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Growing experiments with a medicinal mushroom *Agaricus blazei* (MURRILL)

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Summary: The demand of natural and medicinal products has been increased for the past years. These products are often made from herbal and medicinal plants, and recently the mushroom products are much called. Nearby some well known species (like *Lentinula edodes* or *Ganoderma lucidum*) some not respected biomedicines are available in Hungary. *Agaricus blazei* (Murrill) is a Basidiomycota fungus, with almond-like taste and nice texture. This medicinal mushroom proved to be useful in cancer therapy and against some bacterial and viral diseases. In our experiment we tested 8 *Agaricus blazei* strains on fermented mushroom compost. The yields, average mushroom size, productivity and biological efficiency of the species were measured. Our data demonstrate that it is possible to get fruit bodies less than 2 months, and strain "837", "2603" and "MaHe" are suggested for further experiments. These strains gave the highest yield and efficiency in the cultivation.

Key words: cultivation, strain, *Agaricus,* almond, efficiency, productivity

Introduction

The mushroom production accounts for a dominant portion of the Hungarian vegetable sector. The total mushroom production in Hungary is around 20–35.000 tons/year, and more than 90% is the champignon (*Agaricus bisporus*). In some area oyster mushroom (*Pleurotus ostreatus*) and shiitake (*Lentinula edodes*) are frequent. (*Győrfi*, 2005). The most widely and intensively cultivated *Agaricus* species need 16–20 °C during the pinning and cropping period. The forming and maturing is possible at higher temperature as well, but the quantity and quality lags behind the crop which can be realized potentially. From this fact turn up that the white button mushroom growing is available in countries belong to the temperate zone, but on the tropes only big power and expenditures can cultivate it, or on much lower growing standard. Hot summer months constitute a problem in the temperate zone countries, when the exterior temperature rises above the optimal range. The hot days in summer might cause problems for the growers in Hungary and some European countries, because the mushroom farms, which have not a proper air-conditioning technology cannot compensate the expenses in the price of the crop. To solve this problem it is a possibility to settle a heat tolerance culture in the growing structure, and moreover can achieve higher profit. A potential candidate for this project is *Agaricus blazei* (Murrill), which – hopefully – can be cultivated with similar technology as *Agaricus bisporus*. This mushroom benefits from warm temperatures, making it an ideal candidate for the warmest months.

Agaricus blazei was first discovered and described in 1945 by an American mycologist, William Alphonso Murrill (*Murrill*, 1945). Nowadays many scientists are working on improve the cultivation technology (*Chen*, 2001; *Andrade* et al., 2007). The popularity of this mushroom is increasing rapidly, especially by Japanese, Brazilian and Chinese cultivators who are exporting dried fruitbodies and fresh mushroom (*Stamets*, 2000b). The production of *Agaricus blazei* is raising continually worldwide, due to exotic, almond-like taste and very important curative effect (*Mizuno*, 1995; *Iwade & Mizuno*, 1997; *Stamets*, 2000a). In the past decade a lot of reports were available about the positive effect of different *A. blazei* extracts. *Agaricus blazei* gets to customers in fresh form or after drying in a pulverized form, in special package in capsules, in pills or in the form of solution followed by hot water extraction. The fruiting body contains 89–91% water, less than *Agaricus bisporus*. Almost 48% of total dry matter consists of crude proteins, 18% of carbon hydrates and the lipid content is 0.5%. It contains a greater amount of water-soluble polysaccharides than the white button mushroom (*Győrfi*, 2007). Already, a lot of information is available nowadays on its therapeutic effect and it is a well-known exceptional healthy food. The extract of the mushroom may be useful as additional prophylactic and possibly therapeutic treatment against *Streptococcus pneumoniae* bacteria in mice, and possibly useful in other human infections (*Bernardshaw* et al., 2005). The antiviral activity was observed against human and bovine herpes viruses in cell culture, due to the mushroom extract (*Bruggemann* et al., 2006). The variation of the antimutagenicity effects of simple water extracts of mushroom was although identified (*Guterreza* et al., 2004).

A patent was announced onto the cultivation technology in artificial mushroom cultivation bed (*Makoto* et al., 2002), but the complex and safety cultivation method is not clarified. We cannot get definite answer for this question: are there any differences between the different strains of *A. blazei* in optimum temperature during spawn run, pinning and cropping period. The cultivation of *Agaricus bisporus* differ from *A. blazei,* because *A. blazei* produces the fruitbodies only in light, and requires higher compost and air temperature during pinning and cropping. If the temperature is decreasing less than 20°C or increasing above 35°C, it can slow down the mycelia growing *in vitro* (*Győrfi,* p.c.). *Agaricus blazei* is a saprobiontic species of the same sort as *Agaricus bisporus*, i.e. requires broken-down nutrients which are ready. The preparation of the compost is almost identical to that of the button mushroom with the difference that the compost must be neutral (pH=7) and at the same time the moisture content is also higher, 68–70% (*Stamets*, 2000b).

Material and method

Different *A. blazei* strains were compared *in vivo* in our experiments. The strains originated from Germany, Unites States, Canada and Hungary, were reserved by Corvinus University of Budapest, Faculty of Horticultural Science. In our essay the strains which can be potential for big mass cultivation were selected. Cultivation technology, yields and average size of the fruit body were characterized.

Spawn: the mushroom spawn was prepared in the mushroom laboratory of Department of Vegetable and Mushroom Growing. Cooked and sterilized rye was used as grain spawn, inoculated with 8 *A. blazei* strains (837, 838, 853, 1105, 2603, Brazil, Ma-He, Si-2.2). The spawn was incubated at 25°C in dark for 3 weeks, till the spawn run finished. Commercial *A. bisporus* hybrid (A15) and an old variety "LeCh" was used as a control.

Substrate: pasteurized mushroom compost was used as a substrate from Bio-Fungi Ltd (Áporka). The compost was made from wheat straw, chicken manure, gypsum and water. The compost was divided into plastic bags, contained 2 kg compost. 30 gram spawn was added, than mixed carefully. Every treatment included 3 of these bags and 7 repeats were brought out.

Conditions of cultivation: the experiment was carried out in the experimental mushroom building at University. Air and compost temperature and relative air humidity were measured every day during the experiment. On the 14–15th day, the bags were covered with the casing soil, which was prepared from mixture of peat and powdered stone (Premium Terra). The thickness of casing layer was 3 cm. During the spawn run and case run period the compost temperature alternate between 24–28 °C, the air temperature was around 22–24 °C. Irrigation was carried out by hand sprinkling. The watering time and irrigation norm depended on the moisture of casing soil.

Data analysis: mushrooms were harvested, counted and weighted daily when the pilei were closed. The data were recorded in Excel database, than statistical analysis was finished by "R 2.7.0" program (*Reiczigel* et al., 2007). The average mushroom size calculated as fresh mushroom weight divided by the number of mushrooms harvested. The total yield and the biological efficiency (ratio of (g) of fresh mushrooms harvested per dry substrate weight (g) was although determined (*Stamets*, 2000a). The productivity (P) was determined from the relation between mushroom fresh weight (MFW) and compost fresh weight (CFW) at the end of Phase II, according to the equation P=MFW/CFWx100 (*Andrade* et al., 2007).

Results and discussion

During the spawn run, the almond-like smell of mycelia coaxed the ants to the compost, which is not common in mushroom growing. The mycelia colonized the compost at 14th day, the bags switched into white color. During the pinning and harvesting lower temperature was needed to form mushroom fruit bodies (*Figure 1*).

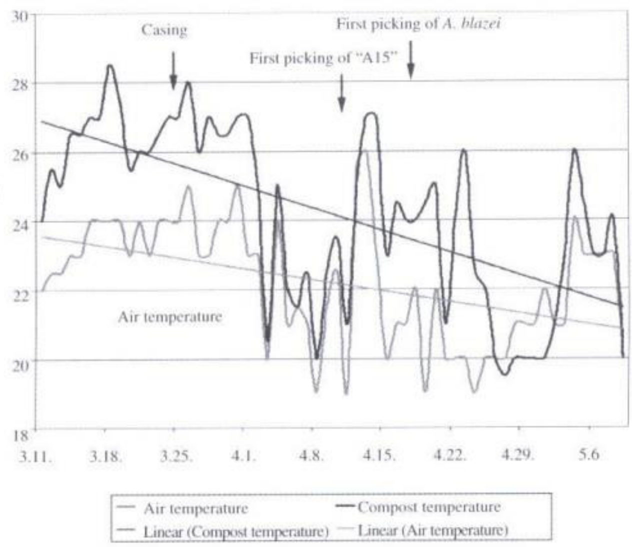

Figure 1 Measured air and compost temperature during the experiment. The trend line can be seen, which represents the decreasing of temperature.

The first mushroom was picked on *A. bisporus* strain A15, exactly 30 days later as spawning. The first flush was picked for 4 days of champignon. On the 38th day the *A. blazei* strain "853", "1105" and "2603" were ready to harvest. The first flush was 6–7 day long, the correct determination is difficult, due to much mushroom between flushes. *A. blazei* in this experiment produce the fruit bodies more or less continuous, we were not able to detect characteristic flushes. The harvesting period was taken till 28 day, during this period 3 flush was picked from A15. The total yield was the highest in case of A15 hybrid strain (*Figure 2*), but it is far from the potential achievable 32–34 kg yield/100 kg compost. This fact probably caused by experimental circumstances and higher compost and air temperature during pinning and picking period as optimum range. The average yields of the 7 repeats demonstrate, that the most bid fair for cultivation *A. blazei* strain is "MaHe"

(*Figure 3*). The mean of strain "MaHe" is high and standard deviation is relative low (*Figure 4*). Tukey-Kramer pairwise comparison of means showed, that "MaHe" had the highest yield at $p<0.01$ of *A. blazei* strains.

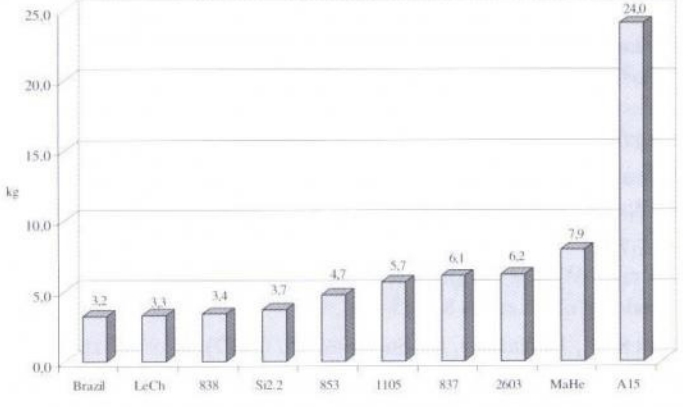

Figure 2 Respective yields of the different treatments expressed per 100 kg compost

We emphasize, to our knowledge this is the first report of *A. blazei* cultivation in Hungary. Compared with the white button mushroom the crop quantities are lower, some of this reason was presented earlier. On the other hand, the *A. blazei* strains were collected from natural habitat and their claim of nutritional elements is not clarified. The 6–8 kg fresh mushroom on 100 kg compost is less, than in literature (*Andrade* et al., 2007), but total cropping period in this culture was only 2 months, so short cropping cycle was not observed earlier.

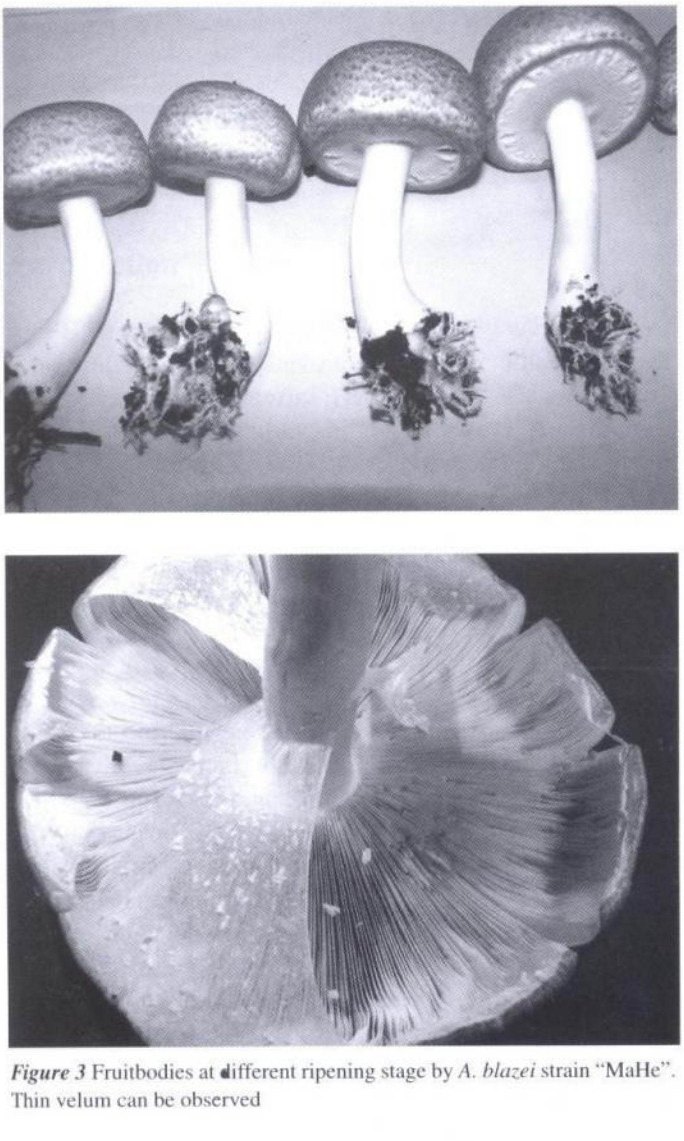

Figure 3 Fruitbodies at different ripening stage by *A. blazei* strain "MaHe". Thin velum can be observed

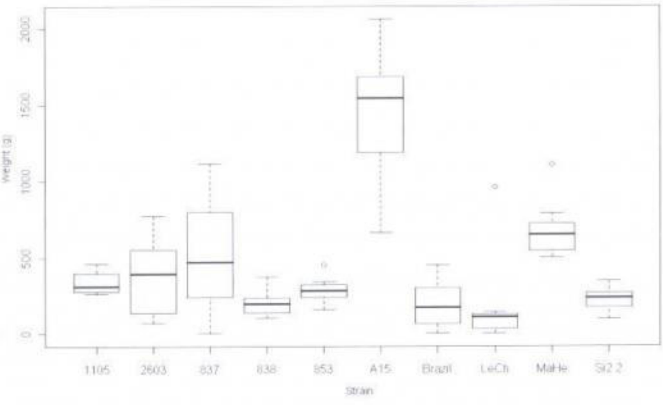

Figure 4 Boxplot of the total yield in gram

Some developments in casing soil and casing technology is necessary, to raise the productivity and biological efficiency of *A. blazei* (*Table 1*). The cultivation of other mushroom species can achieve 120–160% efficiency (*Stamets*, 2000a) with some technological modification it is available with *A. blazei*. In our experiment low efficiency was observed by strain "Brazil", but the average mushroom size was much higher than the others. The strain "MaHe" had high efficiency and the mushroom size was in excess of *A. bisporus*.

Table 1 Calculated biological efficiency, productivity and average mushroom size in the experiment

Strain	*Biological efficiency (%)*	*Productivity (%)*	*Average mushroom size (g)*
A15	91,6	27,5	23,5
MaHe	37,8	11,3	29,2
837	29,0	8,7	27,9
2603	20,6	6,2	26,5
1105	18,8	5,7	18,0
853	15,8	4,8	12,7
Si2.2	12,3	3,7	15,4
LeCh	11,0	3,3	22,3
Brazil	10,7	3,2	71,1
838	9,9	3,0	40,6

Further experiments are needed to raise the yield and select the strains for big mass cultivation. Based on our data "MaHe" 837" and "2603" are suggested for further cultivation. Of course, good management and marketing is required to take a liking to consumers.

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