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Exopolysaccharide from *Agaricus brasiliensis LPB* and its Scale Up Studies in a Stirred Tank Fermenter

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Abstract

Background and purpose: Although the activities of polysaccharides such as beta-glucans are well known in Agaricus brasiliensis, there has been little research on their exopolysaccharides and its characterization or scale up studies. The aim of the study was to study the monosaccharide composition of the exopolysaccharide (EPS) produced by Agaricus brasiliensis LPB3 and its production in a stirred tank fermenter. Materials and methods: Agaricus brasiliensis LPB3 was cultured in submerged fermentation in a medium containing glucose, yeast extract, K_2HPO_4 and $MgSO_4$. Extracellular polysaccharide was precipitated with four volumes of 95% ethanol. For monosaccharide composition, the EPSs were hydrolyzed, derivatized to alditol acetates, and analyzed by gas chromatography (GC) and GC-mass spectrometry (GC-MS). Results and conclusions: The exopolysaccharide produced by Agaricus brasiliensis was predominantly composed by mannose (57,7%) and galactose (28,2%) residues. The studies in a stirred tank fermenter kept the productivity obtained in laboratory scale regarding the production of exopolysaccharides (≈ 1200 mg/L). This result can be considered promising since scale up approach can decrease its yield.

Key words: *Exopolysaccharide, Agaricus brasiliensis, scale up, monosaccharide composition, stirred tank fermenter, basidiomycete*

1. Introduction

Biotechnology *Agaricus brasiliensis* is an eatable basidiomycete known worldwide by its medicinal properties [1]. Although the activities of polysaccharides such as beta-glucans are well known in *A. brasiliensis* [2], there has been little research on their exopolysaccharides (EPSs) and its characterization or scale up studies. The production and characterization of the EPS produced by *A. brasiliensis* in laboratory scale was already evaluated (flask of 250 mL) [3].

The scale up studies evaluate the productivity of EPSs in expanded production conditions [4], [5]. Their studies are extremely important before semi-pilot and pilot scale production

(Figure 1). Furthermore, the productivity of target molecules can be affected negatively by the scale-up process [4].

This study evaluated the expanded production conditions (scale up studies) of EPS produced by *A. brasiliensis* and their monosaccharide characterization contributing with the data obtained in laboratory scale approach. This is also the first time that a scale up study is developed for EPS production by *A. brasiliensis*.

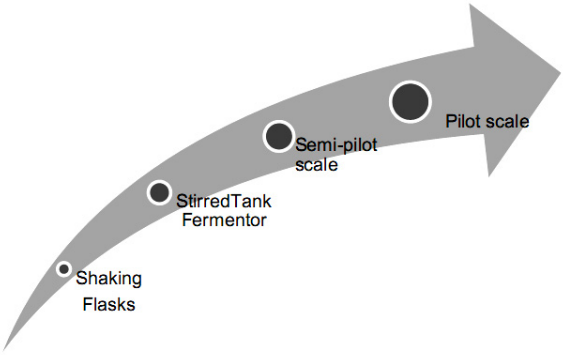

Figure 1. Flowchart for exopolysaccharide production from shaking flasks up to pilot scale

2. Materials and Methods

2.1 Strain of *A. brasiliensis* LPB 03

The strain was maintained on potato-dextrose agar (PDA) medium at room temperature. The inoculum was made with 5 agar block (5 mm in diameter each) in 250 ml Erlenmeyer flasks containing 50 mL of medium (pH 6.0) and incubated at 30°C for 10 days at 120 rpm. The medium was prepared with glucose (20 g/L), yeast extract (4 g/L), K_2HPO_4 (0.6 g/L) and $MgSO_4$ (0.3 g/L) [3].

2.2 Analytical methods

Biomass was determined by dry weight estimation. The culture was filtrated using 5.0 μm filter. The filtrate was concentrated in a rotary evaporator at 55°C under reduced pressure and the extracellular polysaccharide was precipitated with four volumes of 95% ethanol maintained, previously, over-night in the freezer. This mixture was left overnight at -10°C. The precipitated EPS was centrifuged at 6.000 rpm and washed twice with 95% ethanol and acetone, respectively. Polysaccharide concentration was determined by phenol-sulfuric method utilizing glucose as standard [6]. Reducing sugar was measured by Somogy-Nelson method using glucose in the standard curve [7], [8].

2.3 Monosaccharide composition

2.3.1 Acid hydrolysis

EPS (5 mg) was hydrolyzed with 1 M trifluoroacetic acid (TFA, 0.5 mL) for 5 h at 100 °C. After hydrolysis, TFA was completely evaporated at room temperature [9].

2.3.2 Reduction

The resulting monosaccharides were reduced with $NaBH_4$ [10] at room temperature for 3 h rendering the corresponding alditols. Na^+ was removed with cationic resin in the acidic form (H^+, Lewatit S-100). The solution was filtered and evaporated until complete dry. The boric acid was removed by successive co-distillation with methanol (1 mL 5x) as trimethyl borate – a volatile compound eliminated in a rotary evaporator under reduced pressure at 40°C.

2.3.3 Acetylation

The resultant alditols were acetylated [11] by the addition of 0.5 mL of pyridine and 0.5 mL of acetic anhydride in a tube for 24 h at room temperature. Ice was added to stop the reaction by degradation of the excess of acetic anhydride. The alditols acetate were extracted with chloroform (about 3 mL) and the pyridine excess was completed with $CuSO_4$ 5% resulting in pyridine sulphate (eliminated by successive washings with distillated water). The chloroform phase with the alditol acetates was collected and, after the chloroform evaporation, the sample was analyzed by GC-MS.

2.3.4 Gas Chromatography – Mass Spectrometry (GC-MS*)*

It was used a liquid-gas chomatograph VARIAN®, model 3300, coupled to a mass spectrometer FINNINGAM TRAP®, model 410, using a capillary column OV-225. Helium was utilized as array gas with a flux of 1 mL/min with initial temperature of 50°C followed by a gradient of 1°C/min until 230°C [12].

2.3.5 Stirred Tank Fermenter Scale Study

The medium was prepared with glucose 20 g/L, yeast extract 4 g/L, K_2HPO_4 0.6 g/L and $MgSO_4$ 0.3 g/L. Inoculation rate was 40 mL of suspension of mycelium per liter (inoculation rate of 4% v/v). The experiment was conducted at 30°C. The air flow rate used was 1 vvm (air volume/broth volume/min) - 100 rpm.

To evaluate the pH effect during the fermentation period, H_3PO_4 (2N) and NaOH (2N) were used. To avoid the excessive oscillation of the peristaltic pumps, a dead band of 0.05 was used. The pH was controlled at 5.0, 6.0 and 7.0. *A. brasiliensis* was cultivated with three levels of air flow, 0.5, 1.0 and 2.0 vvm. This study was done in an 8 L bioreactor (INCELTECH®) with 4 L of useful volume.

3. Results and Discussion

3.1 Monosaccharide composition

The monosaccharide composition analysis aimed to study the sugar composition of the EPS from *Agaricus brasiliensis* LBP3. The GC-chromatrogram and predominant sugars can be seen in the figures 2 and 3. The EPS is constituted by 57.7% of mannose, 28.2% of galactose, 8.3% of glucose and 5.8% of rhamnose.

This results show that the EPS is predominantly composed by mannose and galactose residues. Previous studies have also reported the presence of mannose and galactose as main residues [3].

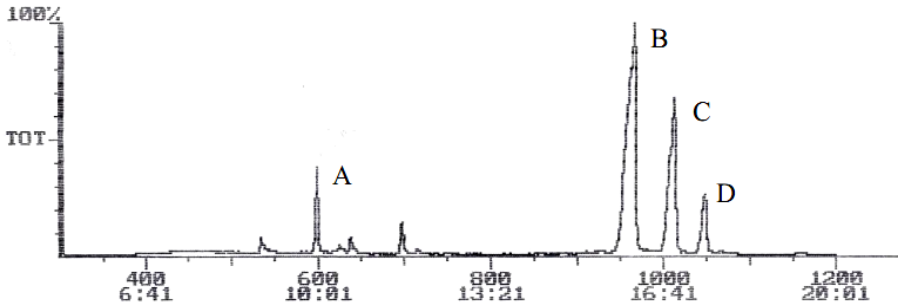

Figure 2. GC-MS analysis of EPS from *A. brasiliensis*.
A - rhamnose, B – mannose, C – galactose and D – glucose

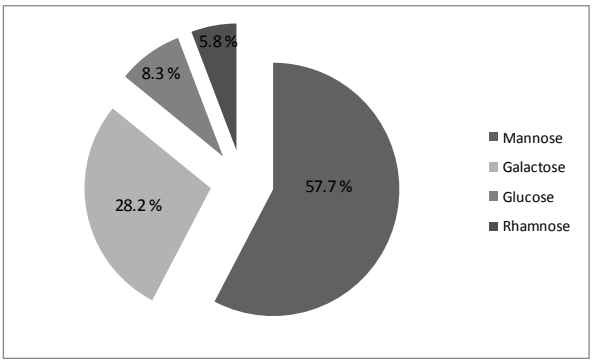

Figure 3. Monosacharide composition of the exopolysaccharide produced by *A. brasiliensis* LPB3

3.2 Stirred Tank Fermenter Scale Study

The scale up studies evaluate the EPS productivity in expanded production conditions. A kinectic was realized to know the maximum EPS production time in bioreactor (Figure 4 and 5). The maximum EPS concentration was observed at 120 h with 409.12 mg/L, 78% higher than the concentration obtained in shake flasks, and remained constant until the 8th day of cultivation.

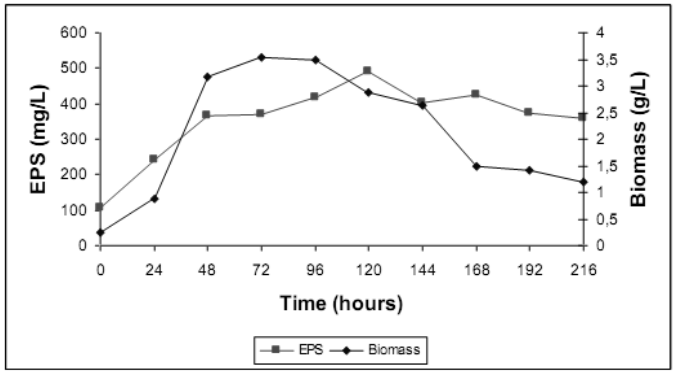

Figure 4. Kinetics of biomass and EPS production in basal media by *A. brasiliensis* LPB3 in stirred tank fermenter

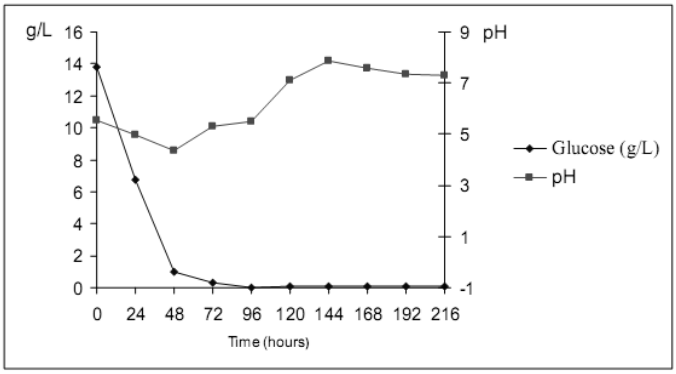

Figure 5. Broth media pH and glucose consumption in basal media by *A. brasiliensis* LPB3 in stirred tank fermenter

The maximum mycelial yield (5.35 g/L) and EPS production (1235.96 mg/L) were obtained with 2.0 vvm. The growth of *A. brasiliensis* LPB3 at different aeration rates is illustrated in Figure 6. This result is similar to that showed by previous studies [13]. They showed that higher aeration (3.5 vvm) resulted in a higher EPS productivity by *Paecilomyces tenuipes*. Other studies also reported that an intensive aeration rate and agitation is important for EPS production in *A. blazei* [14]. The results suggest that oxygen availability is desirable to *A. brasiliensis* in their submerged culture for EPS production. Aeration also results in

better homogeneity of the broth media. This condition helps to maintain constant the nutrients concentration in all points of the bioreactor and can contribute with the EPS production [15].

The effect of controlled pH on biomass and EPS production is represented in figure 7. We can observe that the ideal pH for EPS productivity allows both, biomass growth and optimal enzymatic activity (of the enzymes responsible for the polysaccharide biosynthesis) [16].

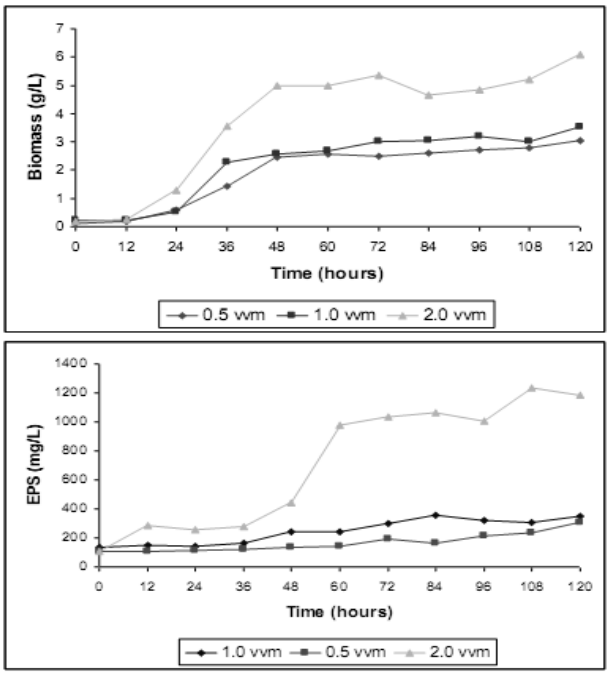

Figure 6. Different aeration rates on biomass and EPS production by *A. brasiliensis* LPB3 in stirred tank fermenter

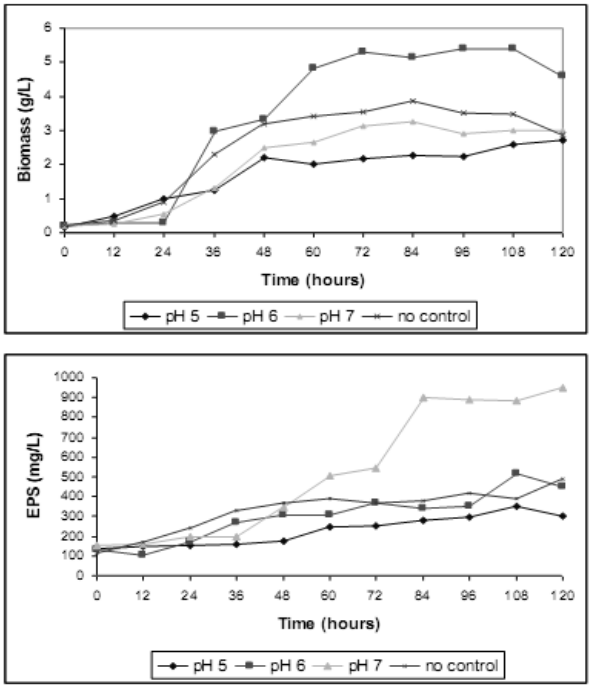

Figure 7. Effect of pH on biomass and EPS production by *A. brasiliensis* LPB3 in stirred tank fermenter

4. Conclusions

This study evaluated the expanded production conditions (scale up studies) of EPS produced by *Agaricus brasiliensis* and their monosaccharide characterization. This is the first time a scale up study is developed for exopolysaccharides produced by *A. brasiliensis*.

The EPS produced by *A. brasiliensis* is predominantly composed by mannose and galactose residues. The studies in a stirred tank fermenter kept the productivity obtained in laboratory scale regarding the EPS production.

These optimizations of production in expanded conditions are considered extremely important due to EPS application in medical field as antitumor agent.

5. Acknowledgements

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Declaration of Interest

The authors report no declaration of interest.

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