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Growth of edible and medicinal mushrooms on commercial agar media

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ABSTRACT: The growth of 30 cultures of edible and medicinal mushrooms was tested at 27 C on commercial malt extract agar (MEA, pH 4.7), potato dextrose agar (PDA, pH 5.8), wort agar (WA, pH 4.7), yeast malt extract agar (YMA, pH 6.2) media, their pH modifications and experimental agar medium GPPA (pH 6.0). Among nutrient agar media used in these experiments, commercial MEA medium provides maximal growth rate only of *Ganoderma lucidum* and PDA only of*Agaricus maskae,* WA-3 and YMA-5 cultures of mushrooms. GPPA provides the maximal growth rate of 15 investigated cultures of *Basidiomycetes* from various ecological groups. This nutrient agar medium is available for comparative or screening investigations with new species and strains.

1 INTRODUCTION

Mushrooms are promising resources of physiologically functional food and as materials for the development of harmless medicines, pharmaceuticals products such as new drugs (protein-bound polysaccharides, terpenoids, steroids, ect.), dietary supplements and healthy beverages, some cosmetics products, etc. (Mizuno 1993; Hobbs 1995; Lorenzen & Anke 1998). Mushrooms are extensive gene pool sources for biotechnology. Therefore it is necessary to preserve different species and strains in culture collections. The culture collection of Higher *Basidiomycetes* was set up at the Mycology Department of the N.G. Kholodny Institute of Botany, NAS of Ukraine (IBK) in 1966. A major function of the collection is to preserve the genofond of macromycetes including the endemic and vanishing species, and to create a data bank on their biological and morphological properties.

The culture collection was used for: the investigation of growth conditions of edible mushrooms in different solid and liquid substrates; the investigation of the dry biomass nutritive value of fruit bodies and the safety for human consumption; the cultivation of some edible and medicinal mushrooms (such as *Pleurotus* spp., *Lentinus edodes)* in submerged cultures to obtain biomass in a short period of time, that can be used as liquid spawn and/or as substrate for the production of some medicine (Buchalo & Solomko 1979; Solomko & Mitropolskaya 1994 a, b; Bisko, Bilay 1996). At the present time, in addition, IBK culture collection used for the investigation of the growth and cultural characters of some medicinal mushrooms if they are not edible, for example *Omphalotus olearius* (Weis, Solomko et al., 1999).

The list of the IBK culture collection includes over 235 species of mushrooms belonging to different taxons and ecological groups. The main part of cultures was isolated from wild fruiting bodies which were collected in Ukraine and various regions of the former USSR and maintained on beer wort agar (Bukhalo, 1998). Part of the cultures was received in exchange from culture collections of other countries. The knowledge of mushroom biology includes nutritious and environment requirements, kinetics of mycelial growth and production of specific metabolites. This is the basis of cultivation and the core of mushroom biotechnology. Due to better

understanding of their biology and the development of advanced cultivation technique new strains and species of *Basidiomycetes* should be investigated.

2 MATERIALS AND METHODS

2.1 *Mushroom cultures*

The strains used in the present work are from the National Culture collection of Higher Basidiomycetes of the N.G. Kholodny Institute of Botany, Academy of Science of Ukraine (Kiev). As the object of studies we chose 30 species of the mushrooms from different ecological groups which are listed alphabetically in Table 1. Stock cultures were maintained in the mycothec in test tubes on beer wort agar media (BWA) at 4° to 8°C and subcultured every year.

2.2 *Tested nutrient media*

For the investigation of growth rate the following standard commercial (Difco) nutrient agar media were used: malt extract agar (MEA, pH 4.7), potato dextrose agar (PDA, pH 5.8), wort agare (WA, pH 4.7), yeast malt extract agar (YMA, pH 6.2); and their modifications: MEA and WA after change of pH to 6.0 by KOH solution, as well as the experimental agar medium, which contained 25 g glucose, 2.5 g KftPO^ 2 ml corn steep liquor, 20 g agar "Difco" and 11 water (GPPA, pH 6.0). The nutritional media was sterilized for 30 min at 121°C.

2.3 *Growth examination*

The master cultures were incubated in plastic Petri dishes at 27°C on BWA. Each dish with diameter 90 mm contained 15 ml of medium. After 7-10 days, standard disks (d = 5.0 mm) were cut from the margin of the mycelial colony using a sterile stainless steel tube. One disk was transferred to the center of new Petri dishes which contained one of the investigated agar media. Freshly inoculated Petri dishes were incubated at 27°C.

The radius of the colony was measured every day. Four measurements were made for each Petri dish. For each variant of medium and species, 3 Petri dishes were used. When the increase of the radius of the colony (R2-Ri mm) was linear in time (t^, days) (see Figure 1), the mean of the radial growth rate (V_R, mm/day) of the colony was calculated. Figure 1 presents the average data on 8 to 12 measurements.

3 RESULTS

The colony growth dynamics of the investigated medicinal mushrooms on various agar media at 27° is presented in Figure 1. All growth rates (Vr, mm/day) are listed in Table 1.

Comparing the growth rate of mushrooms only on standard agar media, it rendered possible to reach the following observations: YMA, WA and PDA provide a higher growth rate of 13, 11 and 9 species respectively. On standard MEA medium maximal growth rate was observed only for *Ganoderma lucidum.* There was no growth of *Macrolepiotaprocera* on this medium. But, it is available medium for *M.procera* growth if the pH of MEA raise to 6.0. We have also observed increases in the growth rate *ofAgaricus arvensis, Agrocybe aegerita, Cyathus striatus, Inonotus obliguus, Pleurotus* spp. and some other species if the pH of commercial media MEA and WA were raised to 6.0 by adding of KOH solution. It is quite natural that MEA and WA with pH 6.0 decrease the growth rate of *Ganoderma lucidum* and *Lentinus edodes.* Among the commercial media and their pH modifications, the better growth rate of many tested mushrooms was on WA medium with pH 6.0. A high growth rate of *Pleurotus cystidiosus, P.ostreatus, P.sajor-caju, Inonotus obliqus, Cyatus striatus, Laetiporus sulphureus* was found on YMA (pH 6.2) medium.

In accordance with the obtained results and preliminary data a new agar medium GPPA was developed, which provides maximal growth rate for 50% of the investigated cultures.

Table 1. Radial growth rate (VR, mm/day) of Basidiomycetes on different agar media at 27°C. Data in bold: maximum growth rate; - not determined.

No	Species	Standard media				Modified media		
		MEA, pH4.7	WA, pH4.7	PDA, pH5.8	YMA, pH6.2	MEA, pH6.0	WA, pH6.0	GPPA, pH6.0
1	*Agaricus arvensis*	1.55	1.69	1.35	1.47	1.16	2.02	1.36
2	*Agaricus bisporus*	0.27	0.94	0.44	0.50	1.72	1.60	1.77
3	*Agaricus bitorquis*	0.50	1.20	0.97	1.14	0.50	1.50	1.22
4	*Agaricus fissuratus*	1.22	1.25	1.05	0.59	0.64	0.53	1.14
5	*Agaricus maskae*	1.25	0.75	1.36	1.36	0.92	-	1.30
6	*Agaricus silvaticus*	0.64	0.64	0.76	0.64	0.67	0.71	0.88
I	*Agrocube aeqerita*	2.80	4.25	4.17	3.83	4.50	4.83	4.75
8	*Armillariella mellea*	0.66	0.88	0.83	-	0.67	0.92	1.00
9	*Auricularia polytricha*	1.33	2.83	3.42	3.58	1.38	4.33	4.50
10	*Cyathus striatus*	1.10	1.20	1.45	1.75	1.65	1.30	1.60
I1	*Daedalea quercina*	1.77	2.22	2.00	2.00	1.68	2.27	3.19
12	*Daedaleopsis confragosa*	1.87	1.63	1.38	2.87	1.63	3.31	2.75
13	*Flammulina velutipes*	3.00	-	3.67	3.72	2.94	-	3.94
14	*Fames fomentarius*	1.96	1.65	2.08	1.23	0.69	2.04	2.23
15	*Ganoderma lucidum*	3.44	3.33	2.17	1.89	2.39	3.17	2.22
16	*Grifola frondosa*	1.58	1.79	1.63	1.25	0.58	1.33	1.58
17	*Hericium erinaceus*	3.33	2.39	3.67	2.44	1.78	2.44	3.94
18	*Inonotus obliguus*	0.96	1.21	1.38	1.46	1.38	1.50	1.79
19	*Irpex lacteus*	2.75	2.42	2.00	3.25	3.17	2.75	4.67
20	*Laetiporus sulphureus*	2.69	2.88	2.88	3.13	2.69	2.38	3.63
21	*Lentinus edodes*	2.65	4.05	3.85	3.80	2.15	2.80	4.05
22	*Lepista nuda*	1.25	1.96	1.79	1.75	1.25	2.00	1.25
23	*Macrolepiota procera*	0.00	1.55	1.45	1.30	1.35	1.65	2.55
24	*Marasmius scorodonius*	1.00	0.96	0.92	1.08	0.83	1.25	1.50
25	*Piptoporus betulinus*	3.33	3.33	3.42	3.42	2.83	3.08	4.67
26	*Pleurotus cystidiosus*	2.00	1.90	2.10	2.55	2.40	2.25	2.20
27	*Pleurotus ostreatus*	2.80	2.85	4.67	5.67	3.00	5.00	5.58
28	*Pleurotus sajor-caju*	2.92	3.17	4.33	3.83	3.17	3.27	6.33
29	*Psilocybe bohemica*	0.88	0.88	0.79	1.33	1.27	1.04	0.71
30	*Stropharia rugoso-annulata*	2.00	1.75	2.20	2.00	2.40	1.60	1.90

4 CONCLUSION

The growth of the mycelia of 30 mushroom species and strains was different. This depends on the type of medium and pH. It is impossible to have one medium suitable for maximal growth rate of different *Basidiomycetes.* As a matter of fact, we find that among nutrient agar media used in this experiments, commercial MEA (pH 4.7) medium provides maximal growth rate only of *Ganoderma lucidium,* PDA only of *Agaricus maskae* and MEA (pH 6.0) only of *Stropharia rugosoanmtlata.* WA (pH 4.7), WA (pH 6.0) and YMA (pH 6.2) provide higher growth rate of 3, 5 and 5 cultures respectively. Among the very common commercial nutrient agar media, good results were obtained on PDA (pH 5.8) medium. This showed average growth rates of all investigated species of *Basidiomycetes.* On the experimental medium GPPA, a maximal growth rate of 50% of the investigated mushrooms from different ecological groups was found. The latter medium will provide investigators with larger opportunities for investigations of new species and strains of mushrooms.

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