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The Effect of Initial ph on Production Mycelial Biomass of Pholiota (Strophariaceae, Basidiomycota) Species In Liquid Static Culture

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Abstract

The article presents the effect of initial pH on production mycelial biomass of Pholiota species in a liquid static culture among seven Pholiota species from the IBK Mushroom Culture Collection of M.G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine. New data on production mycelial biomass in a liquid static culture of Pholiota species are given. The growth characteristics of species depended on initial pH are shown. The most suitable pH values for each Pholiota species were find out, it ranged from 5,3 to 6,5. During cultivation, the initial pH of the nutrient media become lower. The studied Pholiota species differed not only in their ability to synthesize different amounts of biomass at optimal pH of the culture medium, but also demonstrate varying degrees of sensitivity to pH changes.

Key words: Pholiota, pH, biomass, liquid static culture, vegetative mycelium

INTRODUCTION

The genus Pholiota (Fr.) P. Kumm. belongs to the family Strophariaceae, order Agaricales, class Basidiomycetes (http://www.mycobank.org). More than 30 species are known, which are widespread worldwide [4, 16, 19]. These mushrooms are oftentimes wood destroyers, attacking forest trees as well as park trees, and their mycelia continues the destruction after the tree is cut [20]. There are more than 200 genus of mushrooms that include species useful for people, mostly because of their edible properties. There are about 100 species of fungi that can be cultivated [2]. Marketplaces are dominated by Agaricus bisporus (J.E. Lange) Imbach, *Pleurotus spp*. (Fr.) *P. Kumm*., Lentinula edodes (Berk.) Pegler, *Volvariella volvacea* (Bull.) Singer, Flammulina velutipes (Curtis) Singer, and *Pholiota nameko* (T. Ito) S. Ito & S. Imai. On the other hand, other Pholiota species still not cultivated, but the fruiting body of this mushrooms is rich in proteins, essential amino acids, dietary fiber, vitamins [7,15]. Pholiota species show notably pharmacologic activity: antimicrobial [5], antitumor [6, 8], antioxidant [14, 22] etc. There is no evidence about their toxicity so far [20].

A significant effect of environmental factors such as initial pH of nutrient media and temperature on mycelial growth of macromycetes has been documented in literature [3, 11, 21].

The acidity of nutrient media significantly influences the nature of metabolic processes of mushrooms: nutrient supply to cells, enzyme activity, pigment appearance, ability to produce metabolites, etc. The study of cultural characteristics gives the opportunity to find the optimal nutrient media for the cultivation and preservation of Pholiota species in a proper physiological state [1, 12].

Optimal conditions of cultivation can ensure the quantity and quality of the mycelia production of Pholiota species, however, such information is limited in the literature [10, 18]. This explains the relevance of our research.

In the present study, the influence of initial pH values of nutrient media for mycelial biomass production by Pholiota species are investigated and the results are described.

MATERIALS AND METHODS

Seven strains of six Pholiota species from the IBK Mushroom Culture Collection of the M.G. Kholodny Institute of Botany, National Academy of Sciences of the Ukraine were investigated [1]. Some of these strains were obtained in 2017–2018 (Table 1).

Table 1. List of the studied Pholiota species and strains

Species	*IBK* strain
Pholiota adiposa (Batsch) P.Kumm.	2169
Pholiota aurivella (Batsch) P.Kumm.	2605
Pholiota limonella (Peck) Sacc.	2335
Pholiota nameko (T.Ito) S.Ito & S.Imai	2154
Pholiota squarrosa (Vahl) P. Kumm.	2010
Pholiota subochracea (A.H.Sm.) A.H.Sm. & Hesler	2535

Mushroom cultures were grown on glucose peptone yeast (GPY) liquid media; g/l: glucose – 25.0; peptone – 3.0; yeast extract – 3.0; $MgSO_4 \times 7 H_2O$ – 0.25). Modulating additions to GPY media to maintain pH to certain values are, g/l: KH_2PO_4 – 2,0 (pH 4.0-5.5), KH_2PO_4 – 1.0; K_2HPO_4 – 1.0 (pH 6.0), K_2HPO_4 – 2.0 (pH 6.5-7.5). The acidity of all media was adjusted to certain pH values with 2,8 N KOH and 1N HCl solutions before sterilization. Control measurements of the initial pH values of the media were performed after sterilization of the nutrient media using pH-150M (RUE "Gomel Plant of Measuring Instruments", Belarus). Inoculum of studied strains was grown on GPY media with addition of 20 g/l agar. Four discs of mycelia with a diameter of 5 mm were cut with a sterile steel tube at a distance of 8-10 mm from the edge of active growth of the colony and placed in 100 ml flasks with 50 ml of a liquid medium at temperature 26 ± 0,1 ºC.

Mycelial biomass was separated from the culture liquid by filtration through a nylon filter on the 21-st day of cultivation. The final pH value was measured in the filtrate. The biomass after washing twice with distilled water was transferred to branded vials and dried to constant weight at 105 ± 0,1 °C (a.d.w.). The amount of added inoculum was determined by drying the mycelia from 4 disks [9]. The article presents average statistically reliable data from three parallel measurements. Statistical analysis was performed using the program Microsoft Excel (Microsoft Corp., Redmond, WA, USA).

RESULTS AND DISCUSSION

The results of the effect of pH on the mycelial growth of Pholiota species presented in Figure 1.

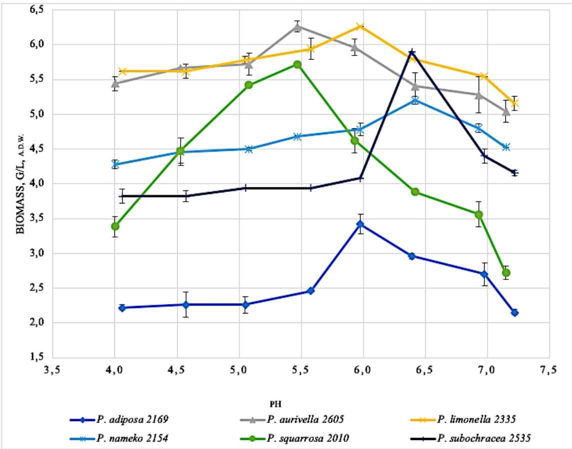

Fig.1. Effect of pH values of nutrient media on the biomass production of Pholiota species 21 day of cultivation, at temperature 26 ± 0,1 ºC, GPY nutrient media.

The results in Figure 1 show that Pholiota species has the ability to grow on a wide range of pH values (4,0-7,22). Maximum biomass amount was obtained in Pholiota cultures grown at an initial pH 5,3-6,5 (fig.1), that coincide with the literature data on the optimal for mushrooms range of acidity values of the medium are 5,0-6,0 [3, 11, 21]. The most favourable for the Pholiota species active growth at optimal pH values biomass yield was from 2,22 g/l (P. adiposa) to 6,26 g/l (P. limonella, P. aurivella) as shown in Table 1.

Table 2. The optimal initial and final pH value for the highest biomass accumulating of Pholiota species, 21 day of cultivation, at temperature 26 ± 0,1 ºC, GPY nutrient media

Species	Optimal initial pH value	Final pH value	Amount of accumulating biomass (g/l, a.d.w.)
Pholiota adiposa 2169	5,98	5,45±0,002	3,42±0,14
P. aurivella 2605	5,47	4,60±0,043	6,26±0,08
P. limonella 2335	5,98	5,30±0,003	6,26±0,00
P. nameko 2154	6,42	5,36±0,000	5,20±0,06
P. squarrosa 2010	5,47	4,98±0,000	5,72±0,00
P. subochracea 2535	6,39	5,09±0,007	5,90±0,00

Data on the effect of initial pH values of the nutrient media on the biomass accumulation of Pholiota species is limited, and available only for two species – P. squarrosa, P. nameko [10, 18], while other five species were investigated for the first time. Results presented by Maziero R. at al. [10] were differed to what we have received for higher P. nameko biomass production (pH 6,0). Wang and Lu [18] suggest appropriate initial pH value as 5,3, that almost matches with the data from our experiment.

In our research, pH always showed a decrease during the cultivation. In cases of maximum mycelia biomass output, species reduced the initial pH value by 0,5-1,3 as shown in Table 2.

The studied Pholiota species differed not only in their ability to synthesize different amounts of biomass at optimal pH (5,5-6,0) of the culture medium but also demonstrate varying degrees of biomass accumulating sensitivity to pH changes.

Only one species *P. squarrosa* showed variability in acidic environment (pH 4,0-5,5) (fig.1). Other species almost did not change the intensity of biomass synthesis at initial pH from 4,0 up to 5,0 (*P. adiposa, P. limonella, P. nameko*) and 6,0 (P. subochracea). In case of an alkaline environment (pH 6,5-7,0) were noticed a considerable decrease in biomass productivity with increasing initial pH of nutrient media.

When comparing the values of biomass accumulation at the optimum pH and while the maximum and minimum pH values the differences between species were found (fig.1). The most notable it was for P. squarrosa. At the optimum pH 5,5 biomass production was 5,72±0,00 g/l, when we compare with the lowest (4,0) and the highest (7,2) pH values, biomass synthesis is reduced by 41,82% and 52,63% respectively. Almost no differences were obtained in case of P. limonella, where the metrics were reduced by only 10,23% (pH 4,06) and 17,50% (pH 7,22) as compared with the biomass yield at optimal initial pH.

CONCLUSIONS

The effect of initial pH on production mycelial biomass of six species of Pholiota in liquid static culture from the IBK Mushroom Culture Collection of the M.G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine were established.

For the first time we conducted a detailed study of production mycelial biomass in liquid static culture of such species as for characteristics of these taxa. New data for Pholiota species in pure culture were obtained.

Pholiota mycelia biomass production was significantly affected by initial pH of the nutrient media. Optimal mycelium production of the studied species was observed at pH 5,3-6,5 and was statistically different from biomass values at other pH levels (from 10,23% to 52,63%). During cultivation, the initial pH of the media become lower. Species showed differing degrees of sensitivity to pH changes.

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