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Isolation of b-glucans from selected Basidiomycetes strains grown in olive oil mill wastewater

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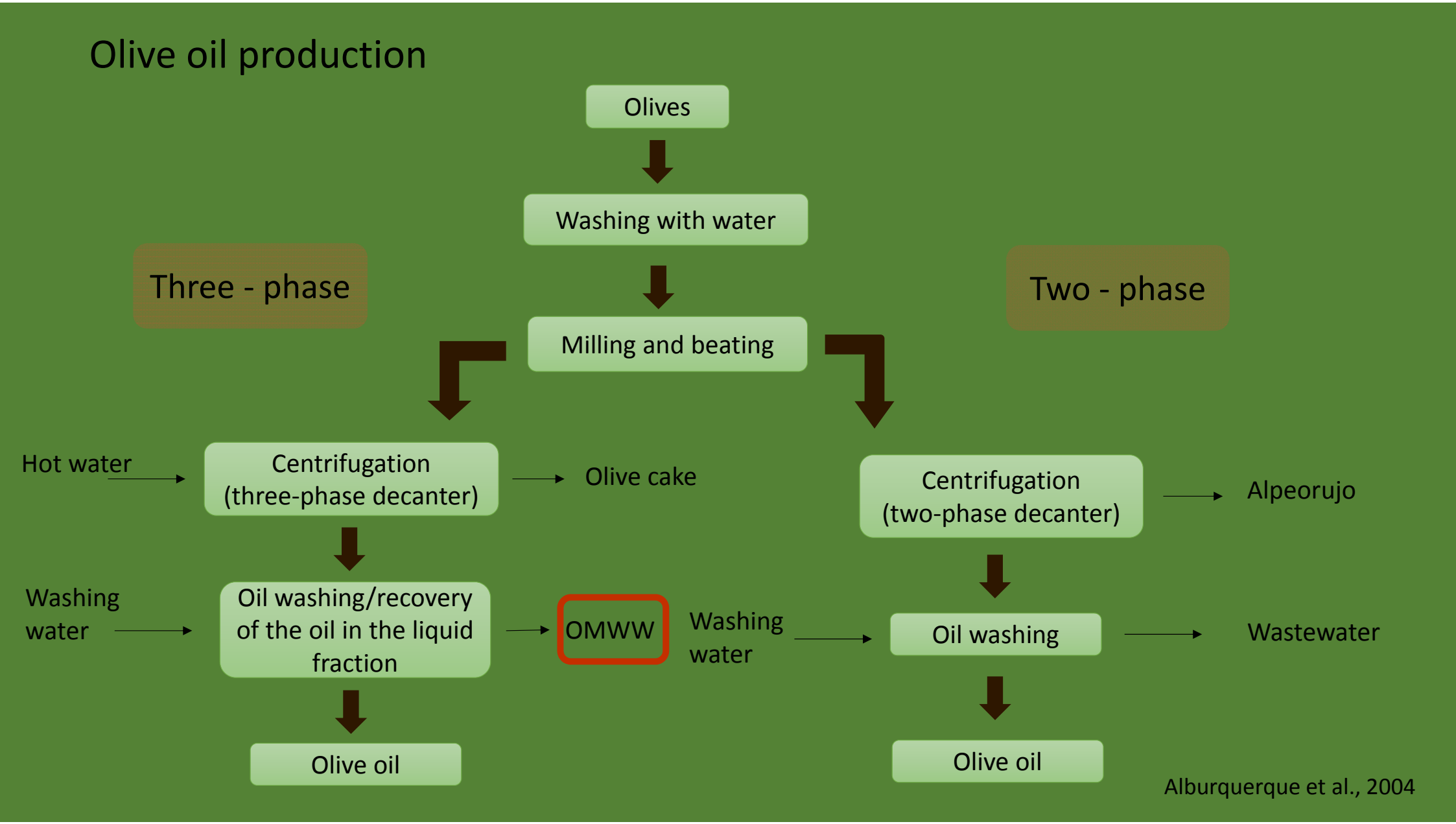
Olive oil production



Alburquerque et al., 2004

Olive oil mill wastewater

- Dark liquid with strong odor
- High organic load
- High concentration of (poly)phenols
- High phytotoxicity
- Toxicity against soil microorganisms
- Deposition in landfills and evaporation

Environmental and aesthetic degradation of the deposition area

OMWW properties	
pH	5.00 ± 0.07
Proteins (g L^{-1})	0.90 ± 0.19
Total N (g L^{-1})	5.00 ± 0.07
Total phenolics (g L^{-1})	3.88 ± 0.92
Total solids (g L^{-1})	43.67 ± 5.43
TOC (g L^{-1})	28.58 ± 3.00

Ntougias, S., et al. (2013).

Basidiomycetes wood-rot fungi

- Potent degraders of lignocellulose
- Degradation of OMWW

⇩

Oxidation of (poly)phenols

⇩

Decrease of toxicity, colour

- Production of a variety of valuable products

Enzymes

Secondary metabolites

Polysaccharides

(α, β- glucans)

AUA, Faculty of crop science,
Associate Prof. G. Zervakis

Pleurotus citrinopileatus

Pleurotus ostreatus

Ganoderma lucidum

Glucans

- Polysaccharides from glucose monomers
- Different bonds among glucose units and branches result in a variety of glucans
- In fungal cell walls, glucans are usually in complex with chitin or proteins
- Basidiomycetes also produce secreted glucans
- α- D- glucans are linear polymers
- β- D- glucans usually branched chains

Biological activities of glucans

- Antitumor
- Immuno-stimulating
- Anti-bacterial, antifungal, antiviral
- Lower blood cholesterol

Aim of the study

- ➢ Biological treatment of OMWW with the use of wood-rot fungi
  - Decolourization
  - Phenols degradation
- ➢ Valorization of the waste through the production of high added-value products
  - Polysaccharides (β-glucans)

Experimental procedure

Mushrooms grown in two different media:

Defined medium
- Xylose 57 g L^{-1}
- Yeast extract 30 g L^{-1}
- K_2HPO_4 1 g L^{-1}
- $MgSO_4(H_2O)7$ 0.2 g L^{-1}

OOMW medium
- OOMW 50 % (v/v)
- Yeast extract 30 g L^{-1}
- Potassium phosphate buffer 100 mM, pH 6

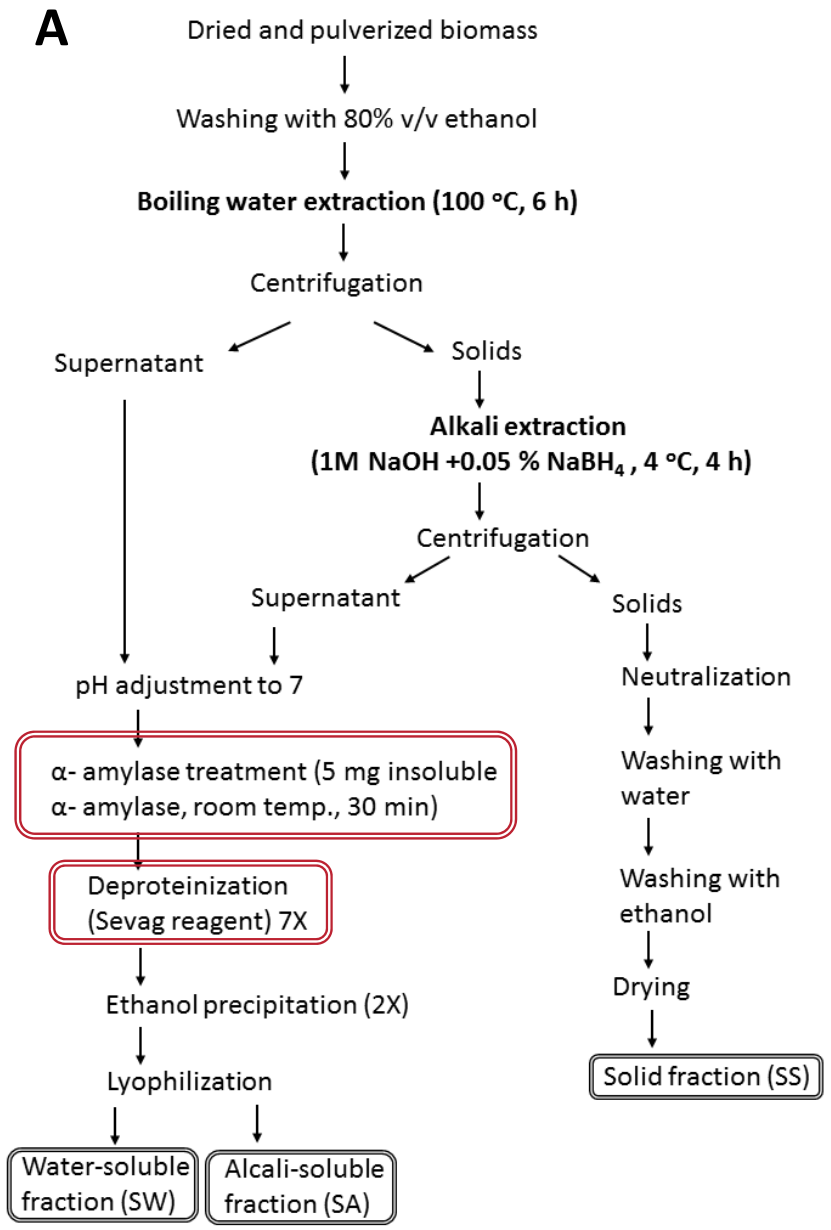
EPS were isolated from the extracellular fluid with ethanol precipitation

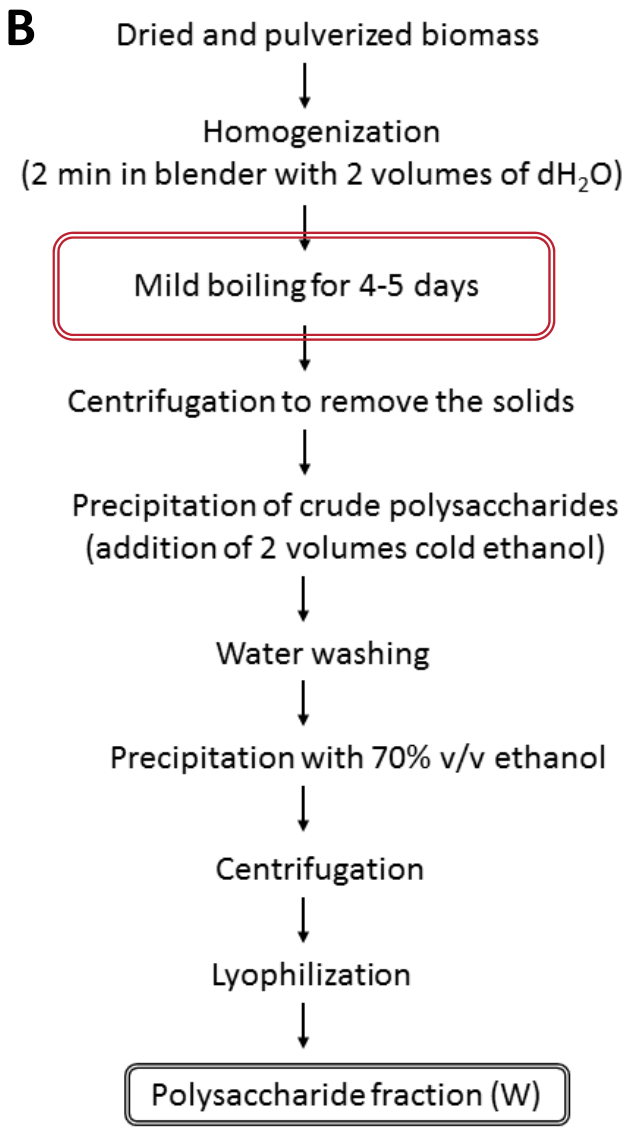
Biomass polysaccharides were purified following one of two protocols, A (Synytsya et al., 2009), or B (Wei et al., 2008).

Glucan content in crude and purified fractions was measured with the Yeast and Mushroom beta-glucan Assay kit from Megazyme ®

Megazyme

Synytsya, A., Míčková, K., Synytsya, A., Jablonský, I., Spěváček, J., Erban, V., Kováříková, E., Čopíková, J.: Glucans from fruit bodies of cultivated mushrooms *Pleurotus ostreatus* and *Pleurotus eryngii*: Structure and potential prebiotic activity. Carbohydr. Polym. 76(4) 548-556 (2009).Wei, S., Helsper, J. P. F. G., Van Griensven, L. J. L. D.: Phenolic Compounds Present in Medicinal Mushroom Extracts Generate Reactive Oxygen Species in Human Cells In Vitro. Int. J. Med. Mushrooms. 10(1), 1-13 (2008).


A
Dried and pulverized biomass
Washing with 80% v/v ethanol
Boiling water extraction (100 °C, 6 h)
Centrifugation
Supernatant
Solids
Alkali extraction
(1M NaOH +0.05 % NaBH4 , 4 °C, 4 h)
Centrifugation
Supernatant
Solids
pH adjustment to 7
Neutralization
α- amylase treatment (5 mg insoluble α- amylase, room temp., 30 min)
Washing with water
Deproteinization (Sevag reagent) 7X
Washing with ethanol
Ethanol precipitation (2X)
Drying
Lyophilization
Solid fraction (SS)
Water-soluble fraction (SW)
Alcali-soluble fraction (SA)


B
Dried and pulverized biomass
Homogenization
(2 min in blender with 2 volumes of dH2O)
Mild boiling for 4-5 days
Centrifugation to remove the solids
Precipitation of crude polysaccharides
(addition of 2 volumes cold ethanol)
Water washing
Precipitation with 70% v/v ethanol
Centrifugation
Lyophilization
Polysaccharide fraction (W)

P. ostreatus

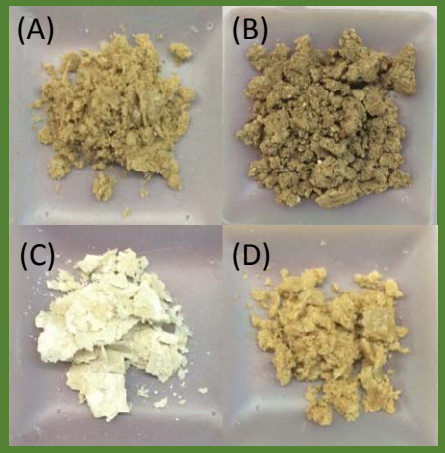
- Phenolics reduction: 43.6%
- Decolourization: 11.1%

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

Defined medium cultures	**Crude biomass (% w/w)**	**Water-soluble fraction (% w/w)**	**Purification (fold)**	**Yield (%)**	**Alkali-soluble fraction (% w/w)**	**Purification (fold)**	**Yield (%)**	**EPS (% w/w)**
Total glucans	8.6	20.4	2.36	21.1	16.4	1.9	1.7	4.6
α- glucans	3.3	11.1	3.29	29.3	11.0	3.3	3	0.27
β- glucans	5.3	9.4	1.77	15.8	5.4	1.0	0.9	4.3
OOMW cultures								
Total glucans	7.6	34.7	4.6	28.6	n.d.	n.d.	n.d.	1.9
α- glucans	1.1	3.0	2.7	16.9	n.d.	n.d.	n.d.	0.5
β- glucans	6.5	31.7	4.9	30.9	n.d.	n.d.	n.d.	1.5

P. citrinopileatus

Significant decolourization and phenols reduction was not observed



Fractions obtained after Protocol A purification of β-glucans from *P. citrinopileatus* biomass grown in OOMW. A) Water-soluble fraction, B) Solid fraction C) Alkali-soluble fraction a, D) Alkali – soluble fraction b.

Table 2: Glucan composition of the isolated fractions from the *P. citrinopileatus* cultures. The isolation was performed following both Protocols A and B.

Defined medium cultures	Crude biomass (% w/w)	Water-soluble fraction (% w/w)	Purification (fold)	Yield (%)	Alkali-soluble fraction (% w/w)	Purification (fold)	Yield (%)	EPS (% w/w)
Total glucans	6.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	6.6
α-glucans	0.6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.45
β-glucans	5.9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	6.2
OOMW cultures								
Total glucans	14.1[1] 7.5[2]	13.1[1] 31.7[2]	0.93[1] 4.2[2]	0.6[1] 1.8[2]	23.8[1]	1.7[1]	1.5[1]	n.d.
α-glucans	3.1[1] 1.7[2]	6.3[1] 15.7[2]	2.03[1] 9.4[2]	1.3[1] 5.2[2]	2.2[1]	0.7[1]	0.6[1]	n.d.
β-glucans	10.9[1] 5.9[2]	6.8[1] 16.0[2]	0.62[1] 2.7[2]	1.4[1] 1.5[2]	21.6[1]	2.0[1]	1.8[1]	n.d.

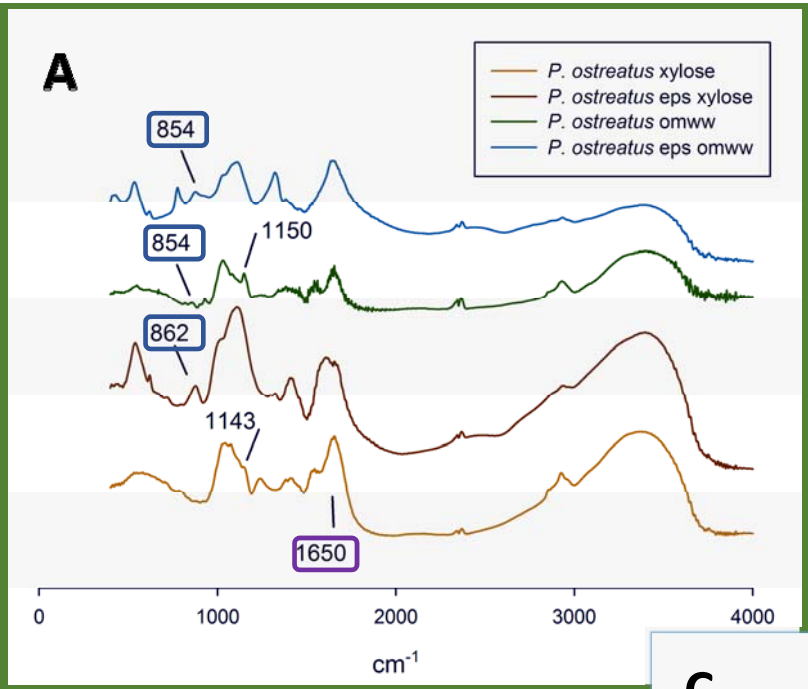
[1] Results from purification protocol A, [2]Results from purification protocol B, n.d. not detected.

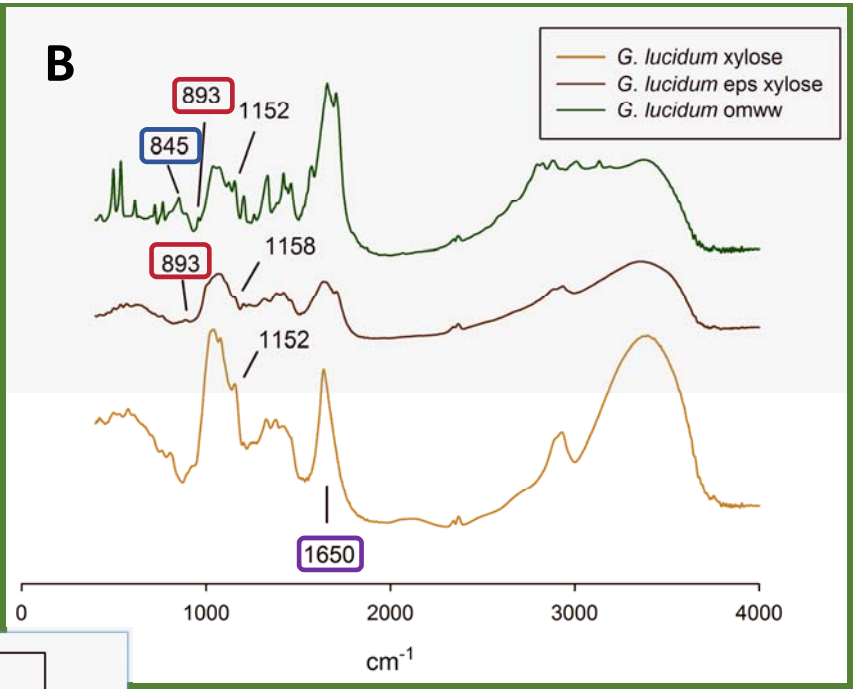
G. lucidum

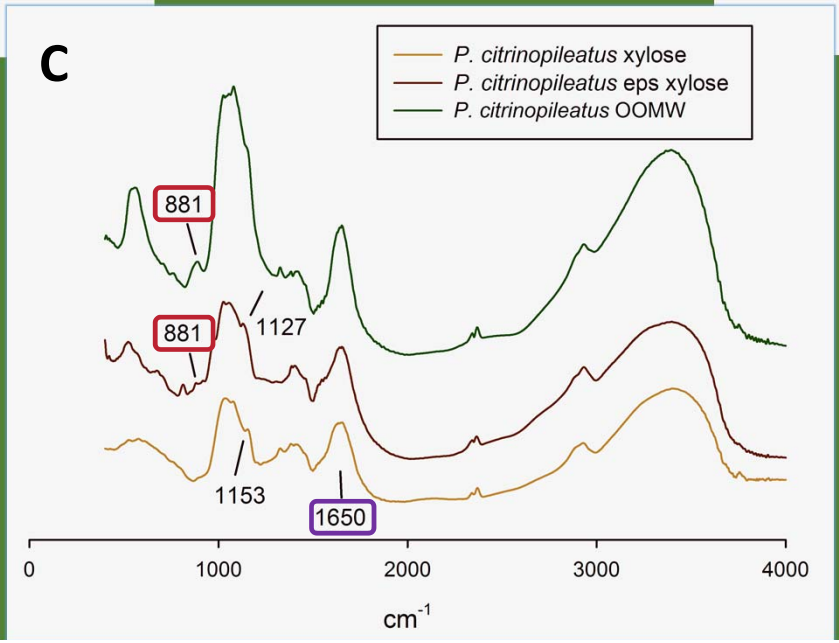
- Phenolics reduction: 19.4%
- Decolourization: 47.5%

Table 3: Glucan composition of the isolated fractions from the *G. lucidum* cultures. The isolation was performed following Protocol B.

Defined medium cultures	Crude biomass (% w/w)	Isolated polysaccharides (% w/w)	Purification (fold)	Yield (%)	EPS (% w/w)
Total glucans	6.2	49.1	7.9	36.3	37.9
α- glucans	0.8	0.5	0.6	2.9	0.3
β- glucans	5.4	48.6	9.0	41.2	37.6
OOMW cultures					
Total glucans	5.5	14.9	2.7	14	n.d.
α- glucans	0.1	0.6	5.0	28.1	n.d.
β- glucans	5.4	14.3	2.6	14.0	n.d.


A
P. ostreatus xylose
P. ostreatus eps xylose
P. ostreatus omww
P. ostreatus eps omww
854
854
1150
862
1143
1650
0
1000
2000
3000
4000
cm-1


B
G. lucidum xylose
G. lucidum eps xylose
G. lucidum omww
893
1152
845
1158
893
1152
1650
0
1000
2000
3000
4000
cm-1


C
P. citrinopileatus xylose
P. citrinopileatus eps xylose
P. citrinopileatus OOMW
881
881
1127
1153
1650
0
1000
2000
3000
4000
cm-1

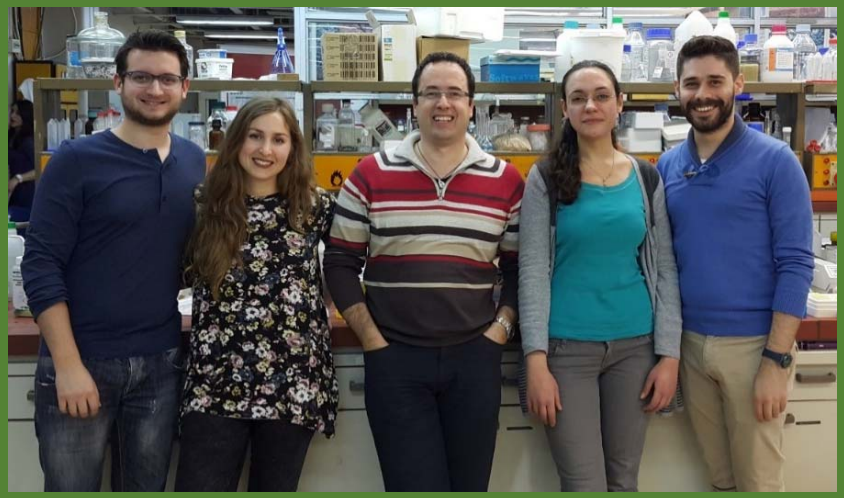
Conclusions

- Satisfactory biomass growth in OOMW, nitrogen- supplemented medium from all three strains
- Submerged fermentation is faster than solid state approaches.
- *G. lucidum* produced gel-like EPS, consisting mainly of beta-glucans.
- The two purification protocols tested did not appear to be very different in terms of yield and product recovery

- Time-consuming and costly steps of a-amylase and Sevag treatments could be omitted, without significant loss
- Protein contamination was evident in the FT-IR spectra of all samples → proteoglycans?
- Overall, the combination of Basidiomycete OOMW treatment and concomitant production of glucans with pharmaceutical and nutritional value could lead to the valorization of the liquid waste.

NTUA IndBioCat Group

http://www.chemeng.ntua.gr/indubiocat/index.html



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Ευρωπαϊκή Ένωση
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ΕΣΠΑ 2007-2013
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Με τη συγχρηματοδότηση της Ελλάδας και της Ευρωπαϊκής Ένωσης

Thank you for
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attention!