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MYCELIAL BIOMASS PRODUCTION OF THE SUN MUSHROOM (*AGARICUS BLAZEI* MURRILL)

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Abstract
Agaricus blazei Murrill has indeed generated a lot of interest in the scientific community. One of the components of mushroom was a polysaccharide complex with a low molecular weight called α-1.4-Glucan, β-1.6-Glucan. The scientists reported that this polysaccharide had the strongest antitumor effect. It was selectively kill tumour cells without affecting normal cells.
Mycelian biomass is a sterile grain substrate that has been inoculated and colonized with fungal mycelium. After the substrate is fully cured, it is dried and ground, and then used for chemical determinations. Mycelian biomass contains: residual cereals, fungal mycelium, metabolites and enzymes secreted by fungal mycelium.
Mushroom mycelial biomass products contain a wide range of bioactive molecules and key immunomodulating beta-glucans and related hetero-polysaccharides. The beta-glucan levels in the studied mycelial biomass ranging from 1.17% in millet mycelial biomass to 1.79% in grain mycelial biomass.

Keywords: Agaricus blazei Murrill, polysaccharide, α-1.4-Glucan, β-1.6-Glucan.

1. INTRODUCTION

Worldwide, specialists are looking for new products from different plants as well as mushrooms, containing vitamins, minerals, enzymes to improve people's health (Firenzuoli et al., 2008).
Viewed from this point of view, mushrooms are a food of high nutritional value, containing essential amino acids in the structure of complex proteins, and some species also have real therapeutic and medicinal virtues (Farnet et al., 2013).
Nowadays, nutritional supplements and natural medicines are also provided by many mushroom-based, cultivated and medicinal basidiomycetes. *Agaricus blazei* Murrill, is a mushroom which belongs *Agaricaceae* family (*Agaricales*, *Basidiomycetes*). It is also called "almond mushroom" due to its almond odour (Firenzuoil et al., 2008; Zied et al., 2012).
A. blazei was first cultivated in the late 1800s in Eastern North America and described by Peck C.H. in 1893 (Kerrigan, 2005).
Compared with *A. bisporus* mushrooms, the water content of *A. blazei* mushrooms is lower, 89-92%, and the total dry matter is made up of crude proteins and carbohydrates. *A. blazei* mushrooms show high content of minerals eg. calcium, magnesium, zinc phosphorus and potassium (Gyorfi et al., 2010).

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Besides the macromycetes fruit bodies, bioactive compounds can also be extracted from pure mycelium culture (Chang and Miles, 2004). It has been scientifically demonstrated that *A. blazei* fungus contains bioactive compounds that can treat many diseases ex. prevention of various cancers, prevention of diabetes and hepatitis, stimulation of the immune system (Takaku et al., 2001, Firenzuoli et al., 2008).

2. MATERIALS AND METHODS

The media used for maintenance, multiplication and preservation of *Agaricus blazei* Murrill mushroom culture are PDA (potatoes-dextrose-agar), MEA (malt-extract-agar) and CEA (compost-extract-agar).

Mycelial biomass (spawn) production mainly consists of three steps: 1. substrate preparation, 2. Substrate inoculation, and 3. Incubation of the inoculated substrate for mycelial biomass production or growth the mycelium on the substrate. Preferably, fresh spawn should be used for mixing with compost for better results (Stamets, 2000).

Materials. Pure culture of *A. blazei*, cereal grains (millet, grain, sorghum), bottles (flask type), cotton (gauze), paper squares 7*7 cm, calcium sulphate (gypsum), calcium carbonate (chalk), glucose bottles / milk bottles / polypropylene bags, cotton, alkathene sheets, autoclave, laminar flow cabinet, incubator / storage room, wire gauge balance, Bunsen burner and water.

Method. Substrate preparation.

Cereal grains were soaked for one night in 2 litres of water per 1 kg of grain, and then cereal grains was washed and strained to remove all water. Sorghum seeds was steamed for 30-45 min to soften grains, then water was drained, and cereal grains were spread to cool down and decrease moisture. Three-fourth of bottle were filled with cereal grains, carefully cotton plug was prepared, tightly plugged in mouth of bottles, and leave out for ventilation. The grain was allowed to surface dry by spreading over alkathene sheets, in shade, for a few hours. The grain was mixed with chemicals (2% calcium sulphate, 0.5% calcium carbonate, on dry weight basis of the grain), to adjust pH of the grain to 7-7.8. The gain must not be coagulated at this stage. The grain-chemical mixture was filled in 500mL bottles, and then the bottles was plugged with non-absorbent cotton. The substrate was sterilized by autoclaving at 121ºC (15psi) for 30 min. The sterilization process was repeated after 24 h of first autoclaving (Stamets, 2000). The substrate was allowed to come to room temperature for making the substrate ready for inoculation (figure 1).

Figure. 1. Agaricus blazei Murrill mycelial biomass production

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Inoculation of the substrate: The substrate was inoculated with the mycelium of *A. blazei* mushroom, by transferring mycelium in agar on the grain, under aseptic (sterile) conditions. The containers were shacked after plugging, to distribute fragments of the mycelium.
Incubation: The inoculated containers were stored at 20-25ºC in darkness for 3 weeks. The containers were shacked for an even distribution of mycelium, after a few days of incubation or as soon as the mycelium is visible on grain (Stamets, 2000).
The appearance of silky whitish growth completely covering the grain, indicates the preparation of mycelial biomass.
The experimental factors and their graduation are shown below:

A - biological material with the following graduations: a1 – millet grains, a2 – wheat grains and a3 – sorghum grains

B - amendments with the following graduations: b1 – calcium sulphate 2% and calcium carbonate 0.5%, b2 – calcium sulphate 2% and b3 – calcium carbonate 0.5%

3. RESULTS AND DISCUSSIONS

The combination of experimental factors resulted in 9 variants shown in Table no. 1, each version having 3 repetitions. The average of repetitions is presented in Table 1.

Table 1. The combination of experimental factors and the average of mycelial growth

Variant	Biological material	Amendments	Mycelial growth mm/day
V1 (a1b1)	Millet grains	calcium sulphate 2% and calcium carbonate 0.5%	1.49
V2 (a1b2)	Millet grains	calcium sulphate 2%	1.27
V3 (a1b3)	Millet grains	calcium carbonate 0.5%	1.15
V4 (a2b1)	Wheat grains	calcium sulphate 2% and calcium carbonate 0.5%	1.62
V5 (a2b2)	Wheat grains	calcium sulphate 2%	1.45
V6 (a2b3)	Wheat grains	calcium carbonate 0.5%	1.37
V7 (a3b1)	Sorghum grains	calcium sulphate 2% and calcium carbonate 0.5%	1.35
V8 (a3b2)	Sorghum grains	calcium sulphate 2%	1.25
V9 (a3b3)	Sorghum grains	calcium carbonate 0.5%	1.02

Taking into account the unilateral influence of biological material on the *Agaricus blazei* Murrill mushrooms beta glucan level, we can be seen as it recorded a difference of 1.52 mg/100g d.m.

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being significant positive, to the average taken as controls (table 2) which registered 1.16 mg/100g d.m. beta-glucans.

Table 2. Unilateral biological material influence on beta-glucan content of Agaricus blazei Murrill mushrooms

Biological material	Beta-glucan mg/100g d.m.		Difference ±D mm/day	Signification of difference
	Value	%		
	1.16	100.0	0.00	Mt
Millet grains	0.95	82.5	-0.20	C (00)
Wheat grains	1.52	131.8	0.37	A (*)
Sorghum grains	0.99	85.7	-0.17	B (0)
DL / LSD (p 5%)			0.12	
DL / LSD (p 1%)			0.20	
DL / LSD (p 0.1%)			0.38	

Summary comparisons by Duncan test, the influence of biological material on the *Agaricus blazei* Murrill mycelial mass beta-glucan content, is presented in Table 3, the highest value of beta-glucan was recorded for wheat seed mycelial biomass with 1.79 mg/100g d.m., in last place was located sorghum seed mycelial biomass with 0.39 mg/100g d.m. beta-glucan content.

Table 3. Summary comparisons by Duncan test, the influence of biological material on the Agarius blazei Murrill mycelial biomass beta-glucan content

Experimental variant	Beta-glucan content Mg/100g d.m.	Significance*
V4 (a2b1)	1.79	A
V5 (a2b2)	1.45	B
V6 (a2b3)	1.33	BC
V7 (a3b1)	1.27	BC
V1 (a1b1)	1.17	CD
V8 (a3b2)	1.01	D
V2 (a1b2)	0.95	DE
V3 (a1b3)	0.74	EF
V9 (a3b3)	0.69	F

DS values 0.24-0.27
* Values marked with different letters are significant

4. CONCLUSIONS

Mushroom mycelial biomass products contain a wide range of bioactive molecules and key immunomodulating beta-glucans and related hetero-polysaccharides. The beta-glucan levels in the studied mycelial biomass ranging from 1.17% in millet mycelial biomass to 1.79% in grain mycelial biomass, on which mycelial growth is faster.
On sorghum seed, the *Agaricus blazei* Murrill mushroom mycelium is more slowest then on grain and millet, and the beta glucan contents of mycelial biomass is also smaller.

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