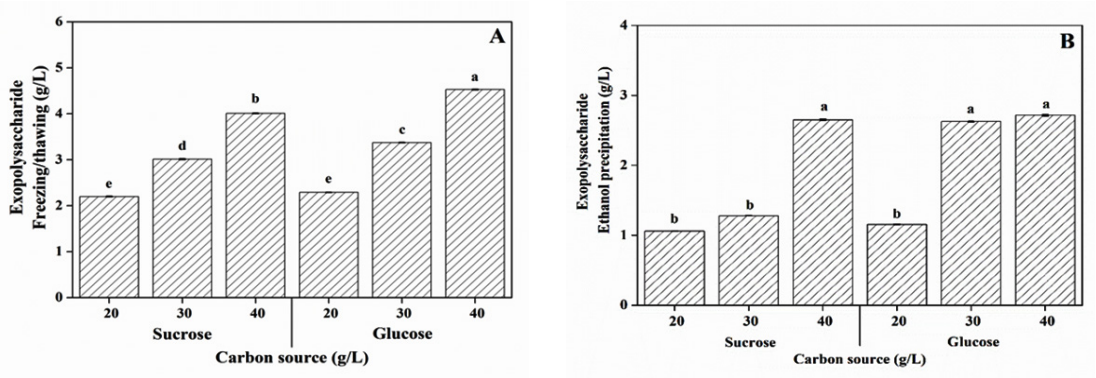


Fig. 3. Precipitation of exopolysaccharides from the fermented broth after the end of the fermentation process using two types of sugars (sucrose and glucose) as a carbon source in different concentrations (20, 30, and 40 g/L): (A) Freeze-thaw cycle, (B) Precipitation with ethanol. Values represent the mean ± standard deviation (n = 3). Different letters above the bars indicate statistically significant differences ($p < 0.05$).

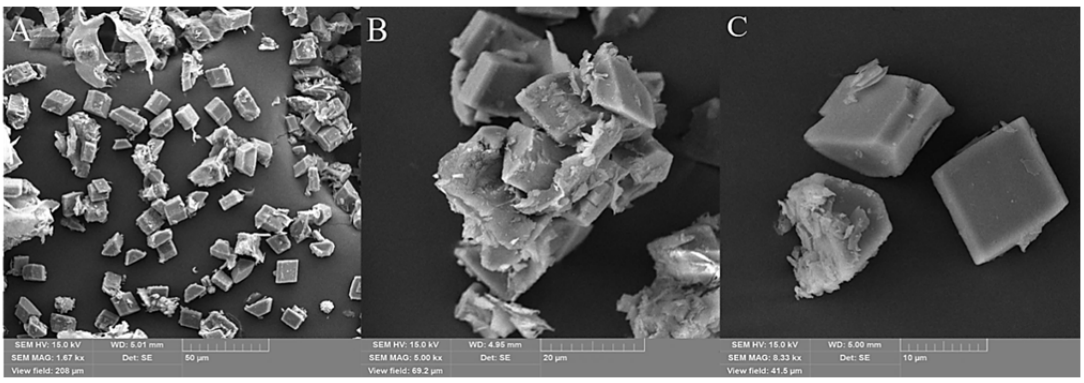


Fig. 4. SEM images of EPS, at amplitudes of 50x (A), 100x (B), and 200x (C).

polysaccharides.[45] Broad, rounded peaks typically indicate the presence of amorphous structures, while sharp diffraction peaks suggest the existence of crystalline structures.[46,47] The overall diffraction peaks of EPS exhibited a broad profile with the presence of some sharp peaks (Figure 6).

Color and UV analysis of EPS

The values for angle L*, a*, b*, chroma, and hue of EPS are shown in Table 1. The L* parameter is an approximate measure of lightness, in which each color can be considered equivalent to a member of the grayscale, between black and white.[50]

The maximum value for luminosity is 100, indicating white, and L* for EPS was 80.08, which is close to luminosity. The a* parameter indicates positive values for red colors and negative values for greenish colors, while b* indicates positive values for yellow colors and negative values for bluish colors. The positive a* (0.53) and b* (10.21) values of EPS indicate reddish and yellowish colors. Chroma (C*) is considered one of the quantitative attributes of color, used to determine the degree of difference of a hue compared to a gray color with the same luminosity. The higher the chroma value, the greater the intensity of the color of the samples perceived by humans.[51]

The chroma values of EPS are closer to the b* values, and the hue angle was found to be 86.13. The hue angle (°) is considered the qualitative attribute of color, in which colors have traditionally been defined as red, green, etc., used to define the difference of a given color with reference to gray with the same luminosity.[52] A low chroma value and a high luminosity value are desired for the product to meet consumer preferences. Thus, in this color study, EPS can be applied to food and/or pharmaceutical products as hydrocolloids due to its higher whiteness (80.08) and low chroma values (10.23). As shown in Figure 7, EPS did not absorb in the ultraviolet spectrum at 260 and 280 nm, suggesting the absence of nucleic acids and proteins.[37,41]

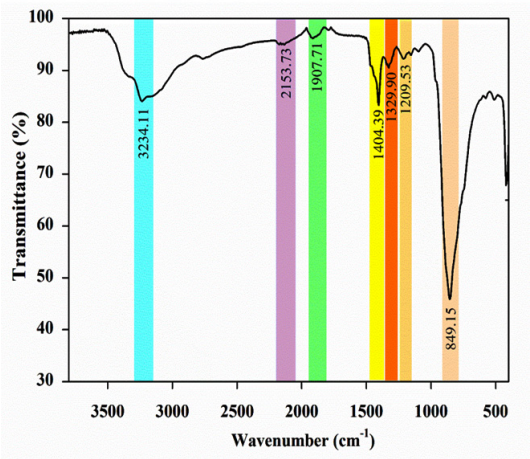


Fig. 5. FT-IR spectra of EPS X-ray diffraction analysis of EPS

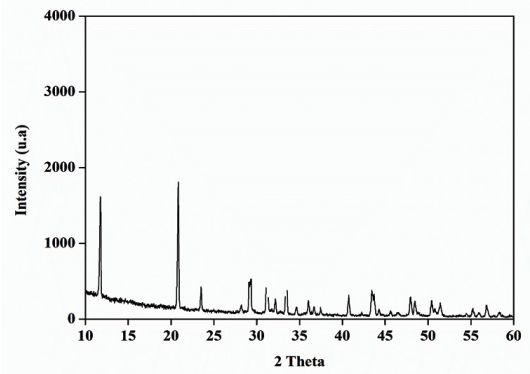


Fig. 6. X-ray diffractogram of EPS

Table 1. CIELAB color parameters (L*, a*, and b*) of EPS

Color analysis	Value
L* = Lightness	80.08 ± 0.59
a* = redness	0.53 ± 0.15
b* = yellowness	10.21 ± 0.58
Chroma (C*)	10.23 ± 0.55
Hue angle (°)	86.13 ± 2.17

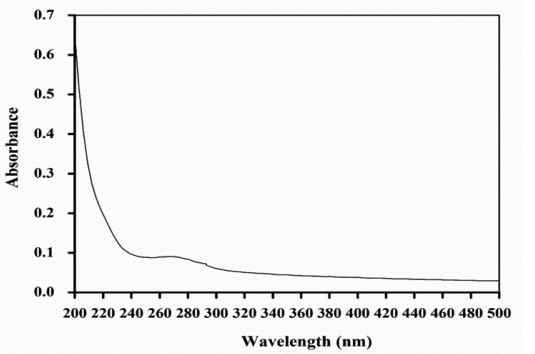


Fig. 7. Ultraviolet spectra of EPS

Table 2. Neutral composition monosaccharide of polysaccharide fractions obtained from the exopolysaccharide from the submerged culture of *G. sichuanense*

Component	Neutral composition monosaccharide (mol%)[a]					
	Rha	Ara	Xyl	Man	Gal	Glc
EPS	2.0	2.0	3.0	52.0	17.0	24.0

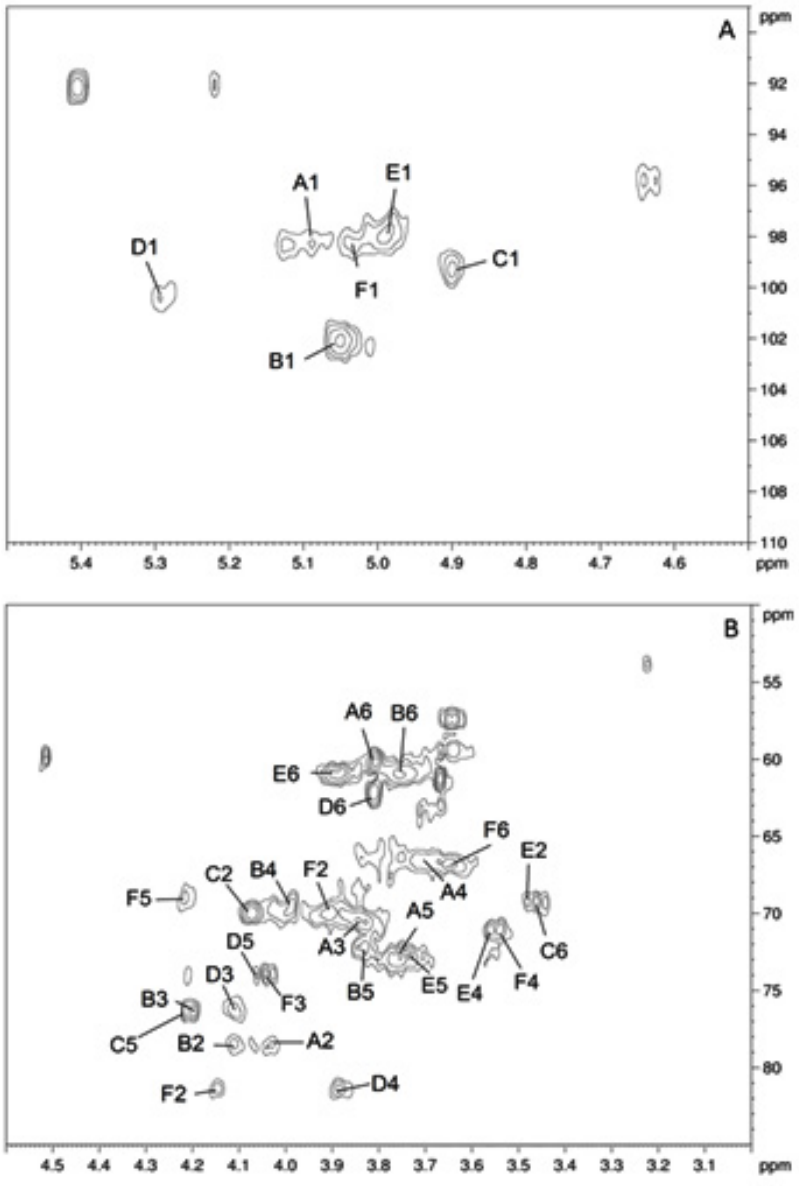


Fig. 8. HSQC of EPS from *Ganoderma sichuanense.*

Table 3. ^{1}H and ^{13}C chemical shifts for EPS from submerged culture of *G. sichuanense*

	Sugar units	Chemical shifts (ppm)					
		C1/H1	C2/H2	C3/H3	C4/H4	C5/H5	C6/H6
A	→2,6)-α-D-Man*p*-(1→	98.35.12	78.44.03	70.43.84	66.53.65	72.63.76	60.23.80
B	→6)-α-D-Man*p*-(1→	102.05.05	78.34.11	76.24.19	69.83.98	72.13.84	60.73.75
C	→4)-α-D-Man*p*-(1→	99.34.90	69.84.02	-	-	76.24.21	69.13.44, 3.46
D	→4)-α-D-Glc*p*-(1→	100.25.28	-	76.24.11	81.23.88	74.04.06	62.13.80
E	→6)-α-D-Glc*p*-(1→	97.74.98	68.93.48	-	70.93.56	72.53.72	60.73.87
F	→6)-α-D-Gal*p*-(1→	98.15.09	69.83.90	73.84.03	70.83.54	69.24.21	66.63.63, 3.65

Analysis of monosaccharide composition

The fraction was identified as a heteropolysaccharide consisting mainly of Mannose (Man) (52%), Glucose (Glc) (24%), and Galactose (Gal) (17%) (Table 2). Many polysaccharides obtained from *Ganoderma* sp. are composed of similar monosaccharides.[46,53] Other monosaccharides were identified as traces (Arabinose (Ara), Xylose (Xyl), and Rhamnose (Rha).[54]

NMR analysis

Comparing the results of monosaccharide analysis and NMR spectroscopy analysis, it can be inferred that the structure of the exopolysaccharide fraction obtained from the culture medium of *Ganoderma sichuanense* is mainly composed of mannose, galactose, and glucose units. The literature reports that polysaccharides composed of mannose and glucose, such as glucomannan, are common in fungi.[57] HSQC analysis was performed, and some signals could be inferred for the units (Figure 8), which are listed in Table 3.

In the anomeric region, six signals could be identified. They were designated as residues from A to F, with the most prominent being at ä 99.3/5.12, 102.0/5.05, 99.3/4.90, 100.2/5.28, 97.7/4.98, and ä 98.1/5.09 ppm. These signals are suggested for ’!2,6)-á-D-Manp-(1’!; ’!6)-á-D-Manp-(1’!; ’!4)-á-D-Manp-(1’!; ’!4)-á-D-Manp-(1’!; ’!4)-á-D-Glcp-(1’!; ’!6)-á-D-Glcp-(1’! and ’!6)-á-D-Galp-(1’!). The units have the á configuration, which is in accordance with that reported by Cao et al.[46]

DISCUSSION

The pH of the medium is a vital intrinsic factor, as it affects the ionic state of the fermentative microenvironment, as well as the structure, morphology, and physiological functions of fungal cells, and can modulate nutrient absorption and product biosynthesis.[19] Studies indicate that pH stability varies according to the culture medium used in the submerged fermentation of *G. sichuanense* CC22. Among the media evaluated, POL showed greater stability throughout the fermentation process, with a minimal reduction in pH at the end of the cultivation (7th day) (data not shown). In contrast, the Modified Melin–Norkrans (MNM) medium underwent a more pronounced acidification, highlighting the influence of the medium composition on pH regulation during fermentation.[21]

The pH values observed in the *Ganoderma* fermented broth at the end of cultivation remained within a slightly acidic range, close to 5.3–5.5, regardless of the carbon source and concentration tested. Such pH maintenance indicates a robust homeostatic capacity of *G. sichuanense* CC22, effectively managing the acidic by-products of carbohydrate metabolism, even with the production of acidic metabolites resulting from carbohydrate consumption. In submerged fermentations of basidiomycetes, moderate pH reductions are common and are associated with the synthesis of organic acids, cellular respiration, and the secretion of extracellular polysaccharides, which contribute to medium acidification during mycelial growth.[22]

It is also observed that both glucose and sucrose resulted in statistically similar final pH values, indicating that both carbon sources were efficiently metabolized by the microorganism. This behavior has been reported in recent studies with *Ganoderma*, in which the assimilation of different sugars did not cause pronounced changes in the final pH, but influenced metabolic rate as well as

biomass and EPS production. Maintaining the pH at moderately acidic levels is considered favorable for the activity of enzymes involved in polysaccharide biosynthesis and for mycelial growth, thereby contributing to fermentation yield.[23]

Thus, the results indicate that *Ganoderma* shows physiological robustness in response to variations in the carbon source of the medium, maintaining suitable conditions for secondary metabolism and exopolysaccharide production. This pH stability is an important factor in biotechnological processes, as it reduces the need for pH adjustments during fermentation and promotes cultivation standardization, which is essential for industrial and pharmaceutical applications involving fungal polysaccharides.[24]

Pessoa et al.[25] evaluated the production of mycelial biomass, proteases, and intracellular protease inhibitors by *G. lucidum* CC22 cultivated under different submerged fermentation conditions, and observed that cultivation under agitated conditions and using glucose as a carbon source (20 g/L) showed the highest biomass production with a value of 7 g/L. Based on these studies, the literature describes that the carbon source metabolized by the fungus is directly related to the monomeric composition of the polysaccharide produced, although not always in the same proportion.[26]

The different extraction methods used in the precipitation of polysaccharides can impact both the yield and purity of the compounds obtained, being an essential step for the characterization and future applications of these biomolecules.[27] The precipitation of exopolysaccharides with ethanol resulted in lower yields than those obtained by the freeze-thaw method. One hypothesis for this difference is the removal of impurities and small lipophilic particles associated with the polysaccharides during the process.[28,29] Additionally, Figure 3b shows the effect of precipitation by ethanol, since it is based on the reduction of the dielectric constant of the medium, forcing the precipitation of EPS due to the reduction of its solubility.

Submerged cultivation using high glucose concentrations results in elevated concentrations of exopolysaccharides (EPS) excreted by fungi.[30-32] Studies demonstrate that the use of glucose is advantageous in EPS yield when compared to other sugars.[18,32] In addition to the carbon source, the presence of Ca^{+2} ions in the culture medium favors the excretion of polysaccharides into the fermented medium, resulting in a significant increase in the production of exopolysaccharides (EPS) by various basidiomycetes.[33,34]

The influence of the initial pH value in the culture medium is another determining factor that can influence EPS production by *G. sichuanense*. According to Kim et al.,[35] pH 6.0 favored optimal EPS production. García-Cruz et al.[36] described pH 4.5 as optimal for EPS production by *Lentinula edodes*. These differences in pH favoring EPS may be linked to the morphology and physiology of *Ganoderma sp*. strains.

Due to its superior yield, the EPS fraction obtained by the freeze-thaw method with 40% glucose was prioritized for further structural characterization.

In SEM analysis, the structure of polysaccharides exhibits great diversity, making it difficult for the same conformation to occur among different compounds. However, structural alterations resulting from treatments, such as precipitation methods, can influence the morphology of polysaccharides.[38-40] Previous studies have shown that ultrasound can alter the apparent structure of *G. lucidum* polysaccharides, significantly increasing their surface area.[37]

The material possibly exhibits both crystalline and amorphous structures, with the amorphous state representing a relatively larger proportion.[48] This pattern reflects the presence of a variety of bioactive components, such as triterpenoids and polysaccharides, which contribute to its unique biochemical composition. This combination indicates that *G. sichuanense* has a more complex and heterogeneous structure with both crystalline and amorphous phases.[46] Due to their large and irregular molecular arrangements, polysaccharides typically form amorphous or semicrystalline regions, findings that align with those of Gao et al.[49] regarding the structural characteristics of glycoproteins in shiitake mushrooms.

The influence of the culture medium composition on structural characteristics can be a factor that expresses the variation in the percentages of monosaccharides that form EPS, as well as the cultivation conditions. Nitrogen sources can affect the ability of *Ganoderma* sp. to produce

EPS under fermentation conditions, as well as combinations of carbon and nitrogen sources that vary the molecular mass of the EPS produced.[18] In the submerged fermentation of *Ganoderma* sp. under various cultivation conditions with glucose as the sole carbon source, the main monosaccharide components in EPS are consistently glucose, galactose, and mannose.[55]

In EPS overexpression experiments in *Ganoderma* species conducted by Zheng et al.[56] and Ai-lati et al.,[57] several fractions were isolated and analyzed according to their monosaccharide composition, which were constructed from glucose, mannose, and galactose, thus corroborating what was found in this study.

According to NMR analyses, a signal is observed at ä 57.4/3.63, which may be associated with the presence of O-Me substitution in galactose units. Thus, the unit described for '!6)-á-D-Galp-(1'!) could be identified as '!6)-3-O-Me-)-á-D-Galp-(1'!).[58]

All signals were assigned by comparing them with literature values for similar polysaccharides.[43,59-62] Unlike cell wall polysaccharides, the exopolysaccharides found in fungi cultivated in liquid medium have a mannose and glucose backbone with galactose substitutions in proportions quite close to those described for the fraction under study, also implying quite similar ^{13}C-^{1}H HSQC signals.

CONCLUSION

Our findings demonstrate that both the choice of carbon source and the EPS recovery strategy are critical determinants of exopolysaccharide yield, structural characteristics, and fungal biomass production by *G. sichuanense*. Glucose proved to be a more effective carbon source than sucrose, promoting greater fungal growth and EPS production, while increasing glucose concentrations in the culture medium were positively correlated with enhanced EPS yields. Furthermore, the freeze–thaw recovery method significantly improved EPS recovery and may have contributed to preserving or enhancing the structural integrity of these macromolecules. Overall, these results demonstrate the potential of *G. sichuanense* as a promising producer of exopolysaccharides with physicochemical characteristics that support their prospective application in pharmaceutical and biotechnological fields.

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Conflict of interest

The authors do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials.

Permission to reproduce material from other sources

Not Applicable.

Author Contributions

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