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Research on the biology of the *Agaricus blazei* Murrill mushroom mycelium

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Abstract For the successful cultivation of any mushroom on a small scale or commercial scale, one of the most important requirements is the mycelium of that species or variety. The spawn is a pure culture of the mycelium grown on a special medium. The main purpose of this paper was to determine the optimal physico-chemical conditions for Agaricus blazei Murill mushroom mycelium growth. We studied the influence of the temperature and the influence of pH on the Agaricus blazei Murill mycelium growth.

Key words

medicinal mushroom, mycelium,spawn, Agaricus blazei, pH, temperature

A rising star in the lexicon of medicinal mushrooms, this unique *Agaricus* was first recognized as a novel species by an American mycologist, W.A. Murrill, who found it on the lawn of Mr. R.W. Blaze in Gainesville, Florida (Murrill 1945) and re-collected by Heinemann (1993) in Brazil. (Stamets, 2005).

Taxonomic Synonims and Considerations: The mushroom that shares the closest resemblance to *Agaricus blazei* Murrill is the slender, but almond-flavored *Agaricus subrufescens* Peck, differing slightly in the shapes of the spores. The spores of *Agaricus blazei* Murrill tend to be more ovoid whereas the spores of *Agaricus subrufescens* Peck are more ellipsoid. The spores of the similar *Agaricus augustus* Fries are much larger, 7.5-10x5-6µ compared to the smaller spores, 5-4µ, seen in *Agaricus blazei* Murrill. Freshly picked, *Agaricus blazei* Murrill usually bruise bright yellowish when cut, while *Agaricus subrufescens* Peck bruises only along the outer cuticle, if at all. (Stamets, 2005, 2010)

Medicinal Properties: Anti-tumor (particularly uterocervical), immune enhancing, interferon and interleukin enhancing, anti-viral, cholesterol reducing, and blood sugar modulating (Mizuno, 1995; Mizuno et al., 1989; Kawagishi et al., 1988.). Induction of alpha tumor necrosis factors, interleukin and nitric oxide expression from macrophages was found by Sorimachi et al. (2001) in the ethanol precipitate from an extract of the mycelium. Kawakami et al. (2002) found that macrophages secreted alpha tumor necrosis factors 8 hours after exposure to A. blazei polysaccharide fractions, and 4 hours thereafter, produced nitric oxide target specific to the now weakened cancer cells.

Material and Methods

For the experiments with the *Agaricus blazei* Murrill mushroom mycelium we used a semisolid agar medium using the following formula/recipes (table no. 1):

Table 1

Recipes for culture media used at Agaricus blazei mycelium multiplication

Recipe	*Components*	*Cantity*	*Sterilization time*
1 (PDA)	Sliced potatoes Dextrose Agar Distilated water	200g 20g 20g 1000ml	1 hour la 121°C
2 (CA)	Dryed *Agaricus* compost extract Agar Distilated water	50g 20g 1000ml	1 hour at 121°C in the first day, repeated after 24 hours and 48 hours

(PDA) – potatoes dextrose agar; (CA) – compost agar.

After adjusting the pH (figure no. 1), sterilization and cooling down, the agar media was repartizated in petri dishes for solidification and inoculation (figure no. 2).

The micelyum inoculation was made under the laminar flow hood.

The biological material used in experience was a pure culture of VAB strain of *Agaricus blazei* Murrill.

Fig. 1. Preparation of culture media at different pH values.

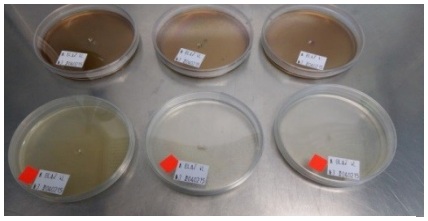

Fig.. 2. Different culture media with inoculum.

The temperature ranges used in the experience were: 10, 15, 20, 25 and 30ºC.

The pH ranges used in the experience were: 4*, 5* (* - ajusted with citric acid $C_6H_8O_7$), 6**, 7** and 8** (** - ajusted with Sodium hydroxide NaOH).

The results of experiences were recorded during 10 days of micelyum growing.

Results and Discussions

Taking into account the unilateral influence of recipe used in experience on the *Agarius blazei* Murrill mycelial growth, we can be seen as it recorded a difference of 1.72 mm/day being very significant positive, to PDA taken as controls (table no. 2) which registered value 7,36mm/day.

Table 2

Unilateral recipe influence on the growth of mycelium of *Agaricus blazei* Murill mushroom

Recipe	Mycelium growth		Difference ±D mm/day	Signification of difference
	mm/day	%		
1. PDA	7,36	100	0,00	Mt
2. CA	9,08	123,4	1,72	***
DL (p 5%)		0,20		
DL (p 1%)		0,46		
DL (p 0,1%)		1,46		

Taking into account the unilateral influence of temperature used in experience on the *Agarius blazei* Murrill mycelial growth, we can be seen that T4-25ºC recorded a difference of 8.08 mm/day being very significant positive, to T3-20ºC taken as controls (table no. 3) which registered value 8,85 mm/day.

Table 3

Unilateral temperature influence on the growth of mycelium of Agaricus blazei Murill mushroom

Temperature ºC	Mycelium growth		Difference ±D mm/day	Signification of difference
	mm/day	%		
T3 - 20ºC	8,85	100	0,00	Mt
T1 - 10ºC	0,58	6,6	-8,27	000
T2 - 15ºC	2,78	31,5	-6,07	000
T4 - 25ºC	16,93	191,3	8,08	***
T5 - 30ºC	11,95	135,0	3,10	***
DL (p 5%)		0,12		
DL (p 1%)		0,17		
DL (p 0,1%)		0.23		

In combining experimental factors, recipe and temperature, on mycelial growth we recorded significant values (table no. 4).

The influence of combined factors, temperature on the growth recipe of mycelium, at temperature T4-25ºC were recorded the highest values, regardless of the recipe used, followed by temperature T5-30º. On the last place was located temperature T1-10ºC. It can be concluded that the recipe used did not affect as much that temperature the mycelium growth.

Table 4

Combining experimental factors, recipe and temperature, on mycelial growth of Agaricus blazei Murill mushroom

Temperature ºC	Recipe	Mycelium growth mm/day	Significance*
T4 - 25ºC	CA	18,67	A
T4 - 25ºC	PDA	15,20	B
T5 - 30ºC	CA	13,17	C
T5 - 30ºC	PDA	10,73	D
T3 - 20ºC	CA	9,90	E
T3 - 20ºC	PDA	7,80	F
T2 - 15ºC	CA	3,10	G
T2 - 15ºC	PDA	2,47	H
T1 - 10ºC	PDA	0,60	I
T1 - 10ºC	CA	0,57	I

DS 0,17-0,20

* Values marked with different letters are significant

Taking into account the unilateral influence of pH value used in experience on the *Agarius blazei* Murrill mycelial growth, we can be seen that pH 6 recorded a difference of 6.08 mm/day being very significant positive, to pH 5 taken as controls (table no. 5) which registered a growth of 12,33 mm/day.

Table 5

Unilateral pH influence on the growth of mycelium of Agaricus blazei Murill mushroom

pH value	Mycelium growth		Difference ±D mm/day	Signification of difference
	mm/day	%		
5	12,33	100,0	0,00	Mt..
4	6,13	49,7	-6,20	000
6	18,42	149,3	6,08	***
7	10,83	87,8	-1,50	000
8	7,45	60,4	-4,88	000
DL (p 5%)		0,15		
DL (p 1%)		0,21		
DL (p 0,1%)		0.29		

The mycelium growing in different phases can be seen in figure no. 3.

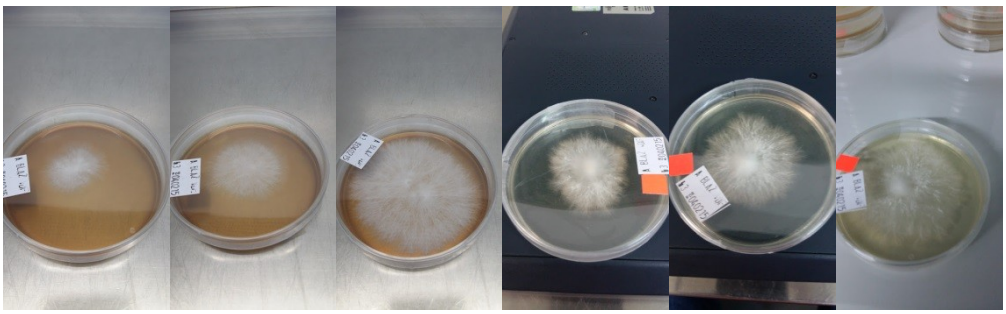

Fig. 3. The mycelium growing in different phases
Source : original photo

In combining experimental factors, recipe and pH, on mycelial growth we recorded significant values (table no. 6).

The influence of combined factors, pH on the growth recipe of mycelium, at pH value 6, were recorded the highest values, on CA recipe, followed by PDA recipe on the same pH value. On the last place was located pH 4. It can be concluded that the mycelial growt is more intensive on CA recipe.

Table 6

Combining experimental factors, recipe and pH, on mycelial growth of Agaricus blazei Murill mushroom

pH value	Recipe	Mycelium growth mm/day	Significance*
6	CA	20,10	A
6	PDA	16,73	B
5	CA	12,87	C
5	PDA	11,80	D
7	CA	10,93	E
7	PDA	10,73	E
8	CA	7,6	F
8	PDA	7,3	G
4	CA	6,8	H
4	PDA	5,47	I

DS 0,21-0,25

* Values marked with different letters are significant

Conclusions

The both recipes may be used for *Agaricus blazei* Murrill mushroom mycelium production, both with result retrieved in foreign scientific literature, wiht 8.22 mm/day mean value.

The mycelial growth is more intensive on CA recipe (13.3-18.8 mm/day) than PDA recipe (10.80-15.20 mm/day).

The *Agaricus blazei* Murrill mushroom mycelium optimal temperature for growing is situated between 25-30ºC and the pH value is 6, demonstrated by the provenance of this mushroom from tropical rain forest of Brazil.

Bibliography

Heinemann, P., 1993, Agarici Austroamericani VIII, Agaricaceae des régions intertropicales d'Amérique du Sud, Bull. Jard. Bot. Belg. 62: 355-384

Kawagishi, H., A. Nomura, T. Yumen, T. Mizuno, T. Hagwara and T. Nakamura, 1988, „Isolation and properties of a lecitin from the fruiting body of Agaricus blazei" Carbohydrate Research Nov 15:183(1):150-4

Kawagishi, H., R. Inagaki, T. Kanao, T. Mizuno, K. Shimura, H. Ito, T. Hagiwara, T. Nakamura, 1989, „Fractionation and antitumor activity of the water insoluble residue of Agaricus blazei fruiting bodies" Carbohydrate Research Mar. 15; 186(2): 267-73

Kawakami, S., K. Minato, T. Hashimoto, H. Ashida, M. Mizuno, 2002, „TNF-alpha and NO production of macrophages is enhanced through up-regulation of NF-kB by polysaccharides purified from Agaricus blazei Murrill", Proceedings of the 7th International Mycological Congress Oslo 11-17 August, pg. 55.

Mizuno, T., 1995, „Mushrooms: The versatile fungus – food and medicinal properties" in Food Reviews International, vol. 11, no. 1., Marcel Dekker, Inc., New York

Mizuno, T., H. Ito, K. Shimura, T. Hagiwara, T. Nakamura, 1989, „Preparation method of antitumor neutral polysaccharides from Himematsutake", Japan Kokai Tokkyo Koho (A), Sho 64-67195, March 13, 565-568

Murrill,W.A., 1945, „New Florida Fungi", Journal of Florida Academy of Science, vol. 8, no. 2, pp. 175-198

Sorimachi, T, T. Hagiwara, T. Nakamura, 2001, Induction of alpha tumor necrosis factors, interleukin and nitric oxide expression from macrophages Japan Kokai Tokkyo Koho (A), Sho 73-67675, Apr 25, 425-432

STAMETS, P., 2005, Mycelium running – How the mushrooms can help save the world, Ten Speed Press, Berkeley

STAMETS, P., 2010, Growing gourmet and medicinal mushrooms, Ten Speed Press, Berkeley