

## ESTABLISHING THE MOST EFFECTIVE GROWTH MEDIUM FOR MYCELIUM FOR THE DEVELOPMENT OF MYCELIAL PRODUCTS

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### ABSTRACT:

The morphology and growth rate of 4 species of edible and medicinal mushrooms from starter cultures purchased from Everything Mushroom (*fig.1*) were tested using new experimental techniques for submerged cultures. The optimal incubation time, temperature, and suitable media were determined for each culture. One homemade and three synthetic liquid media with mineral and organic nitrogen sources were also tested. The effect of medium pH on the mycelial growth was tested using pH probe. In each stage of inoculum preparation we used physiologically active mycelia. Factors affecting mycelial growth of mushrooms in submerged culture were investigated. Inoculum size and composition and nutrient medium pH were important for maximising growth rate and biomass yield and minimising the duration of the lag phase of growth. Experimental techniques were tested.

Species used were: *Omphalotus nidiformis* (ghost mushroom), *Ganoderma lucidum* (Reishi), *Lentinula edodes* (Shiitake), *Laetiporus* (chicken of the wood) (*See fig. 2*)

### INTRODUCTION

Fungi have been valued throughout the world as both food and medicine for thousands of years, but their role is expanding every day in their use as building material, cosmetic product and polysaccharides. The research for this project is towards creating materials, bioplastics and fabrics developing the polysaccharide properties of the mycelial mass. For the extraction and development of a chitinous bioplastics pure mycelium is needed. The submerged pure culture techniques used in the propagation of medicinal mushrooms in China was researched and tested. The technique where the mycelia is grown in liquid media has three main applications: It is possible to obtain more mass in a short period of time, biomass that can be extracted in pure format, it can be used reused to create more culture. Therefore the investigation of the cultivation conditions on liquid media of different species and strains of medicinal mushroom was the aim of this work.

To study alternative methods of increasing biomass and fruit yield three experimental techniques were used:

1. It has been found that lightening increases the fruit yield of shiitake mushrooms. A modified petri dish was created that could take a current through agar. Current was run continuously through culture and the results were tested against a sample culture. (*fig.3*)
2. Attempts were made to contain mycelia in liquid culture inside alginate to reduce the effect of PH and temperature on the growth of the culture. This method is still under investigation. (*fig.4*)

3. Iron fillings and magnetism was used in a modified petri dish to test magnetism on the growth of mycelium. (fig.5)

## MATERIALS AND METHODS

### Culture media

To investigate the influence of pH and growth of mycelia of mushrooms in submerged culture, the following liquid media were used (Table 1) (fig.6):

Table 1. Composition of liquid media.

Nutrient	Concentration of nutrients in medium (g/l)			
	Homebrew	B	C	MEA (Standard)
Glucose (sub dextrose)	0	30	6.25	0
Peptone	0	0	0.625	0.625
Yeast extract	0	10	0	0
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0	0	0	0
MgSO <sub>4</sub> *7H <sub>2</sub> O	0	5	0.625	0
CaCl <sub>2</sub> *2H <sub>2</sub> O	0	0	0	0
K <sub>2</sub> HPO <sub>4</sub>	0	0	0	0
KH <sub>2</sub> PO <sub>4</sub>	0	1.0	1.25	0
Malt Extract	0	0	6.25	5.0
Manuka Honey	20	0	0	0
Bovril	10	0	0	0
Agar	10	0	0	0
H <sub>2</sub> O	1 000g	1 000g	1 000g	1 000g

We referred to growth of some species of medicinal mushrooms in submerged cultures by methods described previously (Solomko and Sasek 1984, Solomko 1992, Weis *et al.* 1999).

The media were sterilised in an autoclave at 121°C in customised mason jars that used polyfil as air filter and a silicon gasket for sterile injection of cultures with syringe.

Submerged cultivation was done in 100 ml of media in mason jar, incubated at 26°C on a rotary shaker at 120 rpm. 10 ml of homogenised mycelium representing a biomass of 0.9 g/l dry matter were used as inoculum. At the end of the mycelium was collected in porous glass funnels, washed with distilled water, and dried at 105°C to a constant weight. (fig.7)

To further investigate growth rate and morphological and physiological characteristics of colonies encouraged with electrical current and magnetism, the following agar media were used:

- 1) Glucose 20g, Agar 20g, Malt Extract 5g, NaCl 0.25g

All agar media were sterilised for 30 min. at 121°C then cooled and poured into sterile petri dishes. In compliance with the current methodology of Weis *et al.* (1999), one agar disk (5 mm diameter) colonised by mycelium in the active physiological state was used to inoculate each

dish.

Copper modifications to petri dishes were also sterilised.

### Statistical analyses

Three variants of medium per species studied were used.

Growth of the colonies was monitored every 4 to 5 days and recorded.

Cultures were either kept on rotatory shaker or removed in stored in sterile incubator.

Petri dishes were kept in a sterile environment and growth monitored.

## RESULTS

The rates of radial mycelial growth of 4 species of e mushrooms on different natural and commercial media were studied and analysis of data is listed in Table 2. (fig. 8)

Table 2.

Media	Started		New Culture	
<b>O. nidiformis</b>	11/10/2014	11/16/2014	11/17/2014	11/27/2014
Medium A		Great		full coverage
Medium B		Medium		in liquid - good growth-almost half culture - fridge
Medium C			Started	No growth
Medium A			Started	great white growth almost full width on solid agar
Medium MEA			Started	
<b>G.lucidum</b>	11/10/2014	11/16/2014	11/17/2014	11/27/2014
Medium A		Good		good growth - top white - in fridge
Medium B		Medium		good growth in liquid - fridge
Medium C			Started	
Medium A			Started	no growth
Medium MEA			Started	Great cloud in liquid on shaker
<b>Laetiporus</b>	11/10/2014	11/16/2014	11/17/2014	11/27/2014
Medium A		Good		medium growth in fridge - white on top
Medium B		Good		Good Growth on solid agar.
Medium C			Started	none visible
Medium A			Started	
Medium MEA			Started	
<b>Lentinula</b>	11/10/2014	11/16/2014	11/17/2014	
Medium A		Poor		none
Medium B		Agar - Good		slow growth but visible on solid agar
Medium C			Started	
Medium A			Started	
Medium MEA			Started	none

## **Liquid Culture results:**

### ***Omphalotus nidiformis* (ghost mushroom):**

Showed best results on homebrew of domestic nutrition. Culture preferred to grow on surface of liquid culture.

(Fig. 3, 3.1, 3.2)

### ***Ganoderma lucidum* (Reishi):**

Showed best result on standard, commercially supplied MEA.

Culture preferred to grow submerged in liquid culture.

(Fig. 4, 4.1, 4.2, 4.3)

### ***Lentinula edodes* (Shiitake)**

Showed no results in liquid culture. Preferred solid agar.

### ***Laetiporus* (chicken of the wood) :**

Showed best results on scientifically researched nutrition media.

Culture preferred to grow on surface of liquid culture. (Show growth results 3 images)

(Fig. 5, 5.1, 5.2)

## **Modified Petri Dish results:**

### ***Electrical Current:***

The petri dish with electrical current produced rapid results, but it is unclear whether it was the result of the current or the nutrition. More studies are necessary. (fig 6) .

### ***Magnetism:***

Test set. No Results (fig.7)

### ***Alginate Containers:***

Initial test were done to establish the viability of creating liquid culture containers with alginate.

The intention was to create gel enclosure of mycelium mixed with Calcium Carbonate ( $\text{CaCO}_3$ ) and then submerged in Sodium Alginate ( $\text{C}_6\text{H}_8\text{O}_6$ )<sub>n</sub>

However the correct ratio of liquid culture to Calcium carbonate was not correct and gellification failed to happen. More research is needed. (fig. 8)

## **CONCLUSION**

Submerged cultivation in liquid media of mushroom mycelium is proving to a promising method which is delivering results better than expected. As with all biological processes growth is slow

and we are hoping to delve deeper into the physiological makeup of the mycelium in the next phase of the project and start exploring the potential of the pure mycelial biomass.

**LIST OF IMAGES:**

**Fig 1: Starter Culture**



**Fig 2: Species used**

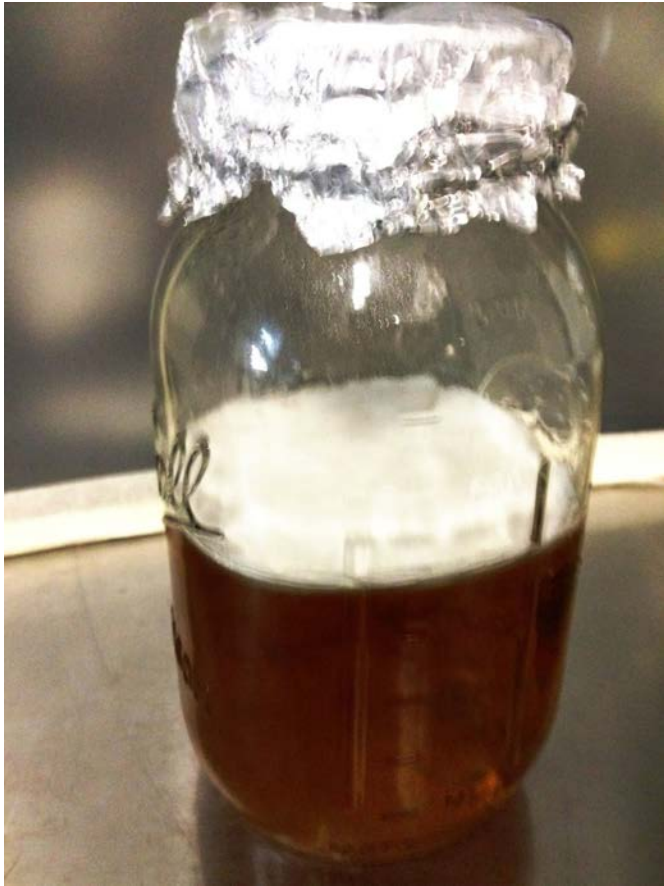




3



3.1



3.2

**Fig. 3, 3.1, 3.2:** Ghost Fungi, liquid culture

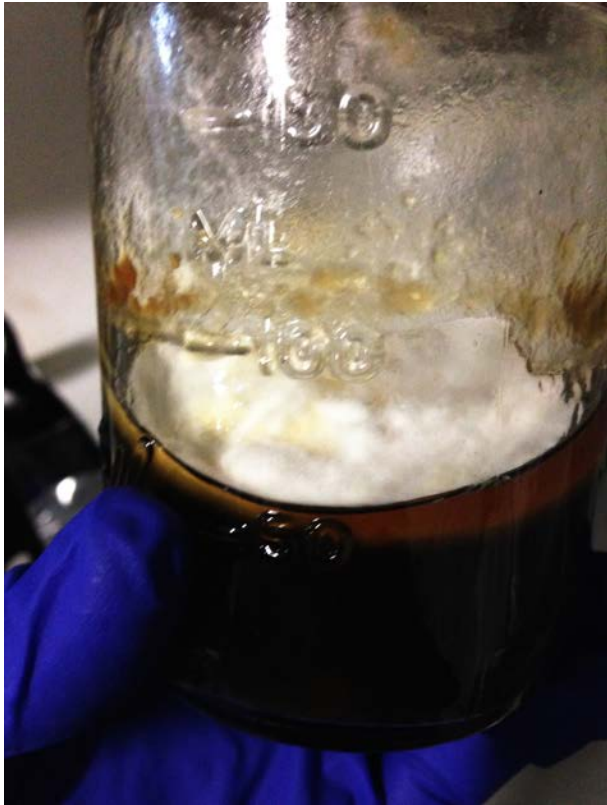


4



4.1





4.2



4.3

**Fig. 4, 4.1, 4.2:** Ganoderma fungi, liquid culture





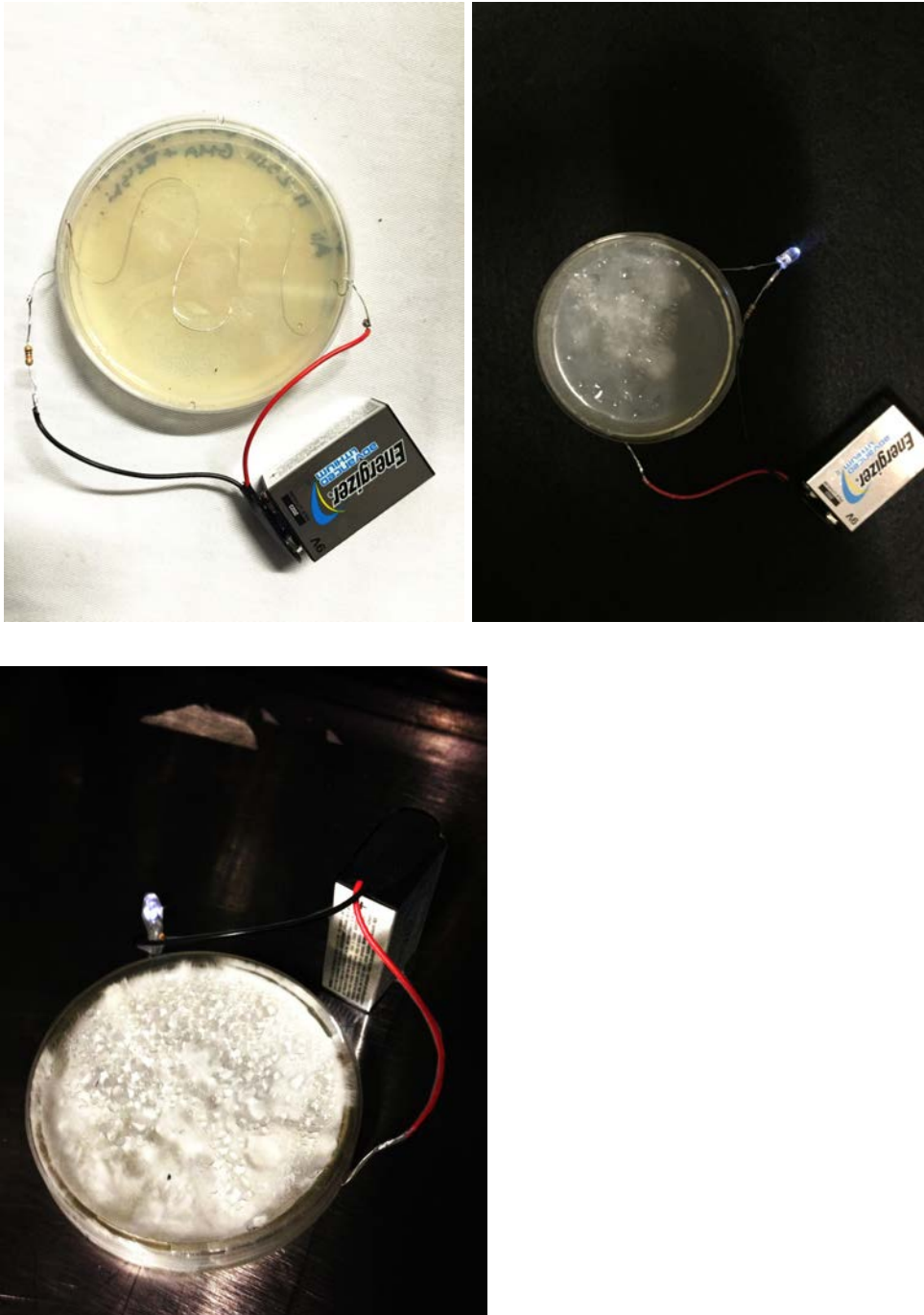
5.1



5.2

**Fig. 5, 5.1, 5.2:** Chicken of the wood fungi, liquid culture

Fig 6 : Electrical Current supplied to Agar



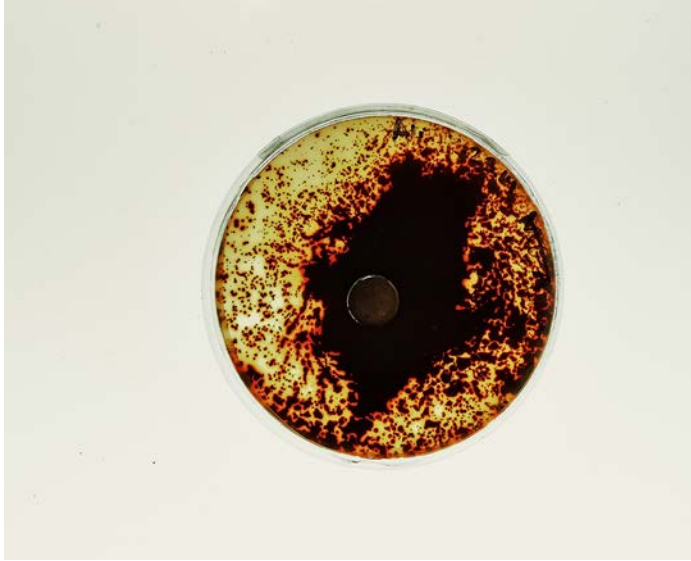


Fig 7: Magnetism effect on mycelial growth



Fig 8: Algininate Container Test.

## REFERENCES:

- M. L. Lomberh, E. F. Solomko, A. S. Buchalo, B. Kirchhoff. 2002. Studies of medicinal mushrooms in submerged cultures. *Acad. Sci. Ukraine, M.G. Kholodny Inst. of Botany, Kyiv*
- Solomko, E.F. and V. Šašek. 1984. The development of technique investigation physiology and growth kinetics of *Pleurotus ostreatus* (Fr.) Kumm. in submerged culture. *Ukr Botan Zurn. 41(4)*: 82-85.
- Solomko, E.F. 1992. A synthetic medium for the cultivation of *Pleurotus ostreatus* (Jacq.:Fr.) Kumm. In submerged culture. *Prepr. Acad. Sci. Ukraine, M.G. Kholodny Inst. of Botany, Kyiv. 23.*
- Solomko, E.F. and N. Yu. Mitropolskaja. 1994. Obtainance of seed material of *Lentinus edodes* (Berk.) Sing. by the deep cultivation method. *Micol Phytopathol. 28(3)*: 34-39.
- Solomko, E.F., M.L. Lomberh, N.Yu. Mitropolska and E.V. Cholovska. 2000. The growth of some medicinal macromycetes species on the different nutritious media. *Ukr.Botan. Journ. 57 (2)*: 119-126.
- Stamets, P. 1993. *Growing gourmet and medicinal mushrooms*. Ten Speed Press. 554
- Wasser, S.P. and A.L. Weis. 1999a. Medicinal properties of substances occurring in higher Basidiomycetes mushrooms: current perspectives (review). *International journal of medicinal mushrooms 1(1)*: 31-62.
- Wasser, S.P. and A.L. Weis. 1999b. Therapeutic effects of substances occurring in higher Basidiomycetes mushrooms: a modern perspective. *Critical Reviews in Immunology. 19*: 65-96.
- Weis, A. L., E.F. Solomko, A.S. Buchalo, S.P. Wasser,, N.Yu. Mitropolskaya, A. Ph. Grigansky and E. L. Gorovits.



**shiitake**