Provided for non-commercial research and education use.
Not for reproduction, distribution or commercial use.

This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

http://www.elsevier.com/copyright

Author's personal copy

Bioresource Technology 101 (2010) 1941–1946

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Effects of nutrients, pH and water potential on exopolysaccharides production by a fungal strain belonging to *Ganoderma lucidum* complex

Leandro Papinutti

Laboratorio de Micología Experimental, Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 29 August 2008
Received in revised form 14 September 2009
Accepted 14 September 2009
Available online 28 October 2009

Keywords:
Doehlert factorial design
Exopolysaccharide
Ganoderma lucidum
Water activity

ABSTRACT

Exopolysaccharide (EPS) production by *Ganoderma lucidum* in response to different culture conditions was studied. Cellulose and glucose, in defined media, resulted in the more efficient enhancers of EPS production among the carbon sources tested. In natural media cultures containing glucose and malt extract exhibited a marked increase (up to 29-fold) respect to defined media. Subsequently, high malt extract and glucose concentrations were tested. *G. lucidum* produced two fractions of EPS, water-soluble and water-insoluble under these culture conditions. The maximum value (15 g L^{-1}) was reached at 21 days in the medium containing 60 g L^{-1} malt extract and 40 g L^{-1} glucose. The incomplete utilization of reducing sugars by the fungus in these media suggested that not only did high malt extract and glucose concentrations play a role in EPS production but also the water activity might be involved. A factorial uniform experimental design to test the effect of malt extract, polyethylene glycol (PEG, as water activity depressor), and initial pH on specific EPS production was applied. *G. lucidum* showed to be a more efficient specific EPS (mg EPS per g mycelium) producer at pH 3.5 in cultures containing the highest PEG and malt extract concentrations.

© 2009 Elsevier Ltd. All rights reserved.

1. Introduction

Production of exopolysaccharides (EPS) is widely distributed among fungi (Selbmann et al., 2003). Most of the EPS produced by fungi are highly hygroscopic β-glucans, suggesting that its production could be related with the tolerance to desiccation; similarly to that observed and described in bacteria (Schnider-Keel et al., 2001). In white rot fungi EPS could also be related with the decay process of wood (Ruel and Joseleau, 1991) and as a protective layer (Vesentini et al., 2007). The EPS consist of wide variety of glucans closely related in structure but possessing very different water solubilities. Purified β-glucans from *Ganoderma lucidum* demonstrated antitumor (Sone et al., 1985) and bactericidal activities, and immunomodulating properties (Tzianabos, 2000). Crude preparations of β-(1–3)-glucans also exhibited a wide range of biologic functions, numerous reports showed the ability of β-(1–3)-glucans to nonspecifically activate cellular and humoral components of the host immune system (Wakshull et al., 1999; Liang et al., 1998). Water extracts from *G. lucidum* fruiting bodies of *G. lucidum* are widely used in oriental medicine for treating various diseases and this fungus has been the preferred organism for performing physiological studies dealing with the regulation and production of EPS (Kim et al., 2006; Tang and Zhong, 2002; Hsieh et al., 2006). These fungi have been used in folk medicine for hundreds of years (Russell and Paterson, 2006), and strains are actually cultivated for preparation of health tablets. Medicinal benefits of *Ganoderma* spp. were reviewed by Jong and Birmingham (1992).

G. lucidum is a group of laccate *Ganoderma* species with uncertain position within the genera because of the inconclusiveness of traditional taxonomic methods for establishing a stable classification of the group, and these methods are useless for characterization of individual strains (Hseu et al., 1996). Isolates used in pharmaceutical and medicinal studies and commercially cultivated isolates are generally named *G. lucidum*. However, as used in the pharmaceutical literature, this name encompasses several laccate *Ganoderma* species that might differ in their production of bioactive compounds. Recently however, there has been a growing interest in studying the EPS production of a wider array of fungi, not only from the standpoint of comparative biology but also with the expectation of finding better EPS-producing systems for use in various biotechnological applications. Besides the health benefits, EPS represent a valid alternative to plant and algal products considering that their properties are almost identical to those employed in food, pharmaceutical and cosmetic industries (Sutherland, 1996). The aim of this work was to explore the regulation by nutrients of growth and EPS production in submerged culture by a new isolate belonging to *G. lucidum* complex.

E-mail address: leandru@bg.fcen.uba.ar

0960-8524/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2009.09.076

Author's personal copy

2. Methods

2.1. Organism and culture conditions

G. lucidum was isolated from *Tipuana tipu* wood, this laccate *Ganoderma* isolate is within *G. lucidum* complex. The fungus was maintained in MEA (malt extract 1.2%, glucose 1%, agar 2%) medium at 4 °C. Inoculum consisted of a 25-mm^2 surface agar plug from a 10-day-old culture grown on MEA. Cultures were performed in 125 mL Erlenmeyer flasks containing 25 mL of liquid medium. The defined media contained: $MgSO_4{\cdot}7H_2O$, 0.5 g; KH_2PO_4, 0.5 g; K_2HPO_4, 0.6 g; $CuSO_4{\cdot}5H_2O$, 0.4 mg; $MnCl_2{\cdot}4H_2O$, 0.09 mg; H_3BO_3, 0.07 mg; $Na_2MoO_4{\cdot}H_2O$, 0.02 mg; $FeCl_3$, 1 mg; $ZnCl_2$, 3.5 mg; thiamine hydrochloride, 0.1 mg; asparagine monohydrate, variable; the carbon source included 10 g each of glucose, lactose, sucrose or crystalline cellulose, and distilled water up to 1 L. The natural media contained variable concentrations of glucose along with the natural component malt extract (ME) or peptone (concentrations are indicated in Table 2). Uninoculated Erlenmeyer flasks served as controls for possible preexisting polysaccharides contained in the natural media.

2.2. Combined effect of PEG, malt extract and pH. Experimental design

A Doehlert uniform shell design was applied to study the effects of PEG, initial pH and malt extract on EPS production. To study the effect of three factors Doehlert (1970) proposed an experimental design based on 13 combinations of the three variables studied. The equally spaced values of each independent variable and the combinations among them were adopted and coded following the Doehlert design (Doehlert, 1970). The coded (in brackets) and real values are shown in Table 1. Specific EPS was defined as mg of water-soluble EPS per gram of dry mycelium. The variable response specific EPS was measured at 14 and 27 days of growth. The values are the means of triplicate cultures. Data processing was performed by direct curvilinear regression, without any prior transformation. The observed values were fitted to a full quadratic equation model with 10 coefficients (Eq. (1)):

$$F = b_0 + b_1X + b_2X^2 + b_3Y + b_4Y^2 + b_5Z + b_6Z^2 + b_7XY + b_8XZ + b_9YZ \quad (1)$$

where F is the variable response and b_i the regression coefficients given by the model; X, Y and Z, are the independent coded variables. Water deficit was applied by adding a non-metabolizable compound, polyethylene glycol 200 (PEG) to the medium to obtain the decrease in water potential (a_w) (Money, 1989).

Table 1
The real and coded (in parenthesis) values adopted for the independent variables.

Run	Malt extract (g L^{-1})	pH	PEG (g L^{-1})
1	16 (0)	7.5 (1)	12.5 (0)
2	16 (0)	3.5 (−1)	12.5 (0)
3	28 (0.866)	6.5 (0.5)	12.5 (0)
4	4 (−0.866)	4.5 (−0.5)	12.5 (0)
5	4 (−0.866)	6.5 (0.5)	12.5 (0)
6	28 (0.866)	4.5 (−0.5)	12.5 (0)
7	20 (0.289)	6.5 (0.5)	25 (0.816)
8	12 (−0.289)	4.5 (−0.5)	0 (−0.816)
9	12 (−0.289)	6.5 (0.5)	0 (−0.816)
10	24 (0.577)	5.5 (0)	0 (−0.816)
11	20 (0.289)	4.5 (−0.5)	25 (0.816)
12	8 (−0.577)	5.5 (0)	25 (0.816)
13	16 (0)	5.5 (0)	12.5 (0)
14	16 (0)	5.5 (0)	12.5 (0)
15	16 (0)	5.5 (0)	12.5 (0)

Table 2
Effect of media composition on biomass and water-soluble EPS (volumetric) production; specific EPS (sp EPS) was calculated per g of dry biomass. Data are the means of triplicate independent cultures. Standard deviation around the mean was less than 5%.

	Biomass (g L^{-1})	EPS (g L^{-1})	sp EPS (g g^{-1})
Cellulose	2.03 (43)[a]	0.55 (43)	0.27
Glucose	3.93 (23)	0.49 (16)	0.12
Lactose	3.18 (23)	0.09 (14)	0.03
Sucrose	1.91 (23)	0.11 (14)	0.06
Malt extract 10 g glc 10 g	4.32 (32)	2.20 (27)	0.51
Malt extract 20 g glc 20 g	4.70 (32)	2.63 (32)	0.56
Asparagine (2 g)	3.45 (19)	1.07 (27)	0.31
Asparagine (0.8 g)	1.92 (23)	0.90 (43)	0.47
Peptone 10 g glc 10 g	4.28 (37)	0.03 (37)	0.01
Peptone 20 g glc 40 g	8.20 (37)	0.07 (37)	0.01

[a] The number in brackets indicates the day in which the highest value was observed.

2.3. Analytical determinations

Glycogen determination: To assess glycogen content of mycelia, the method of Krisman (1962) was used. For this purpose, mycelia samples were dried at 80 °C overnight, weighed to determine biomass and ground in a mortar. Ground mycelia consisting of 100 mg per sample were used to determine the glycogen content. Each ground mycelia sample was boiled in 5 mL of 33% KOH for 20 min. After cooling, samples were centrifuged at 3000×*g* for 15 min and the supernatant was poured through cheesecloth into fresh tubes. Ethanol (6 mL g^{-1} original mycelia sample) was added to the supernatant followed by vigorous agitation. Samples were then subjected to centrifugation at 3000×*g* for 15 min. The supernatant was discarded and pellets were re-suspended in 300 µl distilled water and stored at −20 °C. The reaction was carried out by adding 100 µl HCl 5 N plus 2.5 mL iodine reagent (saturated $CaCl_2$ solution containing 6 mM potassium iodide and 0.8 mM iodine). This mixture was allowed to incubate at room temperature for 7 min and the absorbance was recorded at 460 nm.

EPS determination: Cultures were harvested over time and mycelial mats separated from the culture fluids by two steps of filtration. Initial filtration was done by using filter with a pore size of 250 µm to separate the water-soluble EPS from the mycelial mats. The mycelial mats embedded in the gelatinous water-insoluble EPS were subsequently incubated with 25 mL 1 M NaOH 1 h at 50 °C. After the incubation mycelial mats were separated from NaOH soluble EPS by a second filtration at reduced pressure by using a preweighed glass microfiber filter (Whatman GF/F, Maidstone, England). Mycelial weight to estimate growth was determined after the rinsed and dried (overnight at 80 °C) mycelial mats were obtained from the second filtration. For the determination of both water- and alkali-soluble EPS, 0.5 mL of crude EPS was precipitated with addition of 96% (v/v) ethanol by four times of volume, and then separated by centrifugation at 3000×*g* for 10 min. The insoluble components were re-suspended in 0.5 mL NaOH 1 M at 60 °C for 1 h after EPS solubilization, samples of 5–50 µl were taken (proper dilutions in distilled water were done when necessary) the volume was completed to 0.5 mL with distilled water, then 0.5 mL of 5% (w/v) phenol was added and the mixture shaken thoroughly. Three milliliters of 98% sulphuric acid were also added and shaken. The tubes were allowed to stand 30 min at room temperature. A blank test was prepared by substituting the solubilized EPS with distilled water. The EPS content of supernatants was calculated subtracting the amount of alcohol-insoluble carbohydrate

Author's personal copy

of uninoculated natural media. The absorbance was measured at a wavelength of 490 nm (Dubois et al., 1945). EPS yields were expressed as gram of EPS per litre of culture medium (g L^{-1}) and specific yields as milligram EPS per gram dry mycelium (mg g^{-1} mycelium).

2.4. Statistical analyses

All statistical analyses were performed by using the software STATISTICA 5.1 (StatSoft, Tulsa, Okla). All experiments were done in triplicate. Mean values and standard deviation of the means were determined. Significant differences of means were tested by using Tukey method. The adequate fitting of data to quadratic model was tested with ANOVA.

3. Results

3.1. Screening of culture media for EPS production

In a preliminary step, the effects of selected carbon and nitrogen sources were studied. Cultures were harvested periodically and the time course of fungal biomass and EPS production was determined. The day of maximal growth, volumetric EPS production and specific EPS calculated per g of dry mass of mycelium are shown in Table 2. These factors exhibited different responses in the diverse media assayed. The fungus produced EPS on all the media tested, however, EPS formation was strongly affected by the media used. When adding malt extract to the medium, EPS yields (volumetric and specific) reached maximal values. Best results were obtained by the combined addition of malt extract (10 g L^{-1}) and glucose (10 g L^{-1}) and under such conditions peaks of fungal biomass and EPS were 4.32 g L^{-1} and 2.2 g L^{-1}, respectively. However, despite the high biomass production in the peptone broth, the EPS production was the lowest observed, regardless of the amount of glucose added. When using cellulose, which is more slowly consumed than glucose, similar volumetric EPS yields were obtained, albeit after a longer cultivation time. Nevertheless, a marked high yield of specific EPS was obtained on this medium which was greater than those observed in all other synthetic media. Both lactose and sucrose resulted in low and similar EPS yields. Minimum EPS production was attained from the peptone medium. In an attempt to gain further insight into the growth and EPS production, subsequent experiments proceeded with malt extract and glucose broth, which were the media that exhibited increased EPS production.

3.2. Effect of malt concentration on fungal growth

Fungal growth (Fig. 1) was characterized for more than 50 days by following changes in mycelial biomass, glycogen content and reducing sugars. No growth was observed with ME 80 g L^{-1}. The highest biomass value (8 g L^{-1}) was attained at day 38 in the medium containing 60 g L^{-1} malt extract, lower but similar biomass production (3–4 g L^{-1}) was achieved on the same day in the other two media. In the cultures containing 60 g L^{-1} malt extract a light longer lag period was observed. Changes in reducing sugars and glycogen content of mycelia over time contributed to fungal growth responses on the three substrates. Reducing sugars exhibited a lag period until day 15 followed by a steady decrease to attain a range value of 60–47 g L^{-1} and this pattern was observed for all three media. Glycogen content displayed a pronounced peak at day 12 (100–150 μg mg^{-1} mycelium) in the three media. The prominent and statistically significant difference in response to growth among media was observed in the medium containing the highest malt extract concentration.

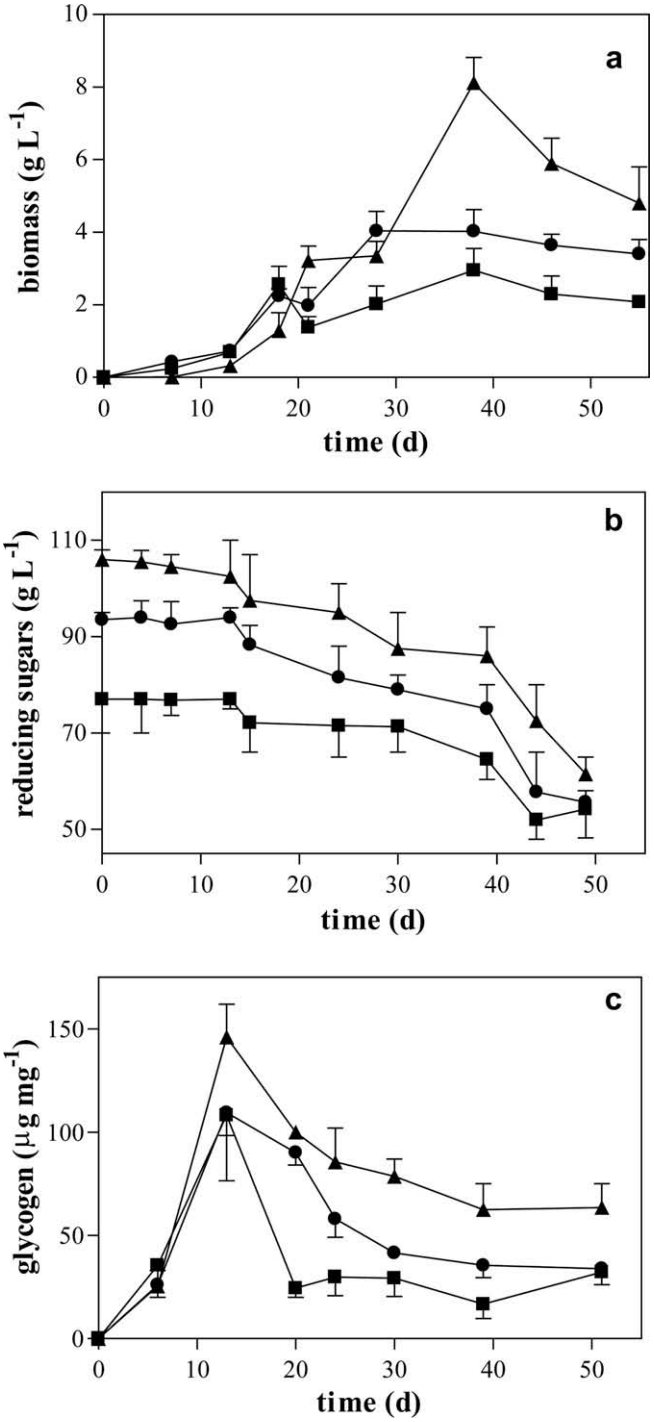

Fig. 1. Effect of malt extract concentration on fungal growth. Time courses of biomass (a), reducing sugars (b) and glycogen (c) in *G. lucidum* growing on different malt extract concentrations and 40 g L^{-1} glucose. Values are the mean of triplicate independent experiments. ■, Malt extract 20 g L^{-1}; ●, malt extract 40 g L^{-1}; and ▲, malt extract 60 g L^{-1}. Standard deviation around the mean is represented by the bars.

3.3. Effect of malt concentration on EPS production

EPS production by *G. lucidum* by using different malt concentrations ranging from 20 to 60 g L^{-1} is shown (Fig. 2). The highest yield of water-soluble EPS was 10.5 g L^{-1} in the cultivations containing ME 60 g L^{-1}, while the increase of ME from 20 to 40 g L^{-1} exhibited minimal effect. This trend was similar for alkali-soluble

Author's personal copy

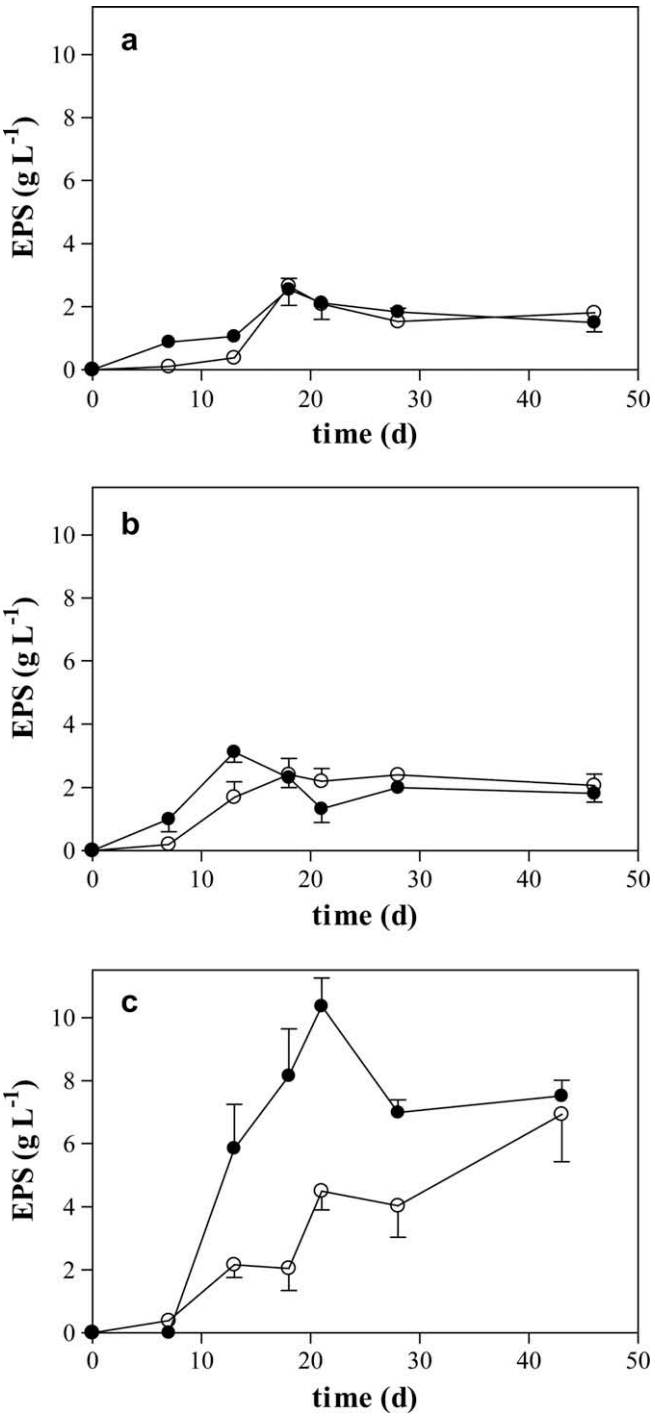

Fig. 2. Effect of malt extract concentration on water- and alkali-soluble EPS production; (a) 20 g L^{-1} (b) 40 g L^{-1}; and (c) 60 g L^{-1}. All media contained glucose 40 g L^{-1}. Values are the mean of triplicate independent experiments. ○, Alkali-soluble and ●, water-soluble. Standard deviation around the mean is represented by the bars.

EPS, which showed the highest yield (6.5 g L^{-1}) on the last sampling day in the cultivations with the highest malt concentration. The stimulatory effect of EPS production in response to ME concentration was markedly higher for water-soluble EPS. In the cultivations with ME 20 g L^{-1} the water-soluble EPS was similar to the alkali-soluble level, while in the cultivations with ME 60 g L^{-1} water-soluble EPS yield was up to 2.4 times higher than alkali-soluble EPS. The maximum level of EPS was 15 g L^{-1} at 21 days.

3.4. Combined effect of PEG, malt extract and pH

Incomplete utilization of reducing sugars occurred regardless of the ME concentrations used, suggesting that the decrease of water activity (a_w) of culture medium due to the high ME and glucose concentration played a role in EPS production. As increasing malt extract concentrations affected the water-soluble EPS more than the alkali-soluble EPS, the effect of decreasing water activity was tested by measuring this variable response. For this purpose a factorial experimental design testing pH, PEG and ME concentration on EPS production was conducted. Values of a_w were not incorporated in the model because such a factor is a result of the combination of the three factors; therefore, it should mask interactions between them. Water activity measures the availability of water for fungal growth. PEG is a non-metabolizable compound commonly used as osmotic solute (water activity depressor) and it was utilized in this study to test pure osmotic effect on EPS production. Since the three variables tested could affect fungal growth, EPS production was expressed as mg EPS per g mycelium. Cultures were harvested on days 14 and 27, to guarantee high levels of water-soluble EPS. Although a peak of EPS was observed at day 21 in the cultivations with ME 60 g L^{-1}, different media composition could delay or move forward such a peak. For this reason, cultures were harvested on two days flanking the peak of EPS production. Only data obtained in the 27-day-old cultures were analyzed and shown because they were higher in specific EPS production compared to values at day 14. The uniform design of the experiment includes different values and combinations of the factors to be tested. To account for the effect of each nutrient, the specific EPS data were fitted to a full quadratic equation. Coefficients for the regression equation are shown in Table 3. The value of R^2 together with the significant effects provide strong evidence that the model accurately reflects the process. The large value for PEG concentration in the linear term illustrates the significant positive effect of this compound. This positive linear coefficient indicates that specific EPS increased with increasing of PEG, whereas a negative effect by pH was suggested by its negative linear term. High negative or antagonistic effects between pH with the other two factors were observed. However the positive coefficient (25.5) revealed a synergistic interaction between PEG and ME. Contour plot is the usual graphic representation of data when the effect of two independent variables on the dependent variable is studied (Fig. 3). Production of EPS reached highest values at pH 3.5 while at the other four pH values, production was lower and the plots are not shown. The zone with the highest EPS production is shadowed on the Fig. 3. It was evident that high PEG and ME concentrations were necessary to achieve high EPS production. Validation of the

Table 3
Model coefficients estimated by multiple linear regression.

Coefficient	
Constant	78.35
Linear	
ME	5.51
pH	−11.61
PEG	18.65
Interaction	
ME × pH	−27.00
ME × PEG	25.50
pH × PEG	−50.34
Quadratic	
ME^2	−27.31
pH^2	−8.36
PEG^2	24.19
R^2	80.74

Author's personal copy

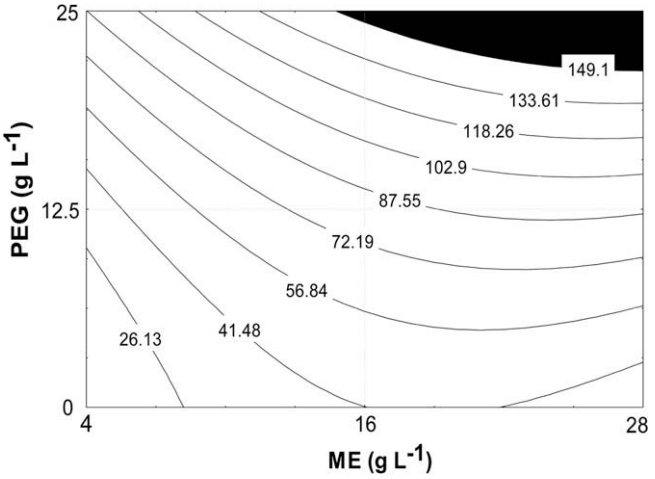

Fig. 3. Contour diagram for specific water-soluble EPS (mg EPS per g mycelium) production by *G. lucidum* as a function of malt extract and PEG concentrations; pH was fixed at 3.5. The plot is based on the equation shown in Table 3 of specific EPS production. Shadowed zones determine the conditions that optimized the production of specific EPS. Constant values are indicated on each curve.

method was also carried out, and the results showed good accordance between the predicted values and the experimental values. The experiment was carried out under the following conditions pH 3.5, 18.5 g L^{-1} ME and 23 g L^{-1} PEG and the observed value under such conditions was 139.01 ± 8.38 mg g^{-1} while the estimated value was 149.63 mg g^{-1}.

4. Discussion

The stimulatory effect of glucose in soluble EPS production was also observed in various wood decaying fungi such as *Agrocybe cylindracea* (Kim et al., 2005) *Coriolus versicolor* (Vesentini et al., 2005) *G. applanatum* (Lee et al., 2007), *Lyophyllum decastes* (Pohkrel and Ohga, 2007) and *Grifola frondosa* (Shih et al., 2008). Regarding the other tested carbon sources, great variation of responses were found in many previous reports.

Most studies dealing with the production of EPS have focused on the water-soluble EPS. The activity of water-insoluble EPS were not studied and they are a possible alternative for reclamation studies involving bioactive metabolites. Several *Pleurotus* species produced a minor water-soluble fraction and a major fraction characterized by its low solubility in water and high molecular weight that amounted to more than 80% of the total extracellular polysaccharide (Gutiérrez et al., 1996). Another fungus, *G. frondosa* also produced EPS that consisted of two fractions of different molecular sizes which varied depending on the influence of different plant oils used as substrate (Shih et al., 2008). *G. lucidum* showed not so marked differences but it yielded more similar production values of water- and alkali-soluble EPS. Total production of EPS attained a value of 15 g L^{-1} and this was lower than the highest value reported for *Aureobasidium pullulans* (Lin et al., 2007) but remarkably high when compared to reports for other high producer fungi (Maziero et al., 1995; Lim and Yun, 2006). In fact this value appears to be thus far the highest for *G. lucidum* reported to date from a low cost natural medium and without controlling any factor during the cultivation period.

The fact that the both growth and EPS production (water- and alkali-soluble) increased with increasing malt extract concentration along with the incomplete utilization of reducing sugars suggested that a_w (due to high malt extract and glucose concentration) and fungal biomass could be two additional variables influencing the EPS yields. *G. frondosa* EPS production was limited by the depletion of the carbon source (Shih et al., 2008) thus a high initial concentration of carbon source in the culture media could increase EPS production. However such high nutrient concentrations typically inhibit fungal growth (Chen et al., 2008). Growth of *G. lucidum* was totally inhibited with 80 g L^{-1} ME, while culture medium with 60 g L^{-1} ME showed not only increased biomass production but a similar lag phase compared to cultures with lower ME concentrations. Regarding the intracellular glycogen content, *G. lucidum* followed a similar pattern to that obtained for *A. pullulans* (Simon et al., 1998) that demonstrated an inverse correlation between glycogen content and exopolysaccharide (pullulan) production suggesting a link between both processes. However the exact connection has not been elucidated and further research is warranted.

Water-soluble EPS was the representative measured response used for the factorial model due to the simple method for its quantification along with its parallel incremental response for the alkali-soluble EPS. Because pH of the culture medium is a key factor that could greatly affect the fungal biomass production and in turn the EPS production, it was examined. Previous reports demonstrated the connection between the production of EPS and biomass; *Antrodia cinnamomea* and *Grifola frondosa* exhibited EPS increase that paralleled growth response (Lin and Sung, 2006; Bae et al., 2005). However, *Coriolus versicolor* and *Gloeophyllum trabeum* showed an inverse relationship between biomass and EPS production; thus, low biomass production was associated with high EPS production and vice versa. For this reason, specific EPS (mg g^{-1} mycelium) provide more information on how different culture conditions could influence the EPS enhancement production.

Since the reduction of a_w due to the high ME concentration of malt extract and glucose could be responsible for the observed EPS increase, effect of a_w reduction by a non-metabolizable compound was tested. Previous reports showed that EPS in bacteria are involved in the tolerance to water stress and such concept was extended to filamentous fungi. Thus, the production of EPS as a response to water stress (achieved after PEG addition) was studied. Although, a_w could affect EPS production, addition of PEG involved interaction with pH and ME that accounted for the obtained lack of fit of the model when a_w was evaluated as another independent factor instead of PEG concentration (data not shown). If protection against desiccation can be attributable to EPS then low values of a_w may trigger its production. Interestingly, it was observed that biomass production was not significantly ($p < 0.05$) affected (data not shown) at high PEG and ME concentrations but the highest specific EPS production was obtained under such conditions. The enhancement of EPS in *Phellinus linteus* as a response to the NaCl addition to the culture medium, which was shown in a previous report, could possible be other example of EPS induction occurring concomitantly with a dramatic decrease of growth by water stress (Zou et al., 2006). Regarding the effect of pH on EPS previous reports showed that *G. lucidum* was dramatically affected by pH of the medium. EPS production was improved in cultivations at pH 6 compared to those at pH 3 (Lee et al., 1999). However growth was negatively affected at pH 6, resulting in cell death after 8 days (Lee et al., 1999). Likely, different culture conditions e.g. bistage pH control, shaken cultures in air-lift fermentor, and a different strain could explain the differences encountered with the results observed in this study.

Although, a protective role against desiccation was associated with EPS produced by fungi; the effect of water activity on fungal EPS production was not previously investigated. Nevertheless, further studies are necessary to resolve regulation and function of EPS under low water availability.

Author's personal copy

Acknowledgements

This work was supported partly by CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas) Argentina, and Universidad de Buenos Aires.

References

Bae, J.T., Sim, J.S., Lee, B.C., Pyo, H.B., Choe, T.B., Yun, J.W., 2005. Production of exopolysaccharide from mycelial culture of *Grifola frondosa* and its inhibitory effect on matrix metalloproteinase-1 expression in UV-irradiated human dermal fibroblasts. FEMS Microbiol. Lett. 251, 347–354.

Chen, W., Zhao, Z., Chen, S.-F., Li, Y.-Q., 2008. Optimization for the production of exopolysaccharide from *Fomes fomentarius* in submerged culture and its antitumor effect in vitro. Bioresour. Technol. 99, 3187–3194.

Doehlert, D.H., 1970. Uniform shell designs. Appl. Stat. 19, 231–239.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1945. Colorimetric method for determination of sugar and related substances. Anal. Chem. 28, 350–356.

Gutiérrez, A., Prieto, A., Martínez, A.T., 1996. Structural characterization of extracellular polysaccharides produced by fungi from the genus *Pleurotus*. Carbohydr. Res. 281, 143–154.

Hseu, R.-S., Wang, H.-H., Wang, H.-F., Moncalvo, J.-M., 1996. Differentiation and grouping of isolates of the *Ganoderma lucidum* complex by random amplified polymorphic DNA–PCR compared with grouping on the basis of internal transcribed Spacer Sequences. Appl. Environ. Microbiol. 62, 1354–1363.

Hsieh, C., Tseng, M.H., Liu, C.J., 2006. Production of polysaccharides from *Ganoderma lucidum* (CCRC 36041) under limitations of nutrients. Enzyme Microb. Technol. 38, 109–117.

Jong, S.C., Birmingham, J.M., 1992. Medicinal benefits of the mushroom *Ganoderma*. Adv. Appl. Microbiol. 37, 101–134.

Kim, H.O., Lim, J.-M., Joo, J.H., Kim, S.W., Hwang, H.J., Choi, J.W., Yun, J.W., 2005. Optimization of submerged culture condition for the production of mycelial biomass and exopolysaccharides by *Agrocybe cylindracea*. Bioresour. Technol. 96, 1175–1182.

Kim, H.M., Park, M.K., Yun, J.W., 2006. Culture pH affects exopolysaccharide production in submerged mycelial culture of *Ganoderma lucidum*. Appl. Biochem. Biotechnol. 134, 249–262.

Krisman, C.R., 1962. A method for the colorimetric estimation of glycogen with iodine. Anal. Biochem. 4, 17–23.

Lee, K.M., Lee, S.Y., Lee, H.Y., 1999. Bistage control of pH for improving exopolysaccharide production from mycelia of *Ganoderma lucidum* in an air-lift fermentor. J. Biosci. Bioeng. 88, 646–650.

Lee, W.Y., Park, Y., Ahn, J.K., Ka, K.H., Park, S.Y., 2007. Factors influencing the production of endopolysaccharide and exopolysaccharide from *Ganoderma applanatum*. Enzyme Microb. Technol. 40, 249–254.

Liang, J., Melican, D., Cafro, L., Palace, G., Fisette, R., Armstrong, R., Patchen, M.L., 1998. Enhanced clearance of a multiple antibiotic resistance *Staphylococcus aureus* in rats treated with PGG-glucan is associated with increased leukocyte counts and increased neutrophil oxidative burst activity. Int. J. Immunopharmacol. 20, 595–614.

Lim, J.-M., Yun, J.-W., 2006. Enhanced production of exopolysaccharides by supplementation of toluene in submerged culture of an edible mushroom *Collybia maculata* TG-1. Process Biochem. 41, 1620–1626.

Lin, E.-S., Sung, S.-C., 2006. Cultivating conditions influence exopolysaccharide production by the edible Basidiomycete *Antrodia cinnamomea* in submerged culture. Int. J. Food Microbiol. 108, 182–187.

Lin, Y., Zhang, Z., Thibault, J., 2007. *Aureobasidium pullulans* batch cultivations based on a factorial design for improving the production and molecular weight of exopolysaccharides. Process Biochem. 42, 820–827.

Maziero, R., Bononi, V.L., Adami, A., Cavazzoni, V., 1995. Exopolysaccharide and biomass production in submerged culture by edible mushrooms. In: Proceedings of the 14th International Congress on the Science and Cultivation of Edible Fungi, pp. 887–891.

Money, N.P., 1989. Osmotic pressure of aqueous polyethylene glycols. Relationship between weight and vapour pressure deficit. Plant Physiol. 91, 766–769.

Pohkrel, C.P., Ohga, S., 2007. Submerged culture conditions for mycelial yield and polysaccharides production by *Lyophyllum decastes*. Food Chem. 107, 641–646.

Ruel, K., Joseleau, J.P., 1991. Involvement of an extracellular glucan sheath during degradation of *Populus* wood by *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. 57, 374–384.

Russell, R., Paterson, M., 2006. *Ganoderma* – a therapeutic fungal biofactory. Phytochemistry 67, 1985–2001.

Schnider-Keel, U., LejbØlle, K.B., Baehler, E., Haas, D., Keel, C., 2001. The sigma factor AlgU (AlgT) controls exopolysaccharide production and tolerance towards desiccation and osmotic stress in the biocontrol agent *Pseudomonas fluorescens* CHA0. Appl. Environ. Microbiol. 67, 5683–5693.

Selbmann, L., Stingele, S., Petruccioli, M., 2003. Exopolysaccharide production by filamentous fungi: the example of *Botryosphaeria rhodina*. Antonie Van Leeuwenhoek 84, 135–145.

Shih, I.-L., Chou, B.-W., Chen, C.-C., Wu, J.-Y., Hsieh, C., 2008. Study of mycelial growth and bioactive polysaccharide production in batch and fed-batch culture of *Grifola frondosa*. Bioresour. Technol. 99, 785–793.

Simon, L., Bouchet, B., Bremond, K., Gallant, D.J., Bouchonneau, M., 1998. Studies on pullulan extracellular production and glycogen intracellular content in *Aureobasidium pullulans*. Can. J. Microbiol. 44, 1193–1199.

Sone, Y., Okuda, R., Wada, N., Kishida, E., Misaki, A., 1985. Structure and antitumor activities of the polysaccharide isolated from fruiting body and the growing culture of mycelium of *Ganoderma lucidum*. Agric. Biol. Chem. 49, 2641–2653.

Sutherland, I.W., 1996. Extracellular polysaccharides. In: Rhem, H.J., Reed, G. (Eds.), Biotechnology, vol. 6. VCH, Weinheim, pp. 615–657.

Tang, Y.J., Zhong, J.J., 2002. Fed-batch fermentation of *Ganoderma lucidum* for hyperproduction of polysaccharides and ganoderic acid. Enzyme Microb. Technol. 31, 20–28.

Tzianabos, A.O., 2000. Polysaccharide immunomodulators as therapeutic agents: structural aspects and biologic function. Clin. Microbiol. Rev. 13, 523–533.

Vesentini, D., Dickinson, D.J., Murphy, R.J., 2005. The production of extracellular mucilaginous material (ECMM) in two wood-rotting basidiomycetes is affected by growth conditions. Mycologia 97, 1163–1170.

Vesentini, D., Dickinson, D.J., Murphy, R.J., 2007. The protective role of the extracellular mucilaginous material (ECMM) from two wood-rotting basidiomycetes against copper toxicity. Int. Biodet. Biodegrad. 60, 1–7.

Wakshull, E., Brunke-Reese, D., Lindermuth, J., Fisette, L., Nathans, R.S., Crowley, J.J., Tufts, J.C., Zimmerman, J., Mackin, W., Adams, D.S., 1999. PGG-glucan, a soluble β-(1,3)-glucan, enhances the oxidative burst response, microbicidal activity, and activates an NF-kappa B-like factor in human PMN: evidence for a glycosphingolipid β-(1,3)-glucan receptor. Immunopharmacology 41, 89–107.

Zou, X., Sun, M., Guo, X., 2006. Quantitative response of cell growth and polysaccharide biosynthesis by the medicinal mushroom *Phellinus linteus* to NaCl in the medium. World J. Microbiol. Biotechnol. 22, 1129–1133.