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ORIGINAL PAPER

Influence of drying methods over in vitro antitumoral effects of exopolysaccharides produced by *Agaricus blazei LPB 03* on submerged fermentation

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Abstract *Agaricus blazei* is a mushroom that belongs to the Brazilian biodiversity and is considered as an important producer of bioactive compounds beneficial to human health. Studies have demonstrated that these compounds present immuno-modulatory, antioxidant and antitumor properties. In order to compare the most used method for fungal polysaccharide drying, lyophilization with other industrial-scale methods, the aim of this work was to submit *A. blazei LPB 03* polysaccharide extracts to vaucum, spray and freeze drying, and evaluate the maintenance of its antitumoral effects in vitro. Exopolysaccharides produced by *A. blazei LPB 03* on submerged fermentation were extracted with ethanol and submitted to drying processes. The efficiency represents the water content that was removed during the drying process. The resultant dried products showed water content around 3% and water activity less than 0.380, preventing therefore the growth of microorganisms and reactions of chemical degradation. Exopolysaccharide extracts dried by vacuum and spray dryer did not showed any significant cytotoxic effect on cell viability of Wistar mice macrophages. Content of total sugars and protein decrease after drying, nevertheless, 20 mg/ml of exopolysaccharides dried by spray dryer reached 33% of inhibition rate over Ehrlich tumor cells in vitro.

Keywords *Agaricus blazei* · Macrophages cytotoxicity · Submerged fermentation · Exopolysaccharides production · Spray drying · Antitumoral effect

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Introduction

Exopolysaccharides (EPS) derived from several mushrooms have been used in traditional Oriental therapies for their antitumor properties. These compounds can be obtained directly from fruiting bodies, mycelia or fermentative broth [7, 29]. Traditionally, fruiting bodies of mushrooms are grown on solid substrates using wastes or ligno-cellulosic compounds and the cultivation is a long-term process requiring from one to several months for the first fruiting bodies to appear. The mycelium growth of mushroom conducted by submerged fermentation is a faster alternative method for obtaining quality biomass [4].

The ability to manipulate culture conditions in submerged fermentation may lead to the production of a wider range of bioactive compounds of therapeutic importance by basidiomycetes. Many new metabolites have been reported from basidiomycetes [9].

Agaricus blazei (*Agaricaceae*) is a Brazilian medicinal mushroom popularly known as the "Sun Mushroom" that it has been traditionally used as medicine [7]. *A. blazei* is used for the prevention of cancer and/or as an adjuvant with cancer chemotherapy drugs after the removal of a malignant tumor [14, 15]. Most studies on the antitumor effect of *A. blazei* have focused on polysaccharide, and the principal antitumor compound is a β-D-glucan [5, 14]. FAN et al. [6] showed that EPS extracted from *A. blazei* reached 72.19% inhibition of Sarcoma 180 in rats. According to Pinto et al. [21], subcutaneous Ehrlich tumor-bearing mice were treated with in situ inoculation of a β-glucan-rich extract of *Agaricus brasiliensis*, which reduced tumor growth.

Often, lyophilization is the method used for drying of EPS extracted from *A. blazei* and other mushrooms, and very few works use other drying methods. There is also little information about the effects over structures, molecular

parameters and bioactivities of the EPS when subjected to different drying methods and high temperatures. In order to evaluate these effects, EPS produced by *A. blazei LPB 03* on submerged fermentation were dried by different methods and evaluated about its in vitro antitumoral activity.

Materials and methods

Microorganisms

The strain of *A. blazei LPB 03* was screened and maintained by the standard stock of Laboratory of Bioprocesses and Biotechnology (LPB) at Federal University of Paraná, Curitiba, PR, Brazil. It was maintained on potato–dextrose agar medium, incubated at 30 °C for 7 days. Sub-cultures were made every 3 months to maintain the strain active.

Flask culture

The culture medium was composed (in grams per liter) of: glucose 20, yeast extract 4, K_2HPO_4 0.6 and $MgSO_4$ 0.3; the initial pH of the medium was adjusted to 6.0 [6].

Erlenmeyer flasks (250 mL) containing 50 mL medium were inoculated and incubated at 30 °C in a rotatory shaker, at 120 rpm, for 10 days, to prepare the inoculum. A 10% (v/v) of inoculum was added to the flask (500 ml) containing 200 ml of medium. The culture was incubated at 30 °C, 120 rpm and for 10 days.

Bioreactor culture

The bioreactor culture was carried out in a 8-l fermentor (Marubishi, CO) under the following conditions: 6 l of medium volume, inoculation rate 4% (v/v), temperature 30 °C, aeration rate 2.0 vvm and agitation 120 rpm. The medium was prepared with glucose 20 g/L, yeast extract 4 g/L, K_2HPO_4 0.6 g/L, $MgSO_4$ 0.3 g/L, $MnSO_4$ 0.3 g/L, $CaCl_2$ 0.05 g/L, $FeCl_3$ 0.05 g/L and olive oil 13.0 ml/L.

The processes pH was controlled at 6.0 during first 2 days and then, the set point was modified to pH 7.0 until the end of the fermentation (7 days), in order to enhance the EPS production.

Exopolysaccharide extraction

In accordance to Rubel et al. [23], the culture was filtered using Whatman 1 filter paper and washed with 20 mL of distilled water to remove metabolites adhering to mycelium. The filtrate was concentrated in a rotary evaporator at 55 °C under low pressure until ¼ of initial volume and EPS was precipitated with four volumes of 95% ethanol. This mixture was left overnight at −10 °C. Precipitated EPS was centrifuged at 4,000 rpm, washed with 95% alcohol and centrifuged twice (Fig. 1).

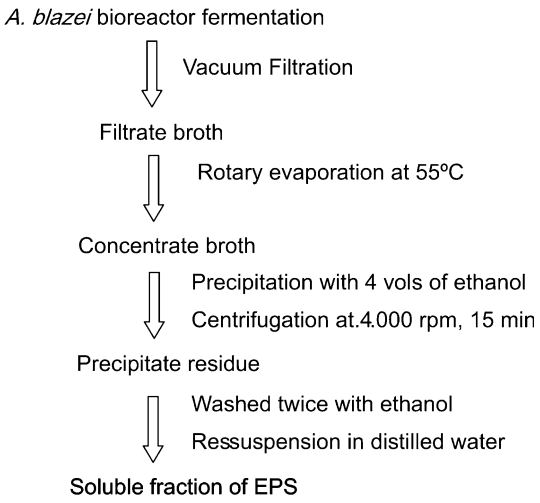

Fig. 1 Schematic extraction system of EPS from *A. blazei LPB 03* bioreactor fermentation

Finally, distilled water was added and only the soluble fraction was used in drying studies.

Drying studies

The EPS were submitted to three different methods of drying: spray drying, vacuum drying, and freeze drying. 150 mL of soluble EPS was used for each drying processes.

EPS freeze drying The sample was processed in a Modulyod Freeze Dryer 230 (Thermo Electron Corporation), with temperature of −45 °C and a negative pressure of 50 mBar.

EPS vacuum drying EPS submitted to Vacuum Cabinet Line Standard Vacucell 22, 55, 111 (MMM Group), with temperature of 50 °C and reduced pressure of 0.1 Bar.

EPS spray drying Soluble fraction submitted in Lab Plant Spray Dryer SD 05 (Labplant, West Yorkshire, UK) with 0.5 mm jet, compressed air pressure of 0.7 Bar, compressed air rate of 45 m^3/h, feed rate of 450 mL/h and inlet temperatures: 120, 180 and 240 °C. This procedure resulted in: *EPS spray drying 120 °C*, *EPS spray drying 180 °C* and *EPS spray drying 240 °C*.

Analytical methods

Analysis of reducing sugars

Reducing sugars were measured using the Somogyi–Nelson method [26], using glucose in the standard curve. Tests were made in triplicate.

Total protein dosage

The dosage of total protein was determined by Lowry method [16], using bovine albumin as standard. Tests were made in triplicate.

Water content

After each drying processes, the water content of the resulting powder was measured following the methodology described by AOAC [1]. Samples were submitted to desiccation in oven-drying at 105 °C until constant weight.

Water activity

Water activity determination was made by direct measurement, following the procedure of equipment Aqualab CX-2 Water Activity-System, making up the calibration with saturated solution of NaCl, at 24 °C. Sequential readings were taken per sample until a maximum variation of 0.003 on the value of water activity was reached.

Biological assays

Macrophages in vitro viability

Peritoneal macrophages were isolated from female Wistar mice of 20 days age by intraperitoneal injection of 10 mL ice-cold PBS (phosphate buffer solution, 10 mM, pH 7.4). The resident macrophages were harvested by peritoneal lavage, and the cells were subsequently cultured in RPMI, supplemented with 10% fetal bovine serum (Cultilab, Materials for Cell Culture Ltda., Campinas, SP, Brazil), 10 μg/mL streptomycin and 10 IU/mL penicillin (Gibco, Invitrogen Corporation, Grand Island, NY, USA). Peritoneal macrophages (3.0×10^5 cells per well) were cultivated in a 96-well plate for 24 h. EPS were added to wells in different concentrations (5, 10 and 20 mg/ml). Control group received only PBS (0.2 M, pH 7.4). Cells were cultured for 48 h at 37 °C, 5% CO_2 and 60–80% humidity in an incubator.

The macrophages viability was checked by MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay method following Mosman [19]. The reduction of MTT in the cell assesses the functional intactness of mitochondria based on the enzymatic reduction of the tetrazolium salt by the mitochondrial dehydrogenase in viable cells [8].

The absorbance was read on a multiwell spectrophotometer (Bio-Tek, Elx 800, US) at 550 nm. The percentage of viability was calculated as $[(A - B)/A \times 100]$, where A and B are the absorbance of control group and treated cells, respectively. The IC_{50} was defined as the concentration that reduced the viability of treated cells to 50% when compared to cell controls. All tests were carried out in triplicate.

In vitro proliferative activity against Ehrlich tumor cells

A single cell suspension of Ehrlich tumor cell was obtained from an ascite-bearing female Wistar mice under aseptic conditions. The suspension was centrifuged at 1,200 rpm for 10 min to obtain a cell pellet. It was resuspended with complete RPMI media. Cell viability was verified using trypan blue. Cell concentration was adjusted to 1.0×10^5 cells/mL and distributed in a 96-well plate. Cells were incubated for 24 h and different concentrations (5, 10 and 20 mg/mL) of EPS were added. The cultures were kept for 48 h at 37 °C in an incubator, which maintained a constant atmosphere of 5% CO_2. No treatment was added to the control group. Proliferation was checked by MTT assay method according to Mosman [19]. The absorbance was read on a multiwell spectrophotometer (Bio-Tek, Elx 800, US) at 550 nm. The percentage of inhibition was calculated as $[(B - A)/A \times 100\%]$, where A and B are the absorbance of control group and treated cells, respectively. The IC_{50} was defined as the concentration that reduced the viability of treated cells to 50% when compared to cell controls. All tests were carried out in triplicate.

Statistical analysis

Data were expressed as means ± standard deviation and were analyzed statistically with ANOVA. The level of significance was at a p value lesser than 0.01. Data in all the bioassays were statistically evaluated by Tukey's test by variance analysis and $p < 0.05$ was considered significant.

Results and discussion

Water content and water activity

EPS not submitted to drying process (*control group*) has a high water content inside, and therefore, present values of water activity very close to value corresponding to pure water, approximately 1.00 (Table 1). According to IAMFES [12] and Beuchat [2], samples with values of water activity above 0.80 are very susceptible to action of pathogenic and non-pathogenic microorganisms. Hence, to avoid chemical degradation and loss of biological properties, EPS must be subjected to drying processes to promote the reduction of water content in the samples.

Table 1 Values of water content and water activity of *A. blazei LPB 03* EPS submitted to different drying methods

Samples	Water content (%)	Water activity
Control group	98.28	0.964
EPS freeze	3.15	0.311
EPS vacuum	3.78	0.291
EPS spray 120 °C	8.58	0.427
EPS spray 180 °C	3.92	0.371
EPS spray 240 °C	3.16	0.336

In pharmaceutical industry, water activity in the formulations of products must be controlled to avoid reactions of darkening, oxidation hydrolytic or microbial proliferation, ensuring the quality and increasing the shelf life of these products [20, 25]. Nevertheless, after the drying processes, all methods tested managed to reduce water content and value of water activity below minimum value required for growth of microorganisms, about 0.80 [2]. This indicates that dried samples are more stable than moist ones.

For EPS drying, the three methods tested, except spray drying with inlet temperature of 120 °C, presented satisfactory results concerning water content and water activity, around 3% of humidity and less than 0.380 of water activity, respectively (Table 1).

Yield and efficiency of drying methods

Yield and efficiency of drying methods were calculated by following relations, respectively. Values of these parameters are showed on Table 2.

$$\text{Yield } (\%) = \left(\frac{\text{dry weight of solids after drying (g)}}{\text{dry weight of solids before drying (g)}}\right) \times 100\% \quad (1)$$

Table 2 Values of yield and efficiency of freeze, vacuum and spray drying of EPS produced by *A. blazei LPB 03*

Samples	Yield (%)	Efficiency (%)
EPS freeze	97.36	96.15
EPS vacuum	93.23	96.79
EPS spray 120 °C	24.11	91.27
EPS spray 180 °C	33.18	96.02
EPS spray 240 °C	42.31	96.79

obtained with 240 °C of inlet temperature. It can be noticed that, regarding the method of spray drying, by increasing the temperature, yield rises. This variation in yield was not expected, because the drying rate of spray does not depend on temperature.

In a comparative analysis, Valduga et al. [28] spray-dried mate extract at 180 °C of inlet temperature, using an equal equipment Lab Plant SD-05, and obtained 28.95% of yield; when these authors added the adjuvant gum arabic at concentration of 0.8% related to the total content of solid, the value of yield increased 25%. Rathananand et al. [22] also using Spray Dryer Lab Plant SD-05 with 140 °C of inlet temperatures dried mucoadhesive microspheres obtaining 20.02% of yield; the addition of adjuvant increased the yield value to 45.86 and 63.44%.

These studies show that the yields obtained in this work are acceptable for this specific equipment, and the addition of adjuvant could raise them. Adjuvants have the capacity to increase the amount of solid adhered to wall of equipment cyclone, through an adhesion effect. As a consequence, less material is removed by exhaust pipe [10].

The efficiency represents the water content that was removed during the drying process. In this case, again, the best result is the one closer to 100%, because the final product (dried polysaccharide) presents smaller water content. For this parameter, all drying methods tested had high results, above 90%.

$$\text{Efficiency } (\%) = \left(\frac{\text{humidity content before drying } (\%) - \text{humidity content after drying } (\%)}{\text{humidity content before drying } (\%)}\right) \times 100\% \quad (2)$$

Through the value of yield, it is possible to quantify the amount of material that was lost during drying process. Most of the values approximate to 100%, larger quantities of final product can be obtained, and the method can be considered more economically viable. Based on this, for extra-cellular polysaccharides drying, the best results were obtained by freeze and vacuum drying.

On the other hand, spray drying presented low yields for all three inlet temperatures tested, while the best result was

Reducing sugars content

Values of reducing sugars percentage present in EPS after submission to drying methods are showed in Fig. 2. *Control group* represents EPS extract not submitted to drying.

All drying methods showed a significant increase in reducing sugar content with respect to extract not subjected to drying (Fig. 2). The probable cause is the lysis of glycosidic linkages of polysaccharide polymer during drying

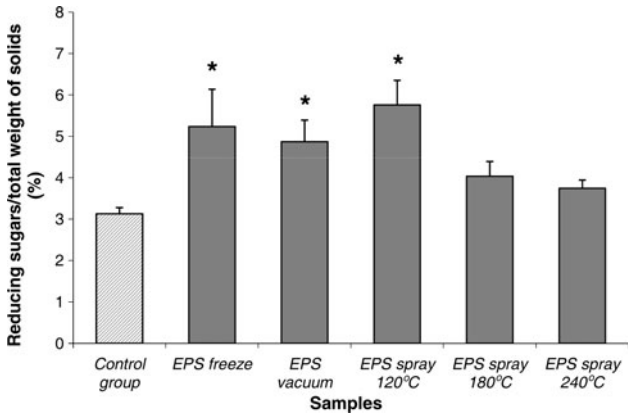

Fig. 2 Percentage of reducing sugars on *A. blazei LPB 03* extracellular polysaccharide extracts submitted to drying process. Results are mean ± standard deviation of three determinations. A difference was considered statistically significant when $*p < 0.05$ against the control group

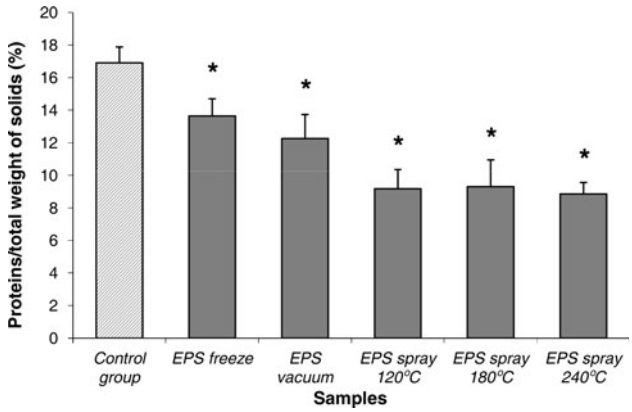

Fig. 3 Percentage of protein content on *A. blazei LPB 03* extracellular polysaccharide extracts submitted to drying process. Results are mean ± standard deviation of three determinations. A difference was considered statistically significant when $*p < 0.05$ against the control group

process, possibly caused by the action of temperature, time of process, solubility of sugars and consequent release of monosaccharides (reducing sugars). Mainly low molecular weight polysaccharides are water soluble. The solubility decreases with increasing molecular weight and capacity of association among molecules. Moreover, the higher solubility of polysaccharides happens by transfers of intermolecular hydrogen bonds between polysaccharide chains and water. This way, soluble polysaccharides can be more susceptible to hydrolyses.

β-Glucans are recognized by cell-surface receptors [3, 7]. Structural features, such as (1 → 3)-β-linkages in main chain of glucan and additional points of (1 → 6)-β-branch, they have been indicated as important factors in antitumor action. However, β-glucans that containing mainly 1 → 6 linkages exhibit less antitumoral activity, possibly due to its inherent flexibility and capacity of generating many possible conformations. Primary structure of polysaccharide is defined by composition and sequence of monosaccharides, and by the position of glycosidic linkages, as well as the nature, number and location of appendice no-carbohydrate groups [3, 30].

Studies suggest that activity of polysaccharides depends of structural conformation, size, and molecular weight. According to Mizuno et al. [18] and Yoshiyuki et al. [30], β-glucans such as lentinan (from *Lentinus edodes*), schizophyllan (from *Schyzophyllan commune*) and grifolan (from *Grifola frondosa*) could induce antitumor activity when they had high molecular weights.

Protein content

A significant decrease of protein content occurred in all polysaccharide extracts after submitted to drying processes. The values of protein percentage are showed on Fig. 3. *Control group* represents EPS extract not submitted to drying.

The decrease of protein content in polysaccharide extracts after drying was an expected result, because denaturation of some proteins that are part of extract is very likely. In addition to temperature effects, changes in the solubility and pH of the extract can modify the secondary and tertiary structures of the protein or disruption of links peptide. Considering the fact that the principal antitumor compound produced by *A. blazei* is a protein-bound polysaccharide, the β-D-glucan [17, 27, 30], a loss of proteins could lead to a lesser antitumor and immuno-modulator activities. For this reason, and analyzing this parameter alone, freeze and vacuum dryer had lower decrease on protein content than spray dryer, being, therefore, the most suitable methods of drying (Fig. 3).

It is interesting to note that the variation in inlet temperature, during the spray drying, had no significant effect on lowering the content of protein; it can be assumed that the rapid evaporation of water maintains the low temperature of the particles so that the high temperature of the drying air does not affect the product. Hong et al. [11] studied the thermal stability of spray-dried powders of extra-cellular polysaccharide from *A. blazei* extracts. According to their results using thermogravimetric analysis, the fractions of three different molecular weights of *A. blazei* extra-cellular polysaccharide showed a range of 200–400 °C of decomposition temperature.

Biological assays

Various concentrations of dried polysaccharide extracts were tested to evaluate its influence over macrophages and

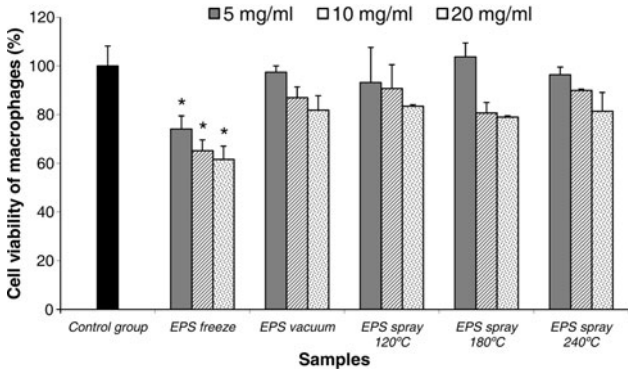

Fig. 4 Cell viability of macrophages treated with *A. blazei LPB 03* extra-cellular polysaccharides submitted to drying process. Concentrations used were 5, 10 and 20 mg/ml. Results are mean ± standard deviation of three determinations. A difference was considered statistically significant when $*p < 0.05$ against the control group

Ehrlich tumor cells proliferation. Control group received only PBS (0.2 M, pH 7.4). The absorbance obtained from control group (untreated cells) was considered as 100% of cell viability. The results were evaluated and plotted below.

Macrophages cytotoxicity evaluation in vitro

Macrophages play critical roles in host defense, including phagocytosis of pathogens and apoptotic cell, cytokines production, proteolytic processing and presentation of foreign antigens. The results showed that the relative cell survival of macrophage is a dose-dependent effect; higher concentrations of polysaccharide extracts presented higher macrophages cytotoxicity (Fig. 4).

EPS extracts submitted to freeze drying showed significant levels of cytotoxicity over macrophages proliferation at the three concentrations tested. A dose of 5 mg/ml caused 25.93% of cell death on macrophages, while at 20 mg/ml, the rate of death on viable macrophages reached 38.35%. Also, this drying process showed the lowest concentration of dried polysaccharide extract necessary to cause 50% of reduction on macrophages proliferation, a value of about 30 mg/ml (Table 3). This means that EPS extracts processed in a freeze dryer are the most damaging to mice macrophages proliferation in vitro, among the drying methods tested. The cellular toxicity can be caused by biological mediators or chemical injuries, resulting in a variety of cellular responses, such as maintenance of normal development and tissue homeostasis leading to cell death. Reducing the number of cells is a consequence of suppressed cell cycle and cell membrane damage, caused by stress from biological and biochemical factors.

On the other hand, EPS extracts dried by vacuum and spray dryer did not show any significant cytotoxic effect on cell viability of macrophages. The results indicate that up to a concentration of 20 mg/ml, EPS extracts submitted to these drying methods do not have any inhibitory influence on mice macrophages viability (Fig. 4). Macrophages are part of innate immune system and they have important role in protecting human body from type of invading cells including cancer cells. β-Glucans bind to toll-like receptors on macrophage and trigger activation processes. The mitogenic activation of macrophages by polysaccharides are characterized by usage of mitogen-activated protein kinases in the intracellular signaling events [24].

Table 3 Cytotoxic effect of *A. blazei LPB 03* extra-cellular polysaccharides over mice macrophages proliferation in vitro

Samples	Macrophages cytotoxicity (%)	CC_{50} (mg/ml)
EPS freeze (5 mg/ml)	25.93	30.03
EPS freeze (10 mg/ml)	34.81	
EPS freeze (20 mg/ml)	38.35	
EPS vacuum (5 mg/ml)	2.54	46.21
EPS vacuum (10 mg/ml)	13.01	
EPS vacuum (20 mg/ml)	18.12	
EPS spray 120 °C (5 mg/ml)	6.81	70.60
EPS spray 120 °C (10 mg/ml)	9.27	
EPS spray 120 °C (20 mg/ml)	16.53	
EPS spray 180 °C (5 mg/ml)	0.00	30.81
EPS spray 180 °C (10 mg/ml)	19.33	
EPS spray 180 °C (20 mg/ml)	21.02	
EPS spray 240 °C (5 mg/ml)	3.61	51.25
EPS spray 240 °C (10 mg/ml)	10.07	
EPS spray 240 °C (20 mg/ml)	18.61	

CC_{50} represents the half maximal effective concentration of polysaccharides that inhibits 50% of macrophages proliferation

According to Table 3, spray dryer with 120 °C of inlet temperature presented the highest CC_{50}, a value of about 71 mg/ml, indicating that high amounts of these EPS extracts are necessary to cause a decrease on mice macrophages proliferation in vitro.

Antiproliferative activity over Ehrlich tumor cells in vitro

EPS extracts submitted to drying by freeze dryer showed significant inhibitory activity over proliferation of Ehrlich tumor cells, at all three concentrations tested (Fig. 5). A rate of inhibition around 28% was verified using a dose of 5 mg/ml, and this value reached 43% when the concentration of polysaccharide used was 20 mg/ml. The concentration of dried EPS necessary to cause 50% of inhibition on cells proliferation was around 34 mg/ml (Table 4), a small dose compared to the other extracts, indicating that the drying of *A. blazei* polysaccharides by freeze dryer produces dried EPS extracts with good ability to inhibit the growth of Ehrlich tumor cells in vitro.

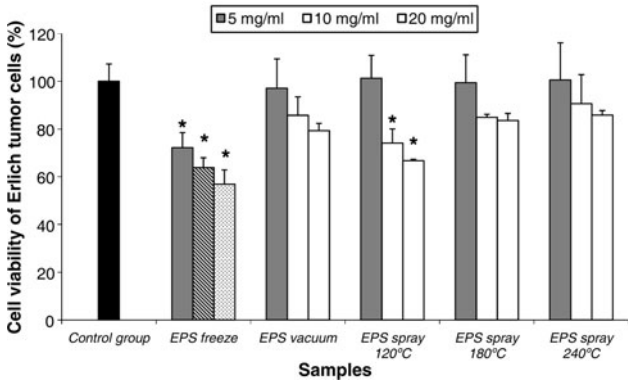

Fig. 5 Cell viability of Ehrlich tumor cells treated with *A. blazei LPB 03* extra-cellular polysaccharides submitted to drying process. Concentrations used were 5, 10 and 20 mg/ml. Results are mean ± standard deviation of three determinations. A difference was considered statistically significant when $*p < 0.05$ against the control group

The method spray dryer with inlet temperature 120 °C generated another EPS extract that also presented significant inhibitory effect over viable cells of Ehrlich tumor. However, unlike *EPS freeze*, this extract did not show any antiproliferative effect at 5 mg/ml (Fig. 5). However, *EPS freeze*, this extract show antiproliferative effect at 5 mg/ml. At the highest concentration used, 20 mg/ml, the value of inhibition rate reached 33%, and IC_{50} was 23 mg/ml, the lowest value among all EPS extracts tested (Table 4). For these reasons, spray dryer with 120 °C of inlet temperature can be considered the best method of drying taking into account the antitumor activity in vitro.

Exopolysaccharides produced by *A. brasiliensis* showed strong inhibition against Sarcoma 180. The complete regression ratio was 50% and the suppression ratio percentage was 72.19% [6]. Kim et al. [14] tested *A. blazei* extracts and that showed significantly inhibited the growth of K-562 cells dose-dependently with an IC_{50} value of 153.8 ± 2.2 g/mL, and a maximum suppression rate of 62.2 ± 4.6%.

Studies showed the influence of weight, degree of branching, conformation and intermolecular associations of β-glucans on antitumor activity and the mechanism of their action [7, 13].

The proposed mechanism by which mushroom polysaccharides exert antitumor effect include cancer preventing activity (oral administration), immune-enhancing activity and direct tumor inhibition activity. Studies indicate that incubation of polysaccharides together with tumor cells could change the expression of signals within tumor cells that could arrest the cell cycle and generate apoptosis, which explains the in vitro antiproliferative effect of polysaccharides [31].

Sarangi et al. [24] demonstrated that two fractions of *Pleurotus ostreatus* can directly kill Sarcoma 180 cells in vitro. Cell–cell adhesion determines the polarity of cells and participates in maintenance of the cell societies called tissues. Adhesion is generally reduced in human cancer cells. Reduced intercellular adhesion allows cancer cells to disobey the social order, resulting in the destruction of histological structure, which is morphological hallmark of malignant tumors. Reduced intracellular adhesiveness is also indispensable for cancer invasion and metastasis.

Table 4 Inhibitory effect of *A. blazei LPB 03* extra-cellular polysaccharides over mice macrophages proliferation in vitro

Samples	Ehrlich tumor cells inhibition (%)	IC_{50} (mg/ml)
EPS freeze (5 mg/ml)	27.80	34.29
EPS freeze (10 mg/ml)	36.12	
EPS freeze (20 mg/ml)	43.08	
EPS vacuum (5 mg/ml)	2.89	41.52
EPS vacuum (10 mg/ml)	14.24	
EPS vacuum (20 mg/ml)	20.64	
EPS spray 120 °C (5 mg/ml)	0.00	23.30
EPS spray 120 °C (10 mg/ml)	25.85	
EPS spray 120 °C (20 mg/ml)	33.25	
EPS spray 180 °C (5 mg/ml)	0.60	39.20
EPS spray 180 °C (10 mg/ml)	15.07	
EPS spray 180 °C (20 mg/ml)	16.41	
EPS spray 240 °C (5 mg/ml)	0.00	53.43
EPS spray 240 °C (10 mg/ml)	9.33	
EPS spray 240 °C (20 mg/ml)	14.12	

CI_{50} represents the half maximal effective concentration of polysaccharides that inhibits 50% of Ehrlich tumor cells proliferation

Among the groups that showed no significant inhibitory activity for any of the concentrations tested, the EPS extract obtained by spray dryer with inlet temperature of 240 °C was the worst method concerning antitumor effect, because it was the group that presented the highest dose required to reduce 50% of Ehrlich tumor cells viability, IC_{50} had a value of about 53% (Table 4).

Researchers suggest that polysaccharides from mushroom have antitumor effects via activation of different immune responses in the host rather than by directly attacking cancer cells. The effects may be indirect pathway, such as activation of immunocytes in vivo. Nonspecific host macrophages and natural killer cell defenses lead to suppression of tumor populations [13].

Conclusion

Extra-cellular polysaccharide produced by *A. blazei LPB 03* on submerged fermentation was extracted successfully with ethanol. The drying of EPS made by freeze, vacuum and spray dryer showed good results in efficiency and yield,

except for spray drying process, but these parameters can be improved by addition of adjuvants, like gum arabic. The values of water content and water activity on polysaccharides extracts submitted to drying were satisfactory, allowing the use of such methods for the production of this product in the pharmaceutical industry, because the values obtained were below the minimum necessary for the development of microorganisms and the triggering of unwanted chemical reactions.

With respect to chemical composition of the extracts submitted to drying, there was a decrease in the levels of proteins and an increase in reducing sugars content, except spray dryer with 180 and 240 °C. Such results are not positive because it can cause a decrease in the biological effect of the product, but variations on these components can be reduced by optimization of the equipments, using other conditions of drying, such as initial and final temperature of the product, water content on the extract and total time of process.

In tests of biological action in vitro, EPS extracts submitted to freeze dryer showed cytotoxic effect over mice macrophages and Ehrlich tumor cells, which may indicate that this method is not recommended for drying of *A. blazei LPB 03* extra-cellular polysaccharides, because even with antitumor activity, this product cannot be administered to patients due to the possibility of damage to the macrophages. Also on the biological aspect, spray dryer with 120 °C of inlet temperature was considered the most suitable method of drying among the tested methods, because the EPS extracts dried by this process showed no significant cytotoxicity over macrophages and presented significant inhibition over Ehrlich tumor cells viability.

This work was an initial study to evaluate the influence of the drying method on some characteristics and properties of the resulting product. Further studies should be made in order to evaluate other parameters that are modified during the process of drying, as well as evaluation of other biological effects, such as antioxidant activity.

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