Scientific Papers. Series B, Horticulture. Vol. LXV, No. 2, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

ASSESSMENT OF THE GROWTH POTENTIAL IN LIQUID CULTURES OF SOME EDIBLE AND MEDICINAL MUSHROOMS

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Abstract

The purpose of this study was to assess the growth potential in liquid cultures conditions of ten edible and medicinal mushrooms named: Flammulina velutipes, Lentinus edodes, Pleurotus eryngii 2600, Pleurotus ostreatus var. Florida, Trametes versicolor, Hericium coralloides, Ganoderma lucidum, Ganoderma applanatum, Agaricus campestris and Laetiporus sulphureus. The evaluation of the mushroom growth potential was carried out using six types of culture media with different chemical composition. After 21 days from the cultures initiation, it was found that Ganoderma species had the greatest potential for the production of biomass in liquid culture conditions on all six media; also, the pH values of culture media have undergone important changes from baseline.

Key words*: fungal biomass, growth conditions, liquid culture, mushrooms.*

INTRODUCTION

Growing mushroom in submerged culture, a procedure applied in the last two decades, may result in a fast production of abundant mycelial biomass. Therefore, this method may present an important advantage over cultures practiced on solid media (Zhong & Tang, 2004; Tang et al., 2007). In addition, submerged cultivation is considered industrially efficient because many bioactive compounds found in liquid culture. Some of these compounds, such as: polysaccharides, proteins and their complexes, phenolic compounds, triterpenoids, steroids, alkaloids, have important medicinal properties (cholesterol-lowering,anti-diabetic, antioxidant, antitumor, immune-modulating, antimicrobial, and antiviral activities, etc.) (Nandi et al., 2019). Since the production of these compounds is usually associated with specific environmental conditions, *in vitro* imitation of these conditions is an effective strategy to influence secondary metabolic pathways.
Most fungi have different growth requirements. The biomass development, as well as bioactive compounds production, are strongly affected by several environmental parameters, including the composition of the culture medium (in particular the nature and concentration of the carbon source) (Lee et al., 2004; Papinutti, 2010). For bioprocessing, not much research has been carried out regarding the effects of the chemical composition of the culture media on the biomass production made by different species of edible/medicinal mushrooms grown under submerged conditions.
In this context, the aim of this study was to investigate the growth potential of some edible/medicinal mushroom species in different liquid culture media conditions for the production of mycelial biomass.

MATERIALS AND METHODS

Mushroom samples. Ten mushroom species, named: *Flammulina velutipes*, *Lentinus edodes*, *Pleurotus eryngii* 2600, *Pleurotus ostreatus* var. *Florida*, *Trametes versicolor*, *Hericium coralloides, Ganoderma lucidum*, *Ganoderma applanatum*, *Agaricus campestris* and *Laetiporus sulphureus* were used for experiments. The mushrooms strains were kept in the collection of Faculty of Biotechnology of the University of Agronomic Sciences and Veterinary Medicine of Bucharest. Stock

cultures were maintained in test tubes on 2% malt extract agar, at 4^0C.

Culture media and performed assays. To investigate the growth potential of mushroom mycelia in submerged culture, the following liquid media were used: malt extract broth (ME) (Merck), potato dextrose broth (PD) (Merck), potato-malt-peptone broth (PMP) (Kim et al., 2002), mushroom complete medium broth (MCM) (Kim et al., 2002), glucose - malt extract - yeast broth (GMY) (Pickard et al., 1999) and yeast – malt extract broth (YM) (Kim et al., 2002). The synthetic media composition are presented in Table 1.

Table 1. Composition of liquid media

Composition of culture media	Culture media and nutrient concentrations (g/l)					
	1 PD	2 ME	3 PMP	4 MCM	5 GMY	6 YM
Potato infusion	**4**	-	-	-	-	-
Dextrose	**20**	-	**24**	-	-	-
Peptone	-	-	**1**	**2**	-	**5**
Glucose	-	-	-	**20**	**10**	**10**
Malt extract	-	**20**	**10**	-	**3,5**	**3**
Yeast extract	-	-	-	**2**	**2,5**	**3**
KH_2PO_4	-	-	-	**0.46**	**2**	-
K_2HPO_4	-	-	-	**1**	-	-
$MgSO_4x7H_2O$	-	-	-	**0.5**	**0.5**	-
pH control	**5.0**	**5.4**	**4.6**	**5.9**	**5.2**	**5.9**

Note: PD: Potato Dextrose Broth; ME: Malt Extract Broth; PMP: Potato- Malt-Peptone medium; MCM: Mushroom complete medium; GMY: Glucose - Malt - Yeast extract medium; YM: Yeast Malt extract.

The inocula were prepared by adding actively growing mycelia from a newly prepared culture (mycelial agar discs with 0.5 cm of diameter) into 100 mL in 250 mL Erlenmeyer flask. For the submerged culture, 100 mL of the each type of liquid medium (see Table 1) were prepared in a 250 mL flask. The cultures were incubated for 3 weeks, with stirring at 110 rpm and 25°C. During the incubation period, once a week, the pH variation in the culture media was measured with a electronic pH meter WTW, inoLab® 730 and compared with the initial pH of inoculated media (pH control). At the end of the experiment, the mycelial mass was recovered from the each liquid medium by filtration and weighed in a wet state. Then, the wet biomass was dried at 70^0C, for 4 hours, and weighed again. Thus, it was established which of the culture medium was most suitable for obtaining an increased amount of fungal biomass.

Statistical analysis. Measurements on the pH value were performed in triplicate and compared with the initial pH values. The data obtained were statistically processed in Excel, by analysis of the variance (ANOVA) using the Student t-test.

RESULTS AND DISCUSSIONS

The investigation of mushrooms growth potential under submerged conditions for biomass production was performed using six variants of liquid culture media, different in terms of chemical composition. After 21 days from fungal mycelium inoculation the developed biomass was filtered, weighed and quantified as wet biomass (Figure 1).

Depending on the composition of the culture medium and the cultivated mushroom species, it can be observed that are differences in the accumulation of mycelial biomass. Also, mushroom mycelium appearance, in terms of color and consistency varies depending on the culture medium. For example, the mycelium of *L. sulphureus,* developed on the MCM and PMP media, was more compact and with spherical agglomerations on the culture medium surface. Contrariwise, the biomass developed on the PD medium had a fluffy appearance with a specific orange color of the mushroom fruiting body. On the GMY and YM culture media, the fungus retained the specific orange color, but the surface developed mycelium has a folded appearance (Figure 2).

The non-isoprenoid polyene laetiporic acid A, recently described from fruit-bodies of the fungus *Laetiporus sulphureus*, was found to be the major orange pigment also in mycelium grown in liquid culture (Davoli et al., 2005).

Between the 10 types of fungi studied, *Ganoderma* species showed the highest potential for biomass production under submerged conditions on all six variants of the tested culture media (Figure 3).

Among *Ganoderma* species, *G. applanatum* developed a significant amount of wet biomass, obtained especially on PD (30.57%), GMY (20.56%) and YM (16.28%) (See Figure 1).

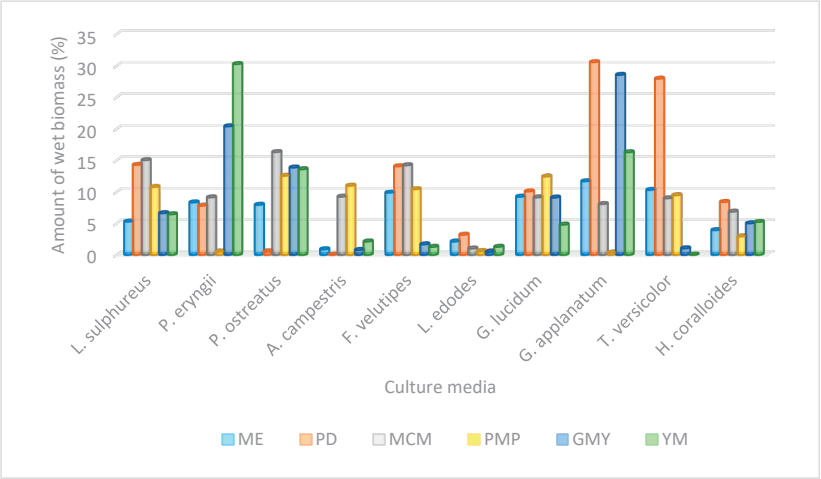

Figure 1. Amount of wet biomass harvested from the six types of liquid media
Abbreviations: ME: Malt Extract Broth; PD: Potato Dextrose Broth; MCM: Mushroom Complete Medium; PMP: Potato- Malt- Peptone medium; GMY: Glucose - Malt - Yeast extract medium; YM: Yeast Malt extract.

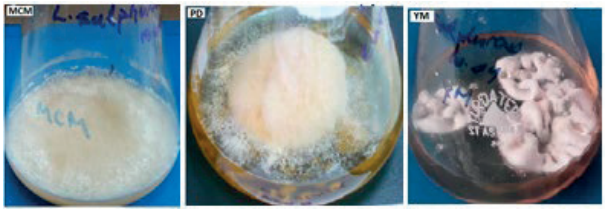

Figure 2. Appearance of *L. sulphureus* mycelia on MCM, PD and YM liquid media

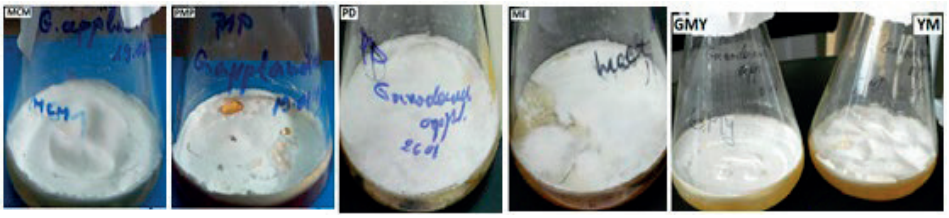

Figure 3. *G. applanatum* - abundant biomass on all types of culture media

After filtering and quantifying, the wet biomass harvested from each variant of culture medium was dried and weighed. Contrary to expectations, it has been found that the dry biomass obtained from a large amount of wet biomass is quite low. For example, *G. applanatum* which had the highest amount of wet biomass on PD (30.58%), GMY (28.56%) and YM (16.28%) media, only 5.33 g, 4.36 g and 6.04 g of dry biomass were obtained after drying. The highest amount of *P. eryngii* wet biomass was obtained on the YM medium (30.27%) and only 6.29 g of dry biomass were recorded after drying (Figure 4).

These differences could be explained due to the particularities of the resulting fungal mycelium (consistency, morphology, etc.), to the media composition and the cultural conditions, and not least of the cultivated species. Therefore, the effect of culture media on mycelium growth varies with the species of fungi.

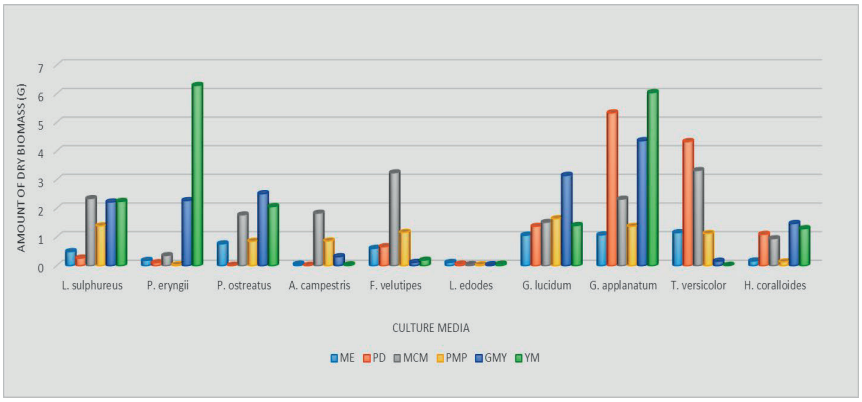

Figure 4. The amount of fungal dry biomass (g) obtained from the six types of liquid media

One of the main factors affecting the biosynthetic potential of fungi in submerged culture is the pH of the medium. This can affect cell membrane functions, uptake of different nutrients from the extracellular environment, solubility of mineral salts, ionic potential of substrates, enzymatic activity and biosynthesis products (Elisashvili, 2012). Environmental pH is an important parameter that has been shown to affect fungal morphology (Gibbs et al., 2000). For basidiomycetes, it has been reported that an optimal pH is around pH 6.0 (Kim et al., 2002). With regard to mushroom species tested, our results shown that the mycelial mass increased quite well at a pH between 4.6 and 7. At the end of the 21 days of culture, performed at the room temperature, study on pH variation in all culture media showed that pH values underwent major changes compared to the initial values. The most drastic decrease was recorded at *Laetiporus sulphureus* grown on the ME (from pH 5.4 to pH 2.1) and PD (from pH 5.0 to pH 2.0) (Figure 5). The values between the initial pH and the final pH in culture media were statistically calculated. As data shown, there are no significant differences for the MCM, PMP, GMY and YM culture media. On the contrary, in the case of ME and PD media, there are significant differences between the initial and final pH (Figure 5). The maximum biomass of *L. sulphureus* (15.04% fresh weight) was harvested from MCM medium at a pH between 5 and 6.9. Acidification of the ME and PD media could be due to the gradual accumulation in the extracellular environment of some acids released by the organism as a result of some metabolic processes which are induced by the composition of culture media (ME or PD). Regarding *P. eryngii*, it was observed that obtaining a maximum quantity of fresh biomass (30.27% on YM and 20.40% on GMY respectively) was produced at a final pH of 6.2; this mean that the fungus has adjusted the medium pH during incubation. As can be seen from the figure below, significant differences between the initial and final pH values exist only in the GMY medium (Figure 5). The strain of *P. ostreatus* var. Florida recorded a significant amount of biomass (16.31%) on MCM medium, with a final pH around 6.9. During the incubation period, the fungus adjusted the initial pH of the culture medium, releasing some alkaline compounds in the extracellular environment. The pH variation was negligible only in the MCM medium (Figure 5). Acidification of the culture medium (pH 3.27) resulted in a reduced amount of biomass (0.57% fresh mass). The maximum amount of biomass obtained from *A. campestris* was recorded on PMP medium (10.97%), at a final pH of 6.8. Although the initial pH of the PMP medium was 4.6, the fungus gradually increased the initial pH during incubation.

The only liquid media in which the pH variation registered insignificant values were: MCM, GMY and YM. *F. velutipes* recorded mycelial biomass values at a final pH between 4 and 6.1, which suggests that this fungus prefers an acidic pH. The pH variation towards neutral values (pH 7.0) resulted in very low quantities of biomass (in the GMY and YM media). The increase of the pH value with the accumulation of biomass may be due to the

elaboration in the culture medium of some basic compounds (e.g. proteins). The difference between the initial pH and the final pH was significant in all tested culture media (Figure 5). In *L. edodes* culture, a well-developed biomass was obtained on PD (3.2%) and ME (2.13%), respectively, at a pH between 3.9 and 4.3. In the case of *G. lucidum*, the pH variation (between 4.2 and 6) during the incubation period does not seem to significantly influence the amount of biomass (Figure 5).

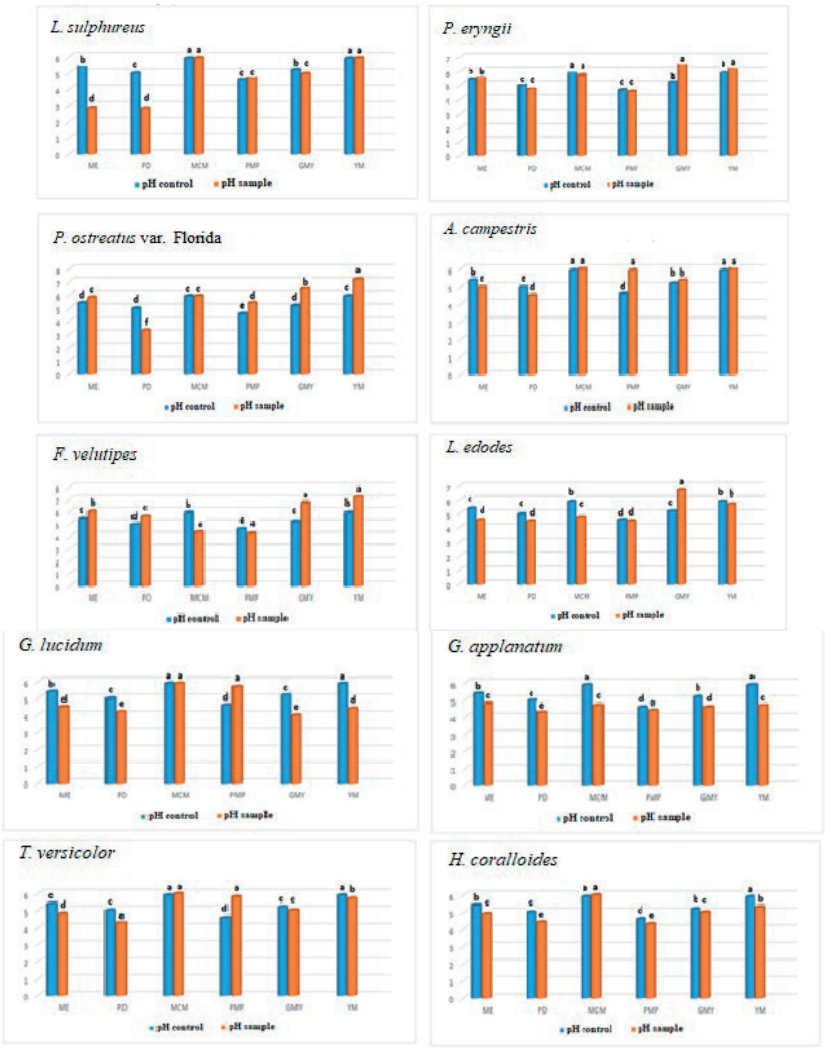

Figure 5. PH variations in submerged culture media during 21 days of incubation, at 25^{0}C

Note: The pH values were calculated based on three measurements to determine if there are significant differences from the initial pH values (pH control). Values with the same letter are not significantly different for $p < 0.05$ (Student t Test)

Insignificant values of pH variation were observed only in the MCM medium. On the other hand, the highest values of *G. applanatum* mycelial biomass were obtained when the pH value was around 4.3. During the culture period, the pH variations were around 4.3-4.7. Significant differences were observed for all culture media. Within the combinations performed for *T. versicolor* and *H. coralloides*, the maximum amount of fresh biomass was obtained on PD medium (27.94% and 8.4%

respectively). The pH variations were around 4.3 (Figure 5).

Many studies developed in recent years have shown that the mycelium growth rate, but also the production of secondary metabolites in submerged culture, depend on the chemical composition of the liquid medium and the conditions of incubation (Zhong & Tang, 2004; Lin & Sung, 2006; Fidler et al., 2013; Popa et al., 2014).

Following these results, it was possible to identify the optimal culture medium for each tested mushroom species, which led to a high production of mycelium biomass. Also, at the beginning and at the end of the experiment, the pH values of the culture media were recorded (Table 2).

Table 2. Optimal liquid media and pH values for maximum fungal biomass yield

Species	Culture media	Initial pH	Final pH	Fresh biomass (g / 100 ml)
Laetiporus sulphureus	MCM	5.9	6.9	15.04
Pleurotus eryngii 2600	YM	5.9	6.2	30.27
P.ostreatus Florida	MCM	5.9	6.9	16.31
Agaricus campestris	PMP	4.6	6.8	10.97
Flammulina velutipes	MCM	5.9	4.1	14.22
Lentinus edodes	PD	5.0	4.3	3.20
Ganoderma lucidum	PMP	4.6	6.2	12.43
Ganoderma applanatum	PD	5.0	4.2	30.57
Trametes versicolor	PD	5.0	4.1	27.94
Hericium coralloides	PD	5.0	4.1	8.40

PD: Potato Dextrose; PMP: Potato- Malt –Peptone; MCM: Mushroom Complete Medium; YM: Yeast-Malt.

CONCLUSIONS

The results showed that the optimal liquid media for maximum fungal biomass yield were: PD for *G. applanatum, T. versicolor, H. coralloides and L. edodes* species; MCM for *P. ostreatus* "Florida", *L. sulphureus* and *F. velutipes*; PMP for *G. lucidum* and *A. campestris* and YM culture medium only for *P. eryngii* 2600 mushroom. Study of the pH variation in the culture media during the incubation period showed that the pH values underwent major changes compared to the initial values. In general, it was found that acidification of the culture medium resulted in a reduced amount of biomass.

Liquid culture of edible and medical mushrooms is viewed as a promising alternative for efficient production of valuable metabolites for the research and development of new pharmaceutical products from mushrooms.

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