Acta Societatis Botanicorum Poloniae

SHORT COMMUNICATION Acta Soc Bot Pol 83(3):239–241 DOI: 10.5586/asbp.2014.020
Received: 2014-02-28 Accepted: 2014-07-11 Published electronically: 2014-07-16

Analysis and glycosyl composition of the exopolysaccharide isolated from submerged fermentation of *Ganoderma lucidum* CG144

Rosália Rubel[1], Leandro Freire dos Santos[1]*, Herta Stutz Dalla Santa[1], Luciana Porto de Souza Vandenberghe[1], Adenise Lorenci Woiciechowski[1], Júlio Cesar de Carvalho[1], Sascha Habu[1], Francisco Menino Destéfanis Vítola[1], Christopher Augur[2], Carlos Ricardo Soccol[1]

[1] Bioprocess Engineering and Biotechnology Division, Federal University of Paraná (UFPR), Jardim das Américas, CEP 81531-990, Curitiba, Paraná (PR), Brazil

[2] Institute of Research for Development, Unite Biotrans – IMEP, Boite 441, Paul Cézanne University, Av. Escadrille Normandie-Niemen, F-13391 Marseille Cedex 20, France

Abstract

The evaluation of glycosyl composition is an essential step to guide future research designs applied in bioactivity. In the same way, the unexplored potential bioactivity of exopolysaccharide from *Ganoderma lucidum* is huge. Therefore, this study investigated the glycosyl composition of the exopolysaccharide isolated from submerged fermentation of *G. lucidum* to serve as guide for future studies on bioactivity. Glycosyl content and composition were evaluated by combined GC/MS of the TMS derivatives of the monosaccharide methyl glycosides produced from the sample by acidic methanolysis. Glycosyl composition analysis showed that the dominant carbohydrate component in all samples of exopolysaccharide isolated from submerged fermentation of *G. lucidum* CG 144 was glucose (58.1%), mannose (26.6%) and galactose (12.5%) which can be referred to as heteroglycan. These results suggest that this *Ganoderma* exopolysaccharide may be a new immunomodulatory agent.

Keywords: glycosyl composition; exopolysaccharide; *Ganoderma lucidum*; submerged fermentation; heteroglycan

Introduction

Ganoderma lucidum (Curtis) P. Karst is a famous fungus (mushroom) in Chinese herbal medicine. Recently, polysaccharides from *G. lucidum* have been identified as active ingredients responsible for its biological activities. For instance, polysaccharides from *G. lucidum* fruiting bodies have been shown to have antimicrobial property [1]. In the same way, *G. lucidum* mycelium have been demonstrated to possess (1,3;1,6)-beta-D-glucans which are recognized for their many health claims [2]. Finally, after the biological evaluation of intracellular polysaccharides (polysaccharides from fruiting bodies and mycelium), studies also have reported biological activities such as antioxidant properties of the exopolysaccharides (extracellular polysaccharides) from *G. lucidum* cultured in submerged fermentation [3]. In addition, there are only a few reports available on biological activities of the exopolysaccharides from *G. lucidum* and new biological activities cannot be carried out randomly.

Similarly, the glycosyl composition is a key factor to understand the underlying biological activities and possible metabolic mechanisms of fungal polysaccharides. For example, studies have been reported the immunostimulatory and antitumor activities of glucans and mannans [4]. Galactose may prevent cognitive deficits associated with glucose hypometabolism in Alzheimer's disease [5]. Recent studies have also focused on the effect of glucans on redox balance [6]. Acetylglucosamine may modulate function of the skin fibroblasts and it also has anti-inflammatory and anti-senescent activity in mesothelial cells [7]. Thus, the glycosyl composition is an essential step to guide future research designs applied in bioactivity. Moreover, a great lack of knowledge exists concerning the glycosyl composition of the exopolysaccharides from *G. lucidum*.

Considering the glycosyl composition as an essential step to guide future researches applied in bioactivity and the unexplored bioactivity of exopolysaccharide from *Ganoderma lucidum*, here we investigated the glycosyl composition of the exopolysaccharide isolated from submerged fermentation of *Ganoderma lucidum* to serve as guide for future studies on bioactivity.

Material and methods

Mushroom strain

The strain of *G. lucidum* CG 144 (botanical family Polyporaceae) was maintained by the standard stock of

* Corresponding author. Email: leandrofreire@onda.com.br

Handling Editor: Łukasz Łuczaj

Bioprocess Engineering and Biotechnology Division at Federal University of Paraná (UFPR), Curitiba – PR, Brazil. It was maintained on potato-dextrose agar (PDA) medium, incubated at 30°C for 10 days and subsequently stored at 4°C. Sub-cultures were made every 2 months to maintain the strain active.

Inoculums preparation

For preparation of inoculums, the *G. lucidum* CG 144 was transferred from agar PDA slants into 125 ml Erlenmeyer flasks containing 20 ml of potate dextrose broth and incubated in a shaker at 30°C, agitated at 120 rpm for 7 days.

Flask culture

The culture medium was composed (in grams per liter) of: glucose 35, peptone 5, yeast extract 2.5, KH_2PO_4 0.88 and $MgSO_4 \cdot 7H_2O$ 0.5; the initial pH of the medium was adjusted to 5.5. Erlenmeyer flasks (250 ml) containing 120 ml medium were inoculated at 30°C in a rotatory shaker, at 120 rpm, for 10 days. A 4% (v/v) of inoculum was added to the flask culture. Cells were removed from the culture medium by centrifugation at 12 000 g for 15 min at 4°C [8].

Isolation and purification of exopolysaccharide

Exopolysaccharide was obtained by diluting the fermented culture medium with 3 volumes of cold 95% ethanol and leaving for 2 h at 4°C for precipitation to be complete, followed by centrifuging (7500 *g* for 10 min at 5°C) and dialysis through a membrane with 10 kDa cutoff. The exopolysaccharide was isolated as a lyophilized material [9].

Glycosyl content and composition

Glycosyl composition analysis was performed by combined gas chromatography/mass spectrometry (GC/MS) of the per-O-trimethylsilyl (TMS) derivatives of the monosaccharide methyl glycosides produced from the sample by acidic methanolysis. An aliquot (10 μg) was taken from the sample and added to separate screw-cap tubes with 40 μg and 60 μg of inositol as the internal standard. The samples were lyophilized. Methyl glycosides were then prepared from the dry sample (0.3 mg) following the mild acid treatment by methanolysis in 1 M HCl in methanol (1 M) at 80°C (16 h), followed by re-N-acetylation with pyridine and acetic anhydride in methanol. The sample was then per-O-trimethylsilylated by treatment with Tri-Sil (Pierce) at 80°C (0.5 h). GC/MS analysis of the TMS methyl glycosides was performed on an AT 6890N GC interfaced to a 5975B MSD (mass selective detector), using a Supelco EC-1 fused silica capillary column (30 m × 0.25 mm ID) [10–12].

Results and discussion

The main objective of this study was to evaluate the glycosyl composition of the exopolysaccharide from *G. lucidum* CG144. Certainly, the glycosyl composition is an essential step to guide future researches applied in bioactivity and must be studied in promising bioactive molecules such as exopolysaccharides, especially from *G. lucidum*.

Glycosyl content and composition were evaluated by combined GC/MS of the TMS derivatives of the monosaccharide methyl glycosides produced from the sample by acidic methanolysis. This technique has been used extensively to evaluate the glycosyl composition of polysaccharides. Detection limits of a few nanograms can be achieved with this methodology [10–12].

Glycosyl composition analysis showed that the dominant carbohydrate component in all samples of exopolysaccharide isolated from submerged fermentation of *G. lucidum* CG 144 was glucose (58.1%), mannose (26.6%) and galactose (12.5%). Traces of fucose (<3%) were detected (Tab. 1). For this reason, this exopolysaccharide may be referred to as heteroglycan.

Tab. 1 Glycosyl content and composition of exopolysaccharide isolated from submerged fermentation of *Ganoderma lucidum* CG 144.

Glycosyl residue	Mass	Mole
Arabinose (Ara)	n.d.	...
Rhamnose (Rha)	n.d.	...
Fucose (Fuc)	0.5 mg	2.8%
Xylose (Xyl)	n.d.	...
Glucuronic acid (GlcA)	n.d.	...
Galacturonic acid (GalA)	n.d.	...
Mannose (Man)	5.2 mg	26.6%
Galactose (Gal)	2.4 mg	12.5%
Glucose (Glc)	11.3 mg	58.1%
N-Acetyl Galactosamine (GalNAc)	n.d.	...
N-Acetyl Glucosamine (GlcNAc)	n.d.	...
N-Acetyl Mannosamine (ManNAc)	n.d.	...
Σ=	19.4 mg	
Total carbohydrate by weight	**3.9%**	

n.d. – none determinated.

Interestingly, the carbohydrate composition of the exopolysaccharide resembles the carbohydrate compositions in other fungal systems (other heteroglycans), which are known about their immunostimulatory activity and antioxidant properties [13,14]. Thus, research focused on exopolysaccharide from *G. lucidum* and all aspects of the immune system is certainly promising and should guide future studies. Exopolysaccharide lyophilization yielded 0.45 g l^{-1} of submerged fermentation. Cell dry weight reached a maximum value of 7.22 g l^{-1}.

Conclusion

In summary, these experiments have shown that the exopolysaccharide from *G. lucidum* CG 144 was composed of glucose (58.1%), mannose (26.6%) and galactose (12.5%), which can be referred to as heteroglycan. Further studies should be made in order to evaluate their immunostimulatory activity.

Acknowledgments

The authors would like to thank the National Research Council (CNPq), and the Paraná's Institute Technology (TECPAR), Brazil, for providing the financial and technical support. We thank Dr. Arailde Fontes Urben (Brazilian Agricultural Research Corporation – EMBRAPA) who kindly provided the *G. lucidum* CG 144 strain.

Authors' contribution

The following declarations about authors' contributions to the research have been made: conceived and designed the experiments: RR, LPSV, ALW, JCC, CA, CRS; performed the experiments: RR, HSDS, SH, FMDV; performed the analysis: RR; wrote the paper: RR, LFS; revised the paper: RR, LFS, CRS.

References

1. Skalicka-Woźniak K, Szypowski J, Łoś R, Siwulski M, Sobieralski K, Głowniak K, et al. Evaluation of polysaccharides content in fruit bodies and their antimicrobial activity of four *Ganoderma lucidum* (W Curt.: Fr.) P. Karst. strains cultivated on different wood type substrates. Acta Soc Bot Pol. 2012;81(1):17–21. http://dx.doi.org/10.5586/asbp.2012.001
2. Wang CH, Hsieh SC, Wang HJ, Chen ML, Lin BF, Chiang BH, et al. Concentration variation and molecular characteristics of soluble (1,3;1,6)-β-d-glucans in submerged cultivation products of *Ganoderma lucidum* mycelium. J Agric Food Chem. 2014;62(3):634–641. http://dx.doi.org/10.1021/jf404533b
3. Yuan B, Zhang W, Yu Z, Zhang R. In vitro evaluation of antioxidant property of the exopolysaccharides peptides from *Ganoderma lucidum* CAU5501 in submerged culture. J Food Agric Env. 2012;10(1):97–101.
4. Batbayar S, Lee DH, Kim HW. Immunomodulation of fungal β-glucan in host defense signaling by dectin-1. Biomol Ther Seoul. 2012;20(5):433–445. http://dx.doi.org/10.4062/biomolther.2012.20.5.433
5. Salkovic-Petrisic M, Osmanovic-Barilar J, Knezovic A, Hoyer S, Mosetter K, Reutter W. Long-term oral galactose treatment prevents cognitive deficits in male Wistar rats treated intracerebroventricularly with streptozotocin. Neuropharmacology. 2014;77:68–80. http://dx.doi.org/10.1016/j.neuropharm.2013.09.002
6. Mihailović M, Arambašić J, Uskoković A, Dinić S, Grdović N, Marković J, et al. B-glucan administration to diabetic rats reestablishes redox balance and stimulates cellular pro-survival mechanisms. J Funct Foods. 2013;5(1):267–278. http://dx.doi.org/10.1016/j.jff.2012.10.016
7. Połubinska A, Cwalinski J, Baum E, Bręborowicz A. N-acetylglucosamine modulates function of the skin fibroblasts. Int J Cosmet Sci. 2013;35(5):472–476. http://dx.doi.org/10.1111/ics.12067
8. Xu P, Ding ZY, Qian Z, Zhao CX, Zhang KC. Improved production of mycelial biomass and ganoderic acid by submerged culture of *Ganoderma lucidum* SB97 using complex media. Enzyme Microb Technol. 2008;42(4):325–331. http://dx.doi.org/10.1016/j.enzmictec.2007.10.016
9. Mandal AK, Yadav KK, Sen IK, Kumar A, Chakraborti S, Islam SS, et al. Partial characterization and flocculating behavior of an exopolysaccharide produced in nutrient-poor medium by a facultative oligotroph *Klebsiella* sp. PB12. J Biosci Bioeng. 2013;115(1):76–81. http://dx.doi.org/10.1016/j.jbiosc.2012.08.006
10. Haas A, Wild C. Composition analysis of organic matter released by cosmopolitan coral reef-associated green algae. Aquat Biol. 2010;10(2):131–138. http://dx.doi.org/10.3354/ab00271
11. Lee HI, Donati AJ, Hahn D, Tisa LS, Chang WS. Alteration of the exopolysaccharide production and the transcriptional profile of free-living *Frankia* strain CcI3 under nitrogen-fixing conditions. Appl Microbiol Biotechnol. 2013;97(24):10499–10509. http://dx.doi.org/10.1007/s00253-013-5277-z
12. Fisher ML, Allen R, Luo Y, Curtiss R. Export of extracellular polysaccharides modulates adherence of the cyanobacterium synechocystis. PLoS ONE. 2013;8(9):e74514. http://dx.doi.org/10.1371/journal.pone.0074514
13. Sen IK, Maji PK, Behera B, Mallick P, Maiti TK, Sikdar SR, et al. Structural characterization of an immunoenhancing heteroglycan of a hybrid mushroom (pfls1h) of *Pleurotus florida* and *Lentinus squarrosulus* (Mont.) Singer. Carbohydr Res. 2013;371:45–51. http://dx.doi.org/10.1016/j.carres.2013.02.004
14. Patra S, Patra P, Maity KK, Mandal S, Bhunia SK, Dey B, et al. A heteroglycan from the mycelia of *Pleurotus ostreatus*: structure determination and study of antioxidant properties. Carbohydr Res. 2013;368:16–21. http://dx.doi.org/10.1016/j.carres.2012.12.003