Valorization of olive oil mill wastewater for the production of β-glucans from selected Basidiomycetes strains

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Abstract

Purpose

The aim of the present study was to investigate the feasibility of polysaccharides production by selected Basidiomycetes in submerged culture. Olive oil mill wastewater (OOMW) was also tested as a potential substrate for polysaccharides production by mushroom strains, focusing on the simultaneous degradation and valorization of the waste material.

Methods

The tested strains were grown in two different substrates, and after biomass harvesting, polysaccharides were isolated using two different methods. The extracellular polysaccharides were isolated from the culture broth, with ethanol precipitation. The isolated fractions were partially characterized with FT-IR spectroscopy.

Results

All three strains performed well in both substrates. Maximum degradation performance of OOMW was achieved by G. lucidum. Extracellular polysaccharides (EPS) production was observed during growth in defined medium only, except from P. ostreatus, where EPS were also isolated from OOMW cultures. In regard to biomass polysaccharides, P. citrinopileatus biomass grown in OOMW was found to be the richer in glucans, with 14.1 % (w/w) total glucan content. After purification of biomass polysaccharides with two methods, the fraction with the highest glucan content was found to be the one from G. lucidum after growth in defined medium cultures, with 49.1% (w/w) total glucans. FT-IR spectra of the isolated samples revealed the bands corresponding to α- and β-glucosidic bonds, but also the existence of protein contamination.

Conclusions

Purification of biomass polysaccharides with two distinct methods revealed that α-amylase and Sevag treatments failed to remove completely α-glucans and proteins respectively, leading to the
suggestion that these two steps could be omitted without significant impact. Moreover, the results imply that the valorization of OOMW might be feasible with the use of mushroom strains, leading to the production of important products, such as glucans.

Keywords: β-glucan, olive oil mill waste valorization, Basidiomycetes submerged culture, polysaccharide isolation

1. Introduction

Glucans are polysaccharides consisting solely of glucose units. The number of different glucosidic bonds with which the glucose monomers can be connected, together with the occurrence of branched chains, results in a variety of different polysaccharide molecules with distinct properties. Among them, β-1,3-D-glucans or β-1,4-D-glucans can be found in the cell walls of higher plants and cereal seeds, while β-1,3,4-D-glucans and β-1,6-D-glucans are usually found in the fungal cell walls [1], together with α-1,3-D-glucans, frequently in complex with chitin or proteins [2]. β-D-glucans are usually branched chains, with varying molecular weight, while α-D-glucans are linear polymers.

Research interest in glucans is steadily growing the last 20 years, due to their important effects in the human health, including antitumor, immunostimulating, (activating phagocytosis) [3], antidiabetic [4], apoptosis-inducing [4], and antioxidant [5] activities. Activity against bacteria, viruses and fungi has also been reported [5]. Moreover, they are found to enhance patient recovery from the toxic effects of chemotherapy, lower blood cholesterol concentration, and participate in blood pressure control [3].

Many fungal strains produce polysaccharides, either as a secreted metabolite, or as a constituent of their cell wall. Among them, the Basidiomycetes species belonging to the genera Pleurotus and Agaricus, with great significance in human nutrition, and some of pharmaceutical importance, such as Ganoderma, are the most promising candidates for polysaccharides production, and thus the focus of most research studies [4, 6, 7, 8, 9, 10]. There are also some studies reporting the detection of the enzymes responsible for the synthesis of polysaccharides, such as β-D-glucan synthases, and their corresponding genes [5, 11].

Apart from their ability to produce glucans with dietary value, many Basidiomycete strains are also very efficient lignin degraders, due to their potent oxidative enzyme system, comprising mainly of laccases (Lac, EC 1.10.3.2), manganese peroxidases (MnP, EC 1.11.1.13) and manganese-independent peroxidases, such as lignin peroxidase (LiP, EC 1.11.1.14), together with a variety of other accessory enzymes. Due to the production of this biocatalytic factory, Basidiomycetes are able to degrade complex phenolic substrates and use them as a carbon source to sustain their growth. Taking this trait into advantage, suitable Basidiomycetes strains could be grown in lignin-containing wastes, and while degradation of phenols is under way, the secreted polysaccharides could be isolated from the extracellular fluid, and the biomass could be harvested for the isolation of cell wall polysaccharides. This way, a combined bioremediation and valorization of the waste can be achieved [12, 13]. A promising phenolic waste for this purpose is olive oil mill wastewater (OOMW), a common effluent from the olive oil production process. OOMW is highly toxic against plants, soil microorganisms and marine organisms, due to its high organic load and phenolic content. Basidiomycetes are able to grow using its components as carbon and energy source, seeing that,
apart from phenol compounds that can be oxidized by their ligninolytic enzyme system, OOMW also contains sugars, lipids and organic acids that promote fungal growth [13, 14].

In the present work, we report the production and isolation of β-D-glucans from three endogenous Greek strains, *Pleurotus ostreatus*, *Pleurotus citrinopileatus* and *Ganoderma lucidum*. The production of biomass polysaccharides and exo-polysaccharides was tested during growth in two different substrates, a defined sugar medium, and a complex wastewater medium, containing OOMW. The isolation of biomass polysaccharides was tested with two different methods. The isolated fractions were further analyzed with FT-IR spectroscopy.

2. Materials and Methods

2.1 Olive oil mill wastewater

OOMW was obtained from an olive oil mill with a three-phase decanter in Kalamata (Peloponnese, S.W. Greece), pretreated and maintained at -20 °C. The composition and physicochemical properties of OMWW was previously assessed [15]. Prior to use, pH was adjusted to 6 with 3N NaOH, and the OOMW was centrifuged at 8000 rpm, 4 °C for 20 min and subsequently filtered through Whatman No. 1, to remove any suspended solids.

2.2 Microorganisms and culture procedures

The *P. citrinopileatus* LGAM 28684, *G. lucidum* LGAM 9720 and *P. ostreatus* LGAM 1123 strains used for this study, were obtained from the culture collection of the Laboratory of General and Agricultural Microbiology (Agricultural University of Athens), and were selected after a screening evaluation including numerous white-rot basidiomycetes [16]. The strains were maintained in Potato Dextrose Agar plates (PDA- Applichem, Germany) at 4 °C. Liquid cultures were prepared as reported previously [17]. Briefly, the 100 mL liquid medium contained xylose 57 g L⁻¹, corn steep liquor 37 g L⁻¹, K₂HPO₄ 1 g L⁻¹, and MgSO₄(H₂O)₇ 0.2 g L⁻¹. Alternatively, for the preparation of liquid cultures, OMWW was diluted at a final concentration of 50% (v/v) with the appropriate buffer solution to a final volume of 100 mL in 250 mL Erlenmeyer flasks with cotton stops, supplemented with 30 g L⁻¹ yeast extract, and autoclaved at 121°C for 20 min. Batch fermentations were also carried out in 2.5 L Bio-Flo 310 bioreactors (New Brunswick Scientific, US), using either two-fold diluted OOMW with 0.1 M potassium phosphate buffer pH 6, or the defined medium described previously. Agitation speed was set to 100 rpm, and the medium was supplemented with 30 g L⁻¹ yeast extract prior to sterilization. The inoculation of 1.5 L of medium was carried out with 200 mL of fully grown precultures, resulting at a starting concentration of 1.5-2.5 g L⁻¹ of dry biomass. The fermentation was maintained at 26 °C, 100 rpm, unless otherwise stated. Samples were taken at selected time intervals, centrifuged (1520 g, 10 min), and the supernatant was used for analysis. At the end of the bioreactor fermentation, biomass was separated from the culture medium by centrifugation at the same conditions, freeze-dried and weighed.

2.3 Determination of OMWW total phenolic content and decolorization

Total phenols content was determined, as described by Waterhouse [18]. Phenols concentration was expressed in ppm of gallic acid equivalents, using the appropriate calibration curve. OMWW
decolorization was estimated spectrophotometrically by measuring the absorbance at 525 nm, as previously described [19].

2.4 Assessment of glucan content in fungal biomass

The content in total glucans and α-glucans were determined in the freeze-dried mycelium of all tested species, obtained from cultures grown in OMWW-based media, according to the Mushroom and Yeast β-glucan Assay Procedure K-YBGL 10/2005 (Megazyme International Ireland, Bray, Ireland). The content in β-glucans was calculated as the difference between total glucans and α-glucans.

2.5 Isolation and partial purification of β-glucans

The purification of β-glucans from the mycelial biomass was tested with two methods: The first was a modified method of Synytsya et al., 2009 (Fig. 1, Protocol A), and the second the method described by Wei et al., 2008 [32] (Fig. 2, Protocol B).

Extracellular polysaccharides (EPS) were isolated as follows: The liquid culture supernatant was concentrated 10-fold in a rotary evaporator (Rotavapor Buchi RE 111, Buchi, Switzerland). In the concentrated supernatant, polysaccharides were precipitated with the addition of 4 volumes cold ethanol (96%) and overnight incubation at 4 °C. The mixture was centrifuged and the precipitate was freeze-dried and weighed.

2.6 FT-IR spectroscopy

FT-IR spectra of the dried glucan preparations were recorded with a Nicolet Magna-IR 560 Spectrophotometer (Thermo Fisher Scientific Inc.) in the 4000-400 cm⁻¹ range in the form of KBr pellets.

2.7 Statistical analyses

Data analysis was performed with the use of SigmaPlot software (Systat Software, Inc., San Jose, CA, USA). Error bars represent the standard error of the mean value.

3. Results

3.1 Fungal growth under submerged fermentation

All three strains showed a satisfactory growth in submerged culture in both media tested. P. ostreatus yielded 4.87 mg mL⁻¹ biomass after 13 days of growth in the 2-L bioreactor in defined medium, and 8.38 mg mL⁻¹ biomass after 13 days under the same conditions in OOMW-based medium. In the latter case, total phenolics reduction reached 43.6 %, and medium decolorization 11.1 %. Biomass production for G. lucidum reached 89.5 mg mL⁻¹ in defined medium, but only 4.2 mg mL⁻¹ in OOMW-based medium, after 6 days of growth. For OOMW- grown cultures, taking into account the low biomass produced, the reduction of total phenolics reached only 19.4%, but decolorization of the medium was 47.56% at the end of the culture.
3.2 Isolation of extracellular polysaccharides

For *P. ostreatus* cultures, the EPS production reached 15.9 mg mL\(^{-1}\) in the defined medium and 18.8 mg mL\(^{-1}\) in the OOMW-based cultures. The crude EPS corresponding to the cultures with the defined medium was found to contain 4.57% total glucans, consisted of 0.27% α-glucans and 4.3% β-glucans. Lower yield was obtained in the EPS fraction corresponding to the cultures grown on OOMW medium, containing 1.91% total glucans consisted of 0.46% α-glucans and 1.45% β-glucans.

In the case of *G. lucidum*, EPS were only produced at an identifiable quantity in the case of defined medium cultures. In this case, EPS concentration was estimated only at 2.53 mg mL\(^{-1}\). Nonetheless, EPS extracted were found to be highly rich in total glucans, reaching the percentage of 37.9%, with only 0.34% accounting for α-glucans and 37.6% for β-glucans. These results are in accordance with previous studies, making *G. lucidum* a promising candidate for large-scale production of valuable β-glucans.

For *P. citrinopileatus* cultures, EPS samples contained 6.6% of total glucans, of which 0.46% were α-glucans and 6.17% were β-glucans during growth in defined medium, with a final concentration of 1.2 mg mL\(^{-1}\). However, EPS were not detected in cultures grown in OOMW medium. For all three strains, it seems that the majority of the exo-polysaccharides isolated accounts for β-glucans.

3.3 Isolation of mycelial biomass-derived glucans

*P. ostreatus* crude biomass samples were analyzed for total glucan content in both fermentation using defined medium or OOMW as growth substrate (Table 1). In the defined-medium cultures, total glucan content was found at 8.68% (w/w), with 3.36% (w/w) accounting for α-glucans and 5.32% (w/w) accounting for β-glucans. Concerning OOMW-based cultures, the corresponding numbers were 7.58% (w/w) for total glucans, 1.1% (w/w) for α-glucans, and 6.48% (w/w) for β-glucans. Isolation of biomass derived polysaccharides from *P. ostreatus*, was carried out following Protocol A, including two isolation steps, one for water soluble polysaccharide molecules and one for alkali-soluble polysaccharide molecules (Table 1). For *P. ostreatus* grown in defined medium, polysaccharide fractions were obtained where the water soluble fraction contained 20.45% (w/w) of total glucans, of which 11.05% (w/w) were α-glucans, and 9.4% (w/w) were β-glucans. Additionally, the alkali-soluble fraction contained 16.39% (w/w) total glucans, composed of 11.02% (w/w) α-glucans and 5.37% (w/w) β-glucans. In the case of OOMW-grown biomass, the alkali-soluble polysaccharide fraction was absent, while the water- soluble fraction contained 34.76% (w/w) total glucans, with 3.02% (w/w) α-glucans, and 31.74% (w/w) β-glucans. From these results it seems that the type of molecules produced may depend on the growth conditions, at least concerning their physicochemical properties.

The isolation of polysaccharides from *P. citrinopileatus* biomass after growth in defined medium was carried out following Protocol B. The obtained polysaccharide fraction was only 37 mg, and thus the determination of its glucan composition was not possible. On the other hand, *P. citrinopileatus* biomass grown in OOMW cultures was treated with both Protocols A and B, allowing the comparison of both methodologies. Isolation of polysaccharides following Protocol B (Table 2), resulted in only one polysaccharide fraction with the composition of 31.71% (w/w) total glucans, 15.72% (w/w) α-glucans and 16% (w/w) β-glucans. On the other hand, the isolation of polysaccharides following Protocol A, yielded a water soluble fraction with 13.13% (w/w) total glucans, composed of 6.33% (w/w) α-glucans and 6.8% (w/w) β-glucans, and an alkali-soluble fraction with 23.76% (w/w) total glucans consisted of 2.21% (w/w) α-glucans and 21.55% (w/w) β-glucans.
3.4 FT-IR analysis of isolated polysaccharide fractions

For the identification of the isolated polysaccharide fractions, FT-IR was employed. All spectra shown in Fig. 3 present a wide peak around 3300-3400 cm\(^{-1}\), corresponding to free –OH groups of a large molecule, indicating the existence of high MW polysaccharide molecules [5]. The peaks in 881 and 893 cm\(^{-1}\) present in EPS from cultures grown in both defined and OOMW medium for the samples derived from the species P. citrinopileatus and G. lucidum, corresponding to β-glucosidic bond, and thus indicating the existence of β-glucan molecules [9, 20, 21]. The peaks at 854, 862 and 845 cm\(^{-1}\) indicate accordingly, the existence of α-glucan molecules in the samples of P. ostreatus and P. citrinopileatus [2, 20, 21]. Moreover, the peaks around 520-550 cm\(^{-1}\) in the P. ostreatus EPS sample, and some of the other samples, also indicate the presence of α-glucans according to several studies [2, 9]. Peaks at 1127, 1153, 1152, 1158, 1150 and 1143 cm\(^{-1}\) represent the stretch of the C-O-C bond of the pyranose ring of sugar moieties [9, 21]. The peak at 1650 cm\(^{-1}\) found in all the samples, corresponds to the C-N bond of the aminoacids, and thus indicates the presence of protein contamination in the samples [9]. The peaks around 2920-2930 cm\(^{-1}\) correspond to –CH\(_2\) groups of lipids, as previously reported [9].

4. Discussion

In the present work, all the studied Basidiomycetes strains grew well in both media, yielding satisfactory amount of biomass in fermentations in defined, as well as in OOMW media. OOMW supplemented with nitrogen source seems to be able to sustain mycelial growth from all strains. However, waste degradation in these cases was not as high as reported previously for other Basidiomycetes strains [13, 22, 23] indicating that further optimization steps are necessary. However, the results suggest that the submerged fermentation might be more efficient in terms of productivity, due to the fact that biomass growth was considerably faster compared to solid state approaches, where complete fruit body formation might take up to two months [24].

P. ostreatus appears to be more productive in terms of EPS compared to the other two strains. In this case, a considerable amount of EPS was isolated in both tested media, albeit with a low concentration of glucans. The other two strains seem to produce a measurable amount of EPS only during growth in the defined medium. EPS obtained from G. lucidum cultures in particular, were isolated as a gel-like substance, as previously reported for Basidiomycete’s EPS [8], consisting almost completely from β-glucans. The yields obtained were comparable to those reported previously for other species [25]. Unfortunately, EPS were not isolated during growth in OOMW-based media. This was also the case for P. citrinopileatus, however, in the defined medium cultures, the EPS yield exceeded those obtained in the previous work by Wang et al. [10], by the same species, where only 0.56 mg mL\(^{-1}\) EPS were isolated.
For *P. citrinopileatus* biomass polysaccharides, the purification yields obtained with the two methods were similar, and comparable to relevant studies [27, 28, 29]. In this case, the two methods tested did not seem to differ in terms of polysaccharides yield and recovery, leading to the conclusion that the time-consuming and costly steps of Protocol A, mainly the incubation with α-amylase and the Sevag treatment steps may be omitted without significant loss in the final product.

Following Protocol A, *P. ostreatus* biomass glucans were found mainly in the water-soluble fraction. The obtained purification yields were surprisingly high, reaching 30% for OOMW-grown mycelium β-glucans. This is quite interesting, taking into account that these yields are considerably higher than others reported in the literature [6, 8, 30]. However, comparable yields have been also obtained previously [26].

On the other hand, for the isolation of glucans from *G. lucidum* biomass Protocol B protocol of Wei et al. [32] was followed. The protocol yields were very high in this case, exceeding 40% for the β-glucans isolated from the mycelium grown in defined medium, as reported previously for other species [26] but quite higher than most relevant studies [6, 8, 30].

FT-IR spectra of the isolated fractions revealed protein contamination, despite multiple treatments with Sevag reagent, as shown in previous studies [9]. Glucans often occur in mushrooms in complex with proteins called proteoglucans and thus complete protein removal from the samples can be challenging [31]. In terms of protein removal, Protocol A does not seem to be superior to Protocol B, despite the Sevag reagent treatments, indicating that this step of the procedure might be omitted without significant changes in the final product.

Moreover, in the case of the glucans isolated following Protocol A, the treatment with α-amylase failed to remove completely α-glucans from the isolated samples. The yield of obtained α-glucans varied from 0.63% in *P. citrinopileatus* alkali-soluble biomass polysaccharide to 29.29% in *P. ostreatus* water-soluble biomass polysaccharide fraction. The corresponding obtained yields in the case of the samples isolated with Protocol B varied from 2.86% to 28.13%, indicating that the use of the commercial α-amylase, a time-consuming and costly step of the procedure, may be omitted without significant change in the α-glucan yields.

5. Conclusions

Overall, the results of the present work support that the production and purification of fungal polysaccharides might be feasible in submerged culture and in large scale, providing an alternative method for the valorization of wastewater as media for fungal growth. The three endogenous Greek Basidiomycetes strains tested in this study performed well during submerged culture in defined medium, but also during growth in OOMW. Although production and purification of Basidiomycete's glucans can be easier and more cost-effective in solid state cultures and in basidiocarp form, submerged culture could offer an interesting alternative due to three main advantages: Firstly, the mycelial growth is much more rapid, leading to higher productivity. Secondly, with this method the use of liquid media is required, leading to a greater variety of growth media choices, including liquid wastes, such as OOMW. Basidiomycetes are known to effectively degrade OOMW, and after the necessary optimization of parameters, the two processes of OOMW degradation and polysaccharides production could be combined in a single procedure, leading to the valorization of a toxic waste. Finally, with the submerged culture, the isolation of extracellular glucans can also be achieved, leading to further increase of the total polysaccharide yield of the procedure.
Acknowledgements

The authors would like to thank Associate Prof. George Zervakis from the Agricultural University of Athens for kindly providing the Basidiomycetes strains used in this work.

6. References


Table 1: Glucan composition of the isolated fractions from the *P. ostreatus* cultures. The isolation was performed following Protocol A.

<table>
<thead>
<tr>
<th>Defined medium cultures</th>
<th>Crude biomass (% w/w)</th>
<th>Water-soluble fraction (% w/w)</th>
<th>Purification (fold)</th>
<th>Yield (%)</th>
<th>Alkali-soluble fraction (% w/w)</th>
<th>Purification (fold)</th>
<th>Yield (%)</th>
<th>EPS (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total glucans</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-glucans</td>
<td>3.36</td>
<td>11.05</td>
<td>3.29</td>
<td>29.29</td>
<td>11.02</td>
<td>3.29</td>
<td>3</td>
<td>0.27</td>
</tr>
<tr>
<td>β-glucans</td>
<td>5.32</td>
<td>9.4</td>
<td>1.77</td>
<td>15.77</td>
<td>5.37</td>
<td>1.01</td>
<td>0.9</td>
<td>4.3</td>
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<tr>
<td>OOMW cultures</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Total glucans</td>
<td>7.58</td>
<td>34.76</td>
<td>4.59</td>
<td>28.57</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>1.91</td>
</tr>
<tr>
<td>α-glucans</td>
<td>1.1</td>
<td>3.02</td>
<td>2.74</td>
<td>16.92</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.46</td>
</tr>
<tr>
<td>β-glucans</td>
<td>6.48</td>
<td>31.74</td>
<td>4.9</td>
<td>30.84</td>
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<td>n.d.</td>
<td>n.d.</td>
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Table 2: Glucan composition of the isolated fractions from the *P. citrinopileatus* cultures. The isolation was performed following both Protocols A and B.

<table>
<thead>
<tr>
<th>Defined medium cultures</th>
<th>Crude biomass (% w/w)</th>
<th>Water-soluble fraction (% w/w)</th>
<th>Purification fold</th>
<th>Yield (%)</th>
<th>Alkali-soluble fraction (% w/w)</th>
<th>Purification fold</th>
<th>Yield (%)</th>
<th>EPS (% w/w)</th>
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<tbody>
<tr>
<td>α-glucans</td>
<td>0.56</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.46</td>
</tr>
<tr>
<td>OOMW cultures</td>
<td>14.06(^1)</td>
<td>13.13(^1)</td>
<td>0.93(^1)</td>
<td>0.6(^1)</td>
<td>23.76(^1)</td>
<td>1.69(^1)</td>
<td>1.5(^1)</td>
<td>n.d.</td>
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<tr>
<td>Total glucans</td>
<td>7.53(^2)</td>
<td>31.71(^2)</td>
<td>4.21(^2)</td>
<td>1.79(^2)</td>
<td>6.79(^2)</td>
<td>9.43(^2)</td>
<td>5.22(^2)</td>
<td>n.d.</td>
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<tr>
<td>α-glucans</td>
<td>3.12(^1)</td>
<td>6.33(^1)</td>
<td>2.03(^1)</td>
<td>1.3(^1)</td>
<td>2.21(^1)</td>
<td>0.71(^1)</td>
<td>0.63(^1)</td>
<td>n.d.</td>
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<tr>
<td>β-glucans</td>
<td>1.67(^2)</td>
<td>15.72(^2)</td>
<td>9.43(^2)</td>
<td>5.22(^2)</td>
<td>1.38(^1)</td>
<td>21.55(^1)</td>
<td>1.97(^1)</td>
<td>1.77(^1)</td>
</tr>
<tr>
<td>Total glucans</td>
<td>10.93(^1)</td>
<td>6.79(^1)</td>
<td>0.62(^1)</td>
<td>1.38(^1)</td>
<td>21.55(^1)</td>
<td>1.97(^1)</td>
<td>1.77(^1)</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

\(^1\) Results from purification protocol A, \(^2\) Results from purification protocol B, n.d. not detected.
Table 3: Glucan composition of the isolated fractions from the *G. lucidum* cultures. The isolation was performed following Protocol B.

<table>
<thead>
<tr>
<th></th>
<th>Defined medium cultures</th>
<th>OOMW cultures</th>
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<td></td>
<td>Crude biomass (% w/w)</td>
<td>(% w/w)</td>
</tr>
<tr>
<td>Total glucans</td>
<td>6.2</td>
<td>5.56</td>
</tr>
<tr>
<td>α- glucans</td>
<td>0.83</td>
<td>0.13</td>
</tr>
<tr>
<td>β- glucans</td>
<td>5.39</td>
<td>5.43</td>
</tr>
<tr>
<td>Isolated polysaccharides</td>
<td>49.1</td>
<td>14.96</td>
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<tr>
<td>Purification (fold)</td>
<td>7.9</td>
<td>2.69</td>
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<td>Yield (%)</td>
<td>36.3</td>
<td>14</td>
</tr>
<tr>
<td>EPS (% w/w)</td>
<td>37.92</td>
<td>n.d.</td>
</tr>
</tbody>
</table>
Figure 1. Outline of polysaccharides purification protocol, as described in Synytsya et al., 2009 (Protocol A).
Figure 2

Dried and pulverized biomass

\[\text{Homogenization (2 min in blender with 2 volumes of } d\text{H}_2\text{O)}\]

\[\text{Mild boiling for 4-5 days}\]

\[\text{Centrifugation to remove the solids}\]

\[\text{Precipitation of crude polysaccharides (addition of 2 volumes cold ethanol)}\]

\[\text{Water washing}\]

\[\text{Precipitation with 70\% v/v ethanol}\]

\[\text{Centrifugation}\]

\[\text{Lyophilization}\]

Polysaccharide fraction (W)

Figure 2. Outline of polysaccharides purification protocol, as described in Wei et al., 2008 (Protocol B) [32].
Figure 3.

Figure 3. FT-IR spectra of partially isolated polysaccharide samples derived from *P. ostreatus* (a), *G. lucidum* (b) and *P. citrinopileatus* (c). Black and dark grey lines represent the FT-IR spectra of the polysaccharides isolated from mycelial biomass and EPS, respectively, isolated both from cultures grown in defined medium. Light grey and dotted lines represent the FT-IR spectra of the polysaccharides isolated from mycelial biomass and EPS, respectively, isolated both from cultures grown in OOMW medium.