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Thesis of Doctoral Dissertation

DEVELOPMENT OF OYSTER MUSHROOM VARIETY-SPECIFIC GROWING TECHNOLOGY

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1. BACKGROUND OF THE RESEARCH AND OBJECTIVES

The oyster mushroom growing is considered a young sector within the horticulture, therefore the development of the intensive cultivation technology has still been happening. Till the middle of the nineties the oyster mushroom growing was in the focus of scientific and practical interest. As result of it Hungary gained international honour. The structure of the sector changed a lot in the nineties. In oyster mushroom cultivation nowadays there is a need arising to modernize the earlier professional knowledge as an effect of the new technological and economical problems.

The cultivation of hybrids grew prevalent, more and more hybrids are appearing beside the reliable HK35 hybrid of Hungarian improvement. In the other hand oyster mushroom growers normally do not use specified growing technology irrespectively which variety is grown. This fact can come from the situation that we have enough available knowledge of the growing conditions at only species level but varieties, hybrids level. Respectively the application of optimalised growing technology for hybrids can increase the yield significantly.

Different pathogens cause great crop losses in the oyster mushroom cultivation time by time. A group of these pathogens is “green molds”, and the most aggressive causative agents are the *Trichoderma* species within the group. *Trichoderma* species can cause smaller and severe damage depending on the virulence of the strains causing the infections. A consortium with members of Hungarian research institutes and universities involved in the research of the *Trichoderma* infections, the identification of the causative agents and the development of biocontrol technology against them since 2004, possesses remarkable scientific results in this field.

Beside green molds, the oyster mushroom cultivation can be infected by heavy crop loss resulting bacterial blotch disease caused by different pathogen bacteria (Győrfi, 1989; Cha, 2004). even though *Pseudomonas tolaasii* is mentioned as the main causative agent in regards of the literature. Probably other fluorescent *Pseudomonas* species (pl. *P. agarici*, a *P. reactans* and *P. gingeri*) can cause similar symptoms (Rainey, Brodey and Johnstone, 1992; Bessette, Kerrigan and Jordan, 1985; Munsch et al., 2002; Szili, 2008). It is possible that mixed infections of some *Pseudomonas* species cause the bacterial blotch disease (Cha, 2004), but all pathogens can be related to these symptoms have not been identified yet.

Although there’re a lot of known pathogens in mushroom cultivation, it is difficult to maintain efficient mushroom protection in mushroom growing than in other crops. The origin

of the problem is that mushroom growing is a marginal field within agriculture; therefore the huge international pesticide companies have no economic interest in developing chemicals for mushroom growing. Recently few fungicides and insecticides can be applied in Agaricus growing, but it is not allowed to use any chemicals for oyster mushroom growing.

The oyster mushroom growing is absolutely not protected from bacterial blotch disease because of the lack of pesticide, therefore oyster mushroom growers sometimes have to endure severe crop loss. Effective biological control against bacterial blotch is not known yet. The first step to develop effective biological control is to identify and characterize all pathogen strains causing the disease and then determine their ecological needs

The objective of my research work is, for this very reason, the examination of cultivation technology for the oyster mushroom (*Pleurotus ostreatus*) hybrids currently being used in commodity production, including the optimization, improvement of efficiency. I aimed to establish variety-specific technology and recommendation for the practice use in support of economically efficient growing of marketable fresh oyster mushroom with high value.

During my research the development of *Pleurotus* hybrids cultivation has been approached in three different ways, therefore I have determined three different goals of my research:

1. To investigate the structure of greatest oyster mushroom growing region by primer data collection, to define the typical unit size, the technological development of the growing units and the growing parameters used by growers.
2. To examine the following aspects of the most applied three hybrids in the intensive mushroom production (HK35, 357 and P70) in the frame of laboratory and low-scale plant experiments:
    - the effect of **incubation temperature on the rate of mycelial growth** and yield,
    - the correlation between the **time period of incubation** (vegetative growth) and the harvesting yield,
    - the effect of **primordium development and fruiting temperature** on yield,
    - the **pH change** in the substrate during mycelial growth,
    - the effect of **temperature and humidity on the growth of oyster mushroom cap**.

3. To expand our knowledge about oyster mushroom related bacterial blotch disease which presence is increasing in oyster mushroom farms. Therefore it is important to examine some environmental factors affecting the growth of *Pseudomonas* species isolated from decaying oyster mushroom. The investigated factors were the following:
    - pH of substrate,
    - temperature,
    - salt tolerance,
    - and copper sensitivity.

The background of my research presented in my doctoral dissertation was the research and development program named „*The optimization of oyster mushroom growing technology towards the competitiveness improvement"*. During the accomplishment of my research I have worked with the Vegetable Growing Research Institute, the Vegetable, Mushroom and Herbs Growing Group of Horticultural Faculty of Kecskemet College, and the TTIK Microbiological Faculty of the Szeged University of Sciences. The special consortium structure gave me the ability to point out and execute three objectives built on each others, but very different by their methods.

2. MATERIALS AND METHODS

2.1. INVESTIGATION OF THE ACTUAL SITUATION OF OYSTER MUSHROOM GROWING IN THE SOUTH-PLAIN REGION

The aim of the survey was to find out the representative size of mushroom farms and growing area in the South-Plain Region, the technical qualification of the growing units, which growing parameters are checked during growing, so to analyze the applied growing technologies in oyster mushroom farms. For the primer survey I have used the questionnaire method including 18 questions. The majority of the questions were closed with one or multiply answers, only in two cases I applied opened questions. The structure of the questionaire was the following:

- General questions (3)
- Questions in regard of growing units (7)
- Questions in regard of growing technology(5 db)
- Economic questions (3 db)

The questionaire included 23 mushroom farms. The geographical layout of the farms: 20 in Bács-Kiskun County, 1 in Csongrád County and 2 in Pest county. Quantitative and qualitative methods were applied during primer data collection and the questionaire creation.

2.2. EXAMINATION OF EFFECTS OF SOME ABIOTIC ENVIRONMENTAL FACTORS ON MYCELIAL GROWTH, PRIMORDIUM FORMATION AND FRUITING OF OYSTER MUSHROOM HYBRIDS

Selection of hybrids

I put under examination those hybrids, which are favoured by farms growing mushrooms by economic purpose. These are: HK35 (Sylvan), No. 357 (Szili) and P70 (Italspawn).

Characteristics of HK35 (Sylvan): Colour of mushroom cap temperature dependent, and it varies from dark grey to lighter grey. Average sized cap is fleshy and thick, it can be stored well. It is fairly tolerant of temperature, the suitable temperature for fruiting is between 8-18°C, but grown on below and above of the optimum, the mushroom quality declines.

Characteristics of No. 357 (Szili): This hybrid is very similar to HK35 by its morphology and cultivation. Fewer caps are in a cluster, the mushroom is more "lumpish", which feature is liked by growers, but its yield is not as high as of HK35 hybrid.

Characteristics of P70 (Italspawn): Sporocarps are larger than average, it can be grown year around. The mushroom cap is thick, brownish-greyish colour, and it develops in clusters. The mycelial run is slower than like HK35 hybrid, therefore it requires longer incubation. The hybrid has a good ability to grow in different seasons; it can be grown all seasons in climatised growing houses.

The effect of temperature on mycelial growth and yield of oyster mushroom hybrids

I examined the effect of temperature on mycelia run in three different laboratory trials: on malt-agar in Petri dish, on pasteurized straw substrate in Petri dish and pasteurized straw substrate in 1000 g bags. The inoculated Petri dishes and 1000g substrate bags were put into 6 different incubation temperatures (22, 25, 28, 31, 34, 37°C) with three repetitions. The actual temperature was adjusted by thermostat. I measured the time of the incubation till the mycelia fully colonized the substrate. In the trial the full colonization means, when the mycelia reaches the side of the Petri dish, or when the colour change done and the mycelia can be recognized in the substrate everywhere.

In the trial with 1000g substrate bags I have examined the yield also. After incubation the completely incubated substrate bags were placed into a cellar. Conditions for fruiting: the

room temperature 16-18°C, RH 80-90% and 300 Lux for 8 hours per day light.

Effect of time length of incubation on yield

In the trial the spawn (HK35, 357, P70 hybrids) was mixed with pasteurized wet straw (3 m/m %) by machine, that the spawned substrate was pressed to blocks (22 kg/block). The blocked substrate was put into special mushroom tents. For the incubation I have applied different incubation periods (14, 17, 20, 23, 26 and 29 days) two blocks per trial with four repetitions. The substrate temperature was set to 28°C. After the end of the certain incubation period I decreased the room temperature to 16°C, I set the RH to 80-90% and I gave 300 Lux light for 8 hours per day. I examined the effect of incubation period on yield.

Investigation of effects of primordium formation and fruiting temperature on yield

I have examined the effect of different primordium formation and fruiting temperature on yield. I used pressed pasteurized straw substrate block 22 kg each. I have applied four different incubation treatments for the substrate blocks (combination of two temperatures: 25 and 28°C, and incubation time periods: 14 and 17 days). For the primordium formation and fruiting I have examined five temperatures: 13, 16, 19, 22, 25°C in 3 repetitions. The incubation and the fruiting took place in special mushroom growing rooms. I investigated the effect of the treatments on the yield.

PH change in substrate during mycelial run

In the first 10 days of the incubation (while the colour change of the substrate completes) every second day I took 12 g substrate sample from 3-3 substrate blocks (always from same). I diluted the samples in 30 ml 0,1 M KCl for 2 hours, than the pH of the filtered liquid was measured.

Investigation of environmental factors influencing mushroom cap growth

The examination of environmental factors happened with PHYTOMONITOR instrument. The end-device of the instrument can be fixed onto the mushroom cap, and it can register the growth of the cap, the temperature of the substrate, and the temperature and humidity of the growing room. The goal of the trial was to examine the effect of different temperatures and relative humidity on the growth of sporocarp. Beside the measurement of the growth of sporocarp I have measured and registered the temperature of the substrate in 10 cm deep, the

RH and temperature of the room. These examinations were carried out in special mushroom tents and cellars with HK35 hybrids on 22 kg pressed substrate blocks.

2.3. EXAMINATION OF SOME ENVIRONMENTAL FACTORS AFFECTING *PSEUDOMONAS* SPECIES GROWTH ISOLATED FROM DECAYING OYSTER MUSHROOMS

The isolation and selection of *Pseudomonas* strains were carried out on selective medium for fluorescent pseudomonads. The isolates were originated from decaying oyster mushroom sporocarps and substrate grown at oyster mushroom farm of Pilze-Nagy Kft. The identification of isolates was carried out with investigation of biochemical characteristics and DNA analysis by TTIK Microbiology Faculty of University of Szeged (Nagy *et al.*, 2008). By the results of the identification the isolates were grouped into seven groups. Both the biochemical assays and the DNA analysis proofed that each group determines a species (Table 1). For further investigation I have selected one representative isolate from each group using random method (the selected isolates are highlighted in the Table 1.) The ecological characterization was carried out only with the selected isolates. The purpose of decreasing the number of investigated isolates was the quite high number of them (44 pieces). I have examined the effect of temperature (on 5, 10, 20, 30, 40°C), pH (between 2-8 pH), NaCl concentration (between 1-10%) and $CuSO_4$ concentration (20, 40, 60, 80 and 100 µg/ml $CuSO_4$) on growth of isolates in liquid culture. I have measured the optical density of the liquid cultures after two days incubation with spectrophotometer on 620 nm.

Table 1: *Grouping isolates by species classification*

Isolates	***Species***
I. group: 9, **10**,13, 14, 55	*P. putida*
II. group: **54**	*P. synxantha*
III. group: **3**	*P. mucidolens*
IV. group: **56**	*P. mandelii*
V. group: **12**, 19	*P. fluorescens* biovar. I.
VI. group: **44**, 45	*P. tolaasii*
VII. group: 1, 2, 4, 5, 6, 7, 8, 11, 15, 16, 17, 18, 36, 37, 38, 39, 40, 41, 42, 43, 46, 47, 48, **49**, 50, 51, 52, 53, 57, 58, 59, 60	*P. fluorescens* biovar. V.

3. RESULTS, DISCUSSION AND RECOMMENDATIONS

23 oyster mushroom growing farm from the South-Plain Region were involved in the primer surveying. The structure of the oyster mushroom growing in the South-Plain Region was considered to be favourable by the survey, because the rate of committed to oyster mushroom growing and experienced growers is high (57%), while the rate of new growers is 1:4, therefore the reform potential is guaranteed. The mushroom growing in the region is represented by commercial type farms (57%) and concentrated farming. It is expected on the basis of these two features, that the practical recommendations developed in my research work can be easily introduced in the practice.

The **technical furnishing of the growing units** is moderate, enough to serve the environmental needs of mushrooms, but falls away from the automatic climate control systems. It means that the growing units are not suitable for setting the environmental parameters precisely, and their operation needs continuous labour control. Measurement and regulation for CO_2 is almost unknown in oyster mushroom growing, while it has been part of the climate control systems in Agaricus growing since decades (Kovács, 1998).

The technical parameters of growing units make it possible to grow oyster mushroom year around. However the partial climatization is not enough to create climate inside in the growing room independent from the outside weather conditions. The result of it is that the **summer and winter growing parameters** differ significantly. In accordance with it we can speak of winter and summer oyster mushroom growing technology (Kovácsné-Gyenes, 1998). Inside in the growing unit the air temperature can reach 18-25°C in the summer, while in wither it is 10-12°C. These values compared with the results of the effect of the temperature on the growth experiment and optimum temperature of the growth of *Trichoderma pleurotum* and some *Pseudomonas* species, I have to point out the following:

1. The HK35 hybrid optimal temperature of cap growth is between 15-18°C with 70% relative humidity concerning the quality also. Neither the winter nor the summer growing conditions meet these requirements absolutely.
2. With the summer growing conditions (18-25°C) applied in practice the oyster mushroom fruit body develops fast, but it is important not to increase the room temperature over 18°C, because higher temperature, decrease the quality of the fruit body. High temperature like 25°C is favorable for the growth of pathogens; it can be the explanation of the phenomenon that in the summer hot temperature

conditions there are a lot of green mold and bacterial infections in oyster mushroom growing.

3. The winter growing temperature applied in practice (10-12°C) is below the temperature optimum of the mushroom cap growth of HK35 hybrid, therefore the growth speed of the fruit body decreases with one-third, which means that the growing circle takes longer. Advantage of the winter growing is, that microorganisms, pathogens among them show very little growth at 10°C, specially *Trichoderma pleurotum* does not grow at 10°C (Kredics *et al.*, 2009) and the growth of examined *Pseudomonas* species is inhibited at this temperature.

Oyster mushroom growers in the South-Plain Region in the present growing units have to work in a very narrow temperature zone in summer and winter in order to grow good quality and marketable oyster mushrooms. Therefore it is very important to get the full climatization in the oyster mushroom growing to happen, like it can be observed in the Agaricus growing.

During the examination of the **effect of incubation temperature on mycelial growth** I have not noticed significant difference among the hybrids. The mycelial growth was the quickest at 25 and 28 ˚C both on agar and on straw substrate in Petri dishes. The optimum temperature of mycelial growth was 28 ˚C in 1000g substrate bags. Temperature over 31°C inhibits the mycelial growth of all investigated hybrids (Figure 1). The optimum temperature of mycelial growth of *Pleurotus ostreatus* determined in the literature is very close to the temperature values I observed in my research (Chang and Miles, 2004; Szabó, 1986; Zadrazil, 1974). Almost half of the growers (48%) keep 32 ˚C or more temperature in the substrate during the incubation by the results of the survey. In my trials I have never observed mycelial growth at these high temperatures, therefore those growers, who follow this kind of practice, inhibit the mycelial growth in the core of the substrate block. For the competitiveness of the oyster mushroom growing it has to analyze while the practice deviates from the ideal temperature of the mycelial growth of the hybrids.

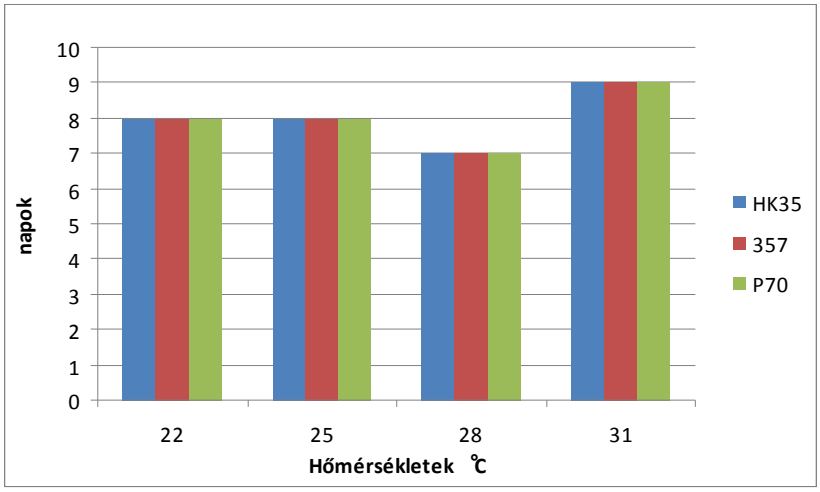

Figure 1: *Mycelial run rate of oyster mushroom hybrids on different temperature in 1000 g substrate bags*

In the trial, where the **temperature requirement of the mycelial growth was investigated by yield**, I have got significant differences among the hybrids, which can come from variety-specific feature. The incubation temperature of 25°C gave the highest yield significantly at all investigated hybrids, while increasing the temperature up to 28 ˚C decreased yield by 25-30%. It has to be pointed out, because the mycelial run was quickest on 28 ˚C. In case of P70 hybrid, both 22 and 25°C incubation temperature resulted high yield, which means that the P70 hybrid can tolerate a bit lower incubation temperature but the two other hybrids. In case of hybrid No. 357, the temperature optimum for incubation is 25 °C, but this hybrid is sensitively reacting onto the increase of substrate temperature, and the yield is decreasing by 50% already at 31 °C (Figure 2). Almost half of the oyster mushroom growers in the South-Plain Region apply a kind of incubation practice, which decrease the yield significantly by my research. Because the *Pleurotus*-pathogenic *Trichoderma pleurotum* (Kredics *et al.*, 2009) and the tested *Pseudomonas* species show maximal mycelia growth at 25-30°C, therefore the incubation temperature of oyster mushroom resulting the highest yields serves the growth of microorganisms, pathogens for example.

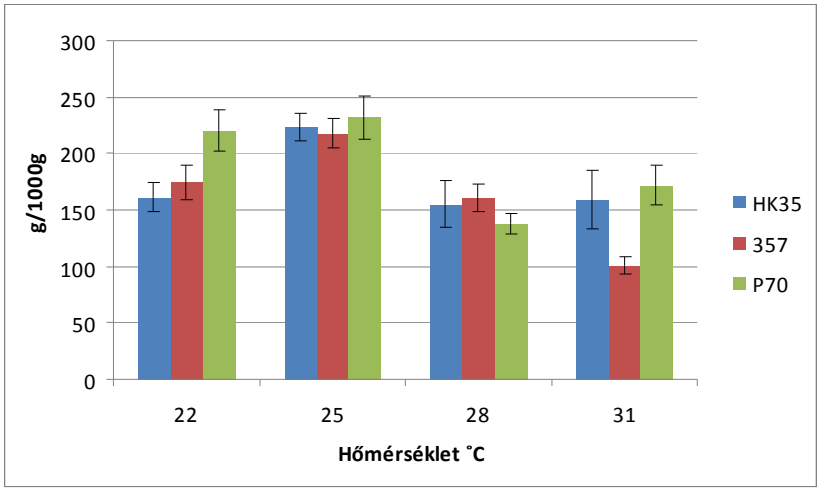

Figure 2: *Yield of oyster mushroom hybrids incubated on different temperature*

In the trial of **correlation between incubation time and yield** I observed no significant difference considering 95% probability level in incubation periods of 20-23-26 days in case of the HK35 hybrid, incubation periods of 20-23-26-29 days in case of No. 357 hybrid and incubation periods of 20-23-26 days in case of P70 hybrid, which means that the difference of values is not proofed to be resulted by the treatments (Figure 3).

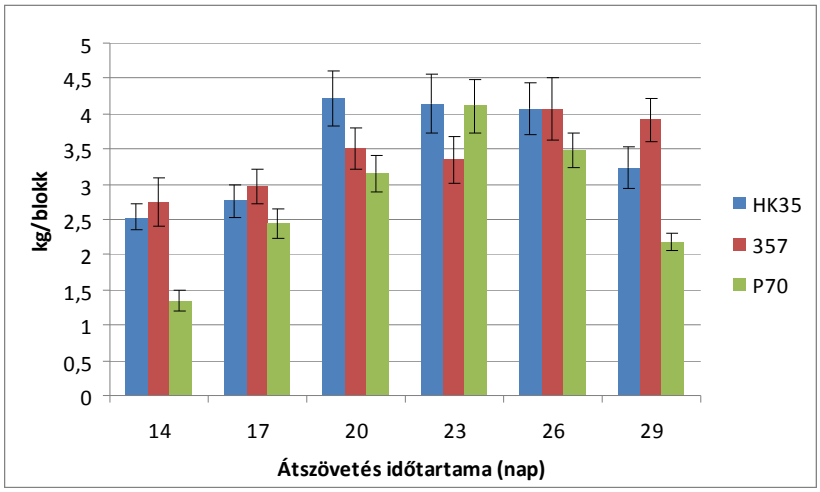

Figure 3: *Yield of oyster mushroom hybrids correlation with incubation time period*

It can be concluded by results, that the No. 357 hybrid can tolerate the incubation period change in the widest spectrum considered the yield among the three examined hybrids. In the point of variety-specific growing technology it is important to keep in mind the following differences: in case of the HK35 and P70 hybrids the incubation period has to reach 20 days, but not more than 26 days to receive the highest yield. The examination of No. 357 hybrid

resulted different profile from HK35 and P70 hybrids in the trial of correlation between incubation time and yield. In the trial I was not able to determine the maximum of the optimal incubation periods, because there was still no significant difference in the highest examined value, 29 days and 20-23-26 days. By the results of the primer survey the 57 percent of the mushroom growers incubates the substrate for 19-25 days, in accordance with my experiment. However 32 percent of the growers differ from the optimal incubation period, while the application of not optimal incubation period can decrease the yield of hybrids by 30-50 percent.

The yield is greatly affected by fact how the mycelia has been prepared to develop primordium in the fruit body induction phase, and the temperature has a key role in it. Significant differences were found among *Pleurotus* hybrids in the **effect of induction of fruit body development and fruiting temperature on yield**. I put in Table 2. the fruit body development and fruiting temperature optimum values of three examined hybrids in consequence with the four incubation treatments. I have excluded those incubation treatments where I have not found significant differences in the fruit body development and fruiting temperatures. I have highlighted the temperature values of fruit body development and fruiting given the highest yield in comparison with all incubation treatments.

Table 2: *Primordium formation and fruiting temperature optimums of examined hybrids related to best yield geared to incubation treatments*

	25°C /14 days		**25°C /17 days**		**28°C /14 days**		**28°C /17 days**	
	°C	**kg/block**	**°C**	**kg/block**	**°C**	**kg/block**	**°C**	**kg/block**
HK35	16	3,93	16 (19)	3,96	16 (19)	3,36		
357	16 (19, 22)	2,95			16 (13, 19)	3,02	19 (22)	3,50
P70	13 (16, 19)	2,92			19 (13, 16)	2,53		

( ) *temperature value significantly not differs from the highest yield giving value*

there is no significant difference among values

treatment resulting highest yield

In case of HK35 the temperature optimum of primordium formation and fruiting is 16°C respectively (Table 2, Figure 4).

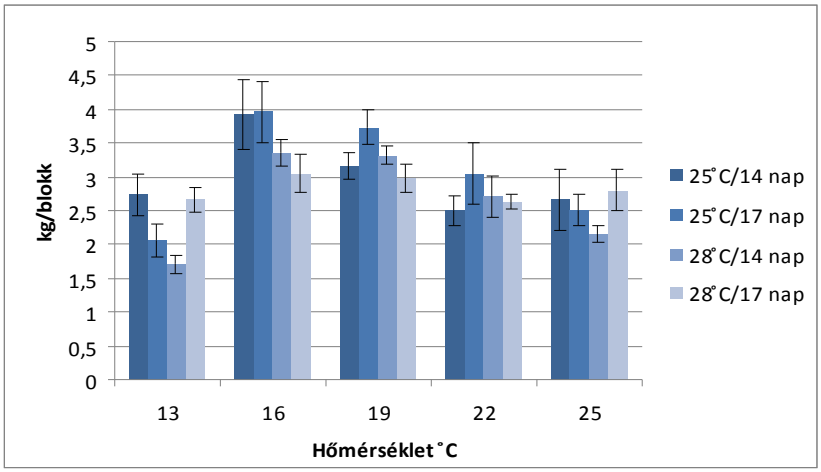

Figure 4: *Yield of HK35 hybrid on different primordium formation and fruiting temperature according to incubation treatments*

In case of No. 357 the temperature optimum of primordium formation and fruiting is a bit higher: 19-22°C serves the highest yield for this hybrid (Table 2, Figure 5).

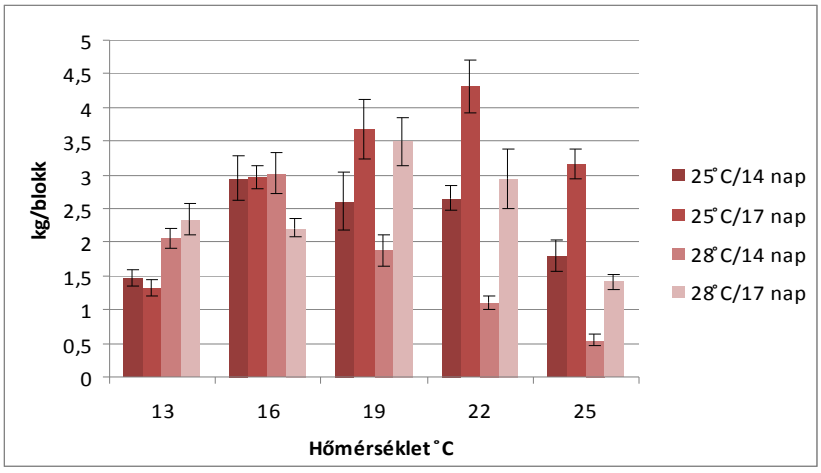

Figure 5: *Yield of No. 357 hybrid on different primordium formation and fruiting temperature according to incubation treatments*

In case of P70 the temperature optimum of primordium formation and fruiting by yield is between 13-19°C, and considering the quality of the mushroom cap, lower fruiting temperature, 13 °C is advised (Table 2, Figure 6).

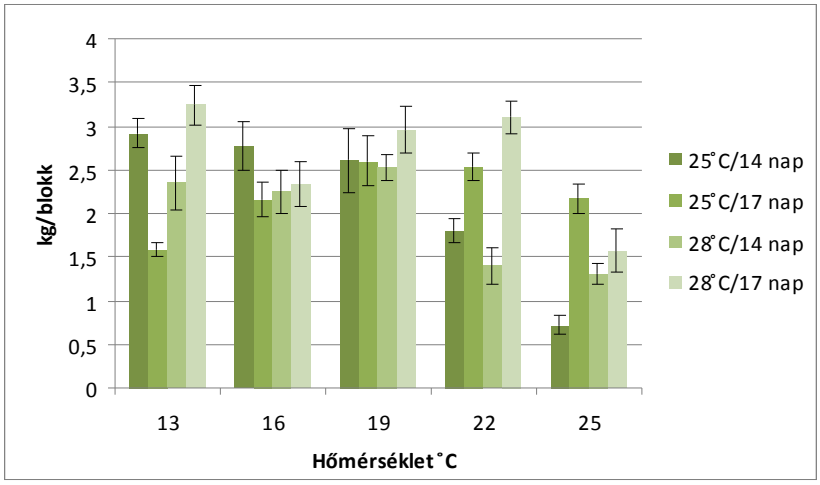

Figure 6: *Yield of P70 hybrid on different primordium formation and fruiting temperature according to incubation treatments*

It can be concluded that with using variety-specific growing technology the yield can be increased by 30-50%.

In case of HK35 hybrid I have studied the **correlation between the growth of sporocarp and the temperature and humidity**. The growth rate of the mushroom cap diameter is varying between 2-8 mm/day on different temperatures (Figure 7). The optimum of the mushroom cap growth is observed on 5-18°C in regard with the quality.

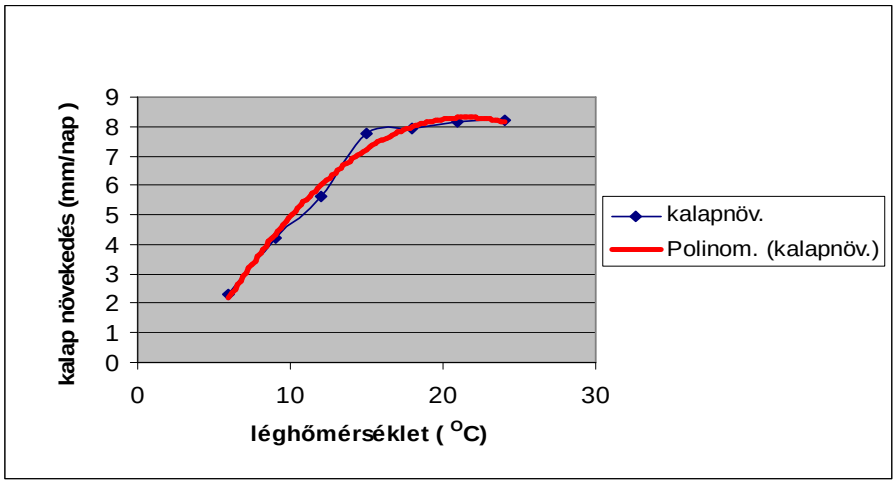

Figure 7: *The average cap growth of HK35 hybrid correlation with room temperature*

Gyurkó (1979) carried out experiences with the growth rate of *Pleurotus ostreatus* sprocarps. It needs 10 days to get harvestable mushroom caps from the appearance of primordia on 14-15°C temperature by his observations. The HK35 hybrid sporocarp growth rate does not differ too much from *Pleurotus ostreatus* in accordance with his findings. The relative humidity of the growing room has also effect on the quality of the harvestable

mushroom. The quality of oyster mushroom cap is better on lower, 70 percent of relative humidity than on 90 percent. I have experienced during the surveying, that oyster mushroom growers do not pay enough attention on setting the suitable environmental parameters in the growing room (temperature and humidity), but the perfect climatisation can serve the equalized high yields, with which the economics of the mushroom growing can be increased.

I have investigated the **pH change of the substrate** in the first 10 days of the incubation (while the colour change of the substrate completes). In case of all examined hybrids the pH value of the substrate quickly decreased parallel with the mycelial growth, for the 9. day of the incubation the substrate reached the pH range of 5-5,6 in all cases. By Chang and Miles (2004) pH optimum for the mycelial growth of *Pleurotus ostreatus* is between 5,4-6, while Gyurkó (1979) determined the pH optimum to 5,5-5,8. In cases of all examined hybrids the pH change during incubation reaches the optimum pH value on the 9. day. There was no difference among the examined hybrids in the kinetics of the pH change, independently of the optimal incubation time period requirement of the hybrids. Oyster mushroom mycelium by the result of the quick pH decrease in the substrate in one hand can help to increase the efficiency its extracellular enzymes, (Rajarathnan, Shashireka and Bano, 1992; Jakucs, 1990) and in the other hand can inhibit the growth of *Pseudomonas* species investigated by me. The pH optimum for the growth of the examined *Pseudomonas* species is neutral or alkaline, which means that beside the first days of incubation the pH value of the oyster mushroom substrate is not preferred by the growth of investigated *Pseudomonas* species. Kerdics *et al.* (2009) has an interesting observation with the pH requirement of *Trichoderma pleurotum*. Beside the pH optimum of *T. pleurotum* is set between 5-6 on synthetic minimal medium, it is not able to grow on *Pleurotus* powder containing medium above pH 5. It means that the pH value favourable to oyster mushroom is not preferred by *T. pleurotum* in the substrate containing oyster mushroom mycelia.

In oyster mushroom sporocarps carrying the symptoms of bacterial blotch disease like in the agarics, not only *P. tolaasii*, but other *Pseudomonas* species can be found. Nevertheless only two of the isolates derived from the decaying oyster mushroom fruit bodies were identified as *Pseudomonas tolaasii*, but most of them belonged to other *Pseudomonas* species (Table 1). It was common characteristic that more than one species could be isolated from any of the ill fruit bodies; which is very important information for the prevention and control of the bacterial blotch disease. I have to note that in this trial I have not investigated the pathogenic feature of the isolates, therefore their virulence and the participation in the disease has not been proofed.

Based on my results the vegetative growth of the examined oyster mushroom hybrids showed a temperature dependence similar to that of the examined *Pseudomonas* species (optimum at 25-30°C). By Palleroni (1984) *Pseudomonas tolaasii* can be grown on 4 and 40°C also, but the minimum and maximum temperature were 10 and 40°C for the growth of the examined *Pseudomonas* species. The growth of examined *Pseudomonas* species is decreasing by the effect of the concentration increase of NaCl, but the higher salt content inhibits the primordium formation of Basidiomycetes also (Kües and Liu, 2000). The trial of copper tolerance has showed, that some of the examined species are sensitive for copper, because the 20 µg/ml $CuSO_4$ concentration can inhibit their growth, while the other species can tolerate twice or three times higher values. As a result of it the copper containing disease control technologies can produce suitable inhibitory effect only applying high doses, which has an effect on mushroom growing also. I suppose that the application of copper in the disease control of mushroom growing cannot be advised, because of the feature of mushrooms, that they can intake metals and concentrate them in their fruit body. It is to be feared, that oyster mushroom fruit bodies contain too much copper in case of these concentration of $CuSO_4$. In conclusion of the *Pseudomonas* experiments, only the H^+ concentration from the examined environmental conditions can be used as a selective ecological factor.

SUMMARY OF PRACTICAL RECOMMENDATIONS

Proposition of forming variety-specific growing technology

Due to the characteristic deviances, and their definitive effects to the harvest yields, I recommend that instead of the generally applied oyster mushroom cultivation technology, considering the different environmental requirements of the hybrids determined by me, producers should apply variety-specific technologies for the purpose of yield increase.

In case of the HK35 hybrid, during incubation, for setting the temperature measured in the substrate, 25 °C is the recommendation; the time period is between 20-26 days. From economical aspect, the shorter incubation temperature is more advantageous, so considering cost efficiency, the recommended time period is 20 days. The optimum of temperature primordium formation and fruiting is 16 °C, but it is important that a temperature level being 3 °C lower than this may decrease harvesting yield by 30-50%.

In case of hybrid No. 357, the temperature optimum for incubation is 25 °C, but this

hybrid is sensitively reacting onto the increase of substrate temperature, and the yield is decreasing by 50% already at 31 °C. For the time period of incubation it is important to minimally reach the 20 days, but the optimum is closer to 26-29 days. The optimum of temperature primordium formation and fruiting is 19 °C, but the initiation at 22 °C still provides acceptable results as well.

The optimal incubation temperature range of P70 hybrid is 22-25 °C, and the time period, similarly to HK35 hybrid, should anyway reach the 20 days, but not more than 26 days. Considering economical aspects, the 20 days range is recommended. The optimum of temperature primordium formation and fruiting is 13 °C, but up to 19 °C only the quality level is decreasing, not the quantity of produce.

I have summarized those environmental values in table 3. with which the development of the growing technology applied in practice can be done, and variety-specific technologies can be formed. Based on the examinations, I propose the seasonal application of hybrids. The HK35 hybrid can be applied well during the spring-time and autumn-time transitive weather conditions, while the 357 hybrid accommodates to the summer cultivation conditions than the HK35. The P70 is a specifically winter type hybrid, requiring lower temperatures both during incubation and the period of fruit body development.

Table 3: *Variety-specific growing conditions of HK35, 357 and P70 hybrids*

hybrid	***mycelial growth***		***Primordium formation and fruiting temperature***
	temperature	***time***	
HK35	25°C	20-26 days	16°C sensitive for lower temperature
357	25°C sensitive for higher temperature *(31°C)*	20-29 days	19°C
P70	22-25°C	20-26 days	13°C

4. NEW OR NOVEL SCIENTIFIC RESULTS

1. I have defined the optimum values of some abiotic environmental factors influencing mycelial growth, primordium formation and fruiting in case of three *Pleurotus ostreatus* hybrids: a HK35, 357 and P70.

2. I have determined that in the case of the three investigated hybrids the effect of incubation temperature on the speed of mycelia growth is not a variety-specific feature, while the effect of incubation, primordium formation and fruiting temperature on yield is variety-specific feature. The optimum of incubation temperature resulting the highest yield is 25°C by HK35 and No. 357 hybrids, and 22-25°C by P70 hybrids. The optimum of primordium formation and fruiting temperature resulting the highest yield is 16°C by HK35 hybrid, 19°C by No. 357 hybrids and 13°C by P70 hybrids.

3. I have observed that the mushroom cap growth rate of HK35 hybrid is varying among 2-8 mm/day between +6°C and +24°C. I concluded that temperature optimum of HK35 hybrid cap growth is between 15-18°C with 70% relative humidity concerning the quality also.

4. A have determined the optimal pH, temperature, NaCl and $CuSO_4$ concentration values of the growth of *Pseudomonas* species isolated from yellowing oyster mushroom fruit bodies. I have concluded that in comparison oyster mushroom environmental parameter requirement with *Pseudomonas* species isolated from yellowing oyster mushroom fruit bodies, only the pH optimum can differ, and can be used as an ecological factor.

5. I have concluded that the pH change in the substrate during mycelial run is not variety-specific feature, and its kinetics is independent from the incubation time requirement of the hybrids.

6. Due to my examinations I recommend that instead of the generally applied oyster mushroom cultivation technology, considering the different environmental requirements of the hybrids, producers should apply variety-specific technologies for the purpose of yield increase.

5. REFERENCES

1. BESSETTE, A.E. – KERRIGAN, R.W. – JORDAN, D.C. (1985): Yellow blotch of *Pleurotus ostreatus*. *Applied and Environmental Microbiology*, 50 (6):1535-1537. p.
2. CHA, JEA-SOON (2004): Brown blotch diseases. In: *Mushroom Gowers' Handbook 1: Oyster Mushroom Cultivation.* Seoul, Corea: Mushworld, 180-192. p.
3. CHANG, S.T. - MILES, P.G. (2004): Mushrooms: Cultivation, Nutritional Value, Medicinal Effect, and Environmental Impact. Boca Raton, Florida, USA: CRC Press LLC, 15. 17. 21. 29. 132. 206. 315. 318. p.
4. GYŐRFI, J. (1989): Present plant protection problems in oyster mushroom (*Pleurotus ostreatus* and its hibrid strains) growing in Hungary. Mushroom Science XII (PartII). Proceedings of the Twelfth Interntional Congress on the Science and Cultivation of Edible Fungi, 603-609. p.
5. GYURKÓ, P. (1979): Laskagomba. In: BALÁZS, S. (Szerk.): *Gombatermesztés.* Budapest: Mezőgazdasági Kiadó, 175-216.
6. JAKUCS, E (1990): A gombák szerepe a cellulóz és lignin lebontásában. *Mikológiai közlemények*, 1-3:13-35. p.
7. KOVÁCS, A. (1998): A környezeti tényezők mérési és szabályozási lehetőségei. *Magyar Gomba*, 2 (8): 14-15. p.
8. KOVÁCSNÉ-GYENES, M. (1998): A laskagomba-termesztés sajátosságai nyáron. *Magyar Gomba*. 2(8): 12-13.
9. KREDICS, L. – KÖRMÖCZI, P. – CSEH, T. – HATVANI, L. – MANCZINGER, L. – NAGY, A. – VÁGVÖLGYI, CS. (2009): Green mould disease of oyster mushroom in Hungary and Romania: ecophysiology of the causative agents. In: KISS, I (ed): Proceedings of the 10th INTERNATIONAL SYMPOSIUM "INTERDISCIPLINARY REGIONAL RESEARCH", Hunedoara, Romania, 23-24 April, 2009, CD-ROM, paper S3-10, 1-6. p.
10. KÜES, U. - LIU, Y. (2000): Fruiting body production in basidiomycetes. Appl. Microbiol. Biotechnol, 54:141-152. p.
11. MUNSCH, P. – ALATOSSAVA, T. – MARTTINEN, N. – MEYER, J-M. – CHRISTEN, R. – GARDAN, L. (2002): *Pseudomonas costantinii* sp. nov., another

causal agent of brown blotch disease, isolated from cultivated mushroom sporophores in Finland. *International Journal of Systematic and Evolutionary Microbiology*, 52: 1973-1983. p.

12. NAGY, A. - SAJBEN, E. - ANTAL, Z. - MANCZINGER, L. - VÁGVÖLGYI, C. (2008): Extracellular enzyme producing abilities of Pseudomonas isolates pathogenic to oyster mushroom. 17th International Conference of the International Society of Mushroom Science, Cape Town, South Africa, 2008. Science and cultivation of edible and medicinal fungi Mushroom Science XVII, 628-637. p.
13. PALLERONI, NJ. (1984): Genus *Pseudomonas*. Migula. In KRIEG, N.R. and HOLT, J.G. BALTIMORE (Szerk): *Bergey's Manual of Systematic Bacteriology* Vol. I, MD, USA: Williams and Wilkins; 141-178. p.
14. RAINEY, P. B. - BRODEY, C.L. - JOHNSTONE, K. (1992): Biology of *Pseudomonas tolaasii*, cause of brown blotch disease of the cultivated mushroom. *Adv. Plant Pathol*., 8:95-117. p.
15. RAJARATHNAM, S. - SHASHIREKA, M.N. - BANO, Z. (1992): Biopotentialities of the Basidiomacromycetes. In *Advances in Applied Microbiology*, 37:233-361. p.
16. SZABÓ, I. (1986): A laskagomba jellemzése és termesztésének története. In SZABÓ (Szerk.): *A laskagomba termesztése*. Budapest: Mezőgazdasági Kiadó, 44-47. p.
17. SZILI, I. (2008): Gombatermesztők könyve. Budapest: Mezőgazda Kiadó, 88. 119. 126. p.
18. ZADRAZIL, F. (1974): The ecology and industrial production of Pleurotus *ostreatus*, *Pleurotus florida*, *Pleurotus cornucopiae* and *Pleurotus eryngii*. IXth International Scientific Congress on the Cultivation of Edible Fungi, Tokyo, on November 4, 1974. *Mushroom Science*, 42-49. p.

6. PUBLICATIONS ON THE THEME OF THE THESIS

REVIEWED ARTICLES IN PAPERS (IF)

Sajben, E., Manczinger, L., **Nagy, A.,** Kredics, L., Vágvölgyi, Cs. (2010): Characterization of pseudomonads isolated from decaying sporocarps of oyster mushroom. Microbiological Research. doi: 10.1016/j.micres.2010.05.002

Kredics, L., Kocsube, S., Nagy, L., Komon´ -Zelazowska, M., Manczinger, L, Sajben, E., **Nagy, A.,** Vágvölgyi, C., Kubicek, P.C., Druzhinina., S.I., Hatvani, L.(2009): Molecular identification of *Trichoderma* species associated with *Pleurotus ostreatus* and natural substrates of the oyster mushroom. FEMS Microbiol Lett 300: 58–67.p.

REVIEWED ARTICLES IN PAPERS

Somosné Nagy, A., Sajben. E., Manczinger. L., Vágvölgyi. C. (2009): Laskagombára patogén *Pseudomonas* nemzetséghez tartozó izolátumok néhány tulajdonságának vizsgálata. Kertgazdaság. 41. (3): 10-18.p.

Somosné Nagy, A., Kovács, A., Kovácsné Gyenes, M. (2007): Az átszövetési idő hatása a *Pleurotus sp*. terméshozamára. Kertgazdaság 39. (2):10-13.p.

Somosné Nagy, A., Kovács, A. (2007): The effect of pre-fruiting temperatures on the yield of *Pleurotus* strains. International Journal of Horticultural Science 13 (4):49-51.p.

Kovácsné Gyenes, M. **Somosné Nagy, A.** Kovács, A. Sándorné Ferenc, K. (2006): Laskagomba törzsek hőmérsékletigényének vizsgálatai az átszövetés időszakában. Kertgazdaság 38 (2):3-7.p.

Nagy, A. (2002): Ungarn: Die Grenzen des ungarischen Pilzmarktes. Der Champignon 426:19-21.p.

CONFERENCE PUBLICATIONS (FULLL PAPERS IN HUNGARIAN)

Nagy, A. (1997): A magyar gombaágazat fejlődési üteme, ágazati stratégiaalkotás SWOT analízis alapján. Vállalati környezet és alkalmazkodás az élelmiszertermelésben - Konferencia, Gödöllői Agrártudományi Egyetem. Tudományos Közlemények III. kötet 41-45.p.

CONFERENCE PUBLICATIONS (ABSTRACTS IN HUNAGRIAN)

Hatvani, L., Kocsubé, S., Nagy, L., Komon-Zelazowska, M; Manczinger, L., Cseh, T., Körmöczi, P., Antal, Zs., **Nagy, A.**; Druzhinina, S.I., Kubicek P.C., Vágvölgyi, Cs., Kredics, L. (2008): A new agricultural pest emerging: the green mould disease of cultivated oyster mushroom. Acta Microbiologica et Immunologica Hungarica 56 (S):58.p.

Sajben, E., Antal, Zs., Manczinger, L., **Nagy, A**., Vágvölgyi, Cs. (2007): Isolation and properties of *Bacillus* strains antagonistic to oyster mushroom pathogen pseudomonads. Lippay János-Ormos Imre-Vas Károly Scientific Symposium, Corvinus University, Budapest 2007. november 7-8. Abstarcts Horticultural Science 350; 351p.

Nagy, A., Kovács, A. (2007): Néhány *Pleurotus* törzs átszövődési idejének vizsgálata. Lippay János – Ormos Imre – Vas Károly Tudományos ülésszak 2007. Összefoglalók 368-369.p.

Sajben, E., Antal, Zs., Manczinger, L., **Nagy, A**., Vágvölgyi, Cs. (2006): Isolation and characterization of apathogenic *Pseudomonas* strains antagonistic to *Pseudomonas tolaasii*. Acta Microbiologica et Immunologica Hungarica 53: 336.p.

Somosné Nagy, A., Kovács, A., Kovácsné Gyenes, M. (2005): A nagyüzemi laskagomba termesztés – lehetőségek és korlátok Magyarországon. Lippay János – Ormos Imre – Vas Károly Tudományos Ülésszak 2005. Összefoglalók 370-371.p.

INTERNATIONAL CONFERENCE PUBLICATIONS (FULL PAPERS IN ENGLISH)

Nagy A, Manczinger L, Hatvani L, Győrfi J, Antal Z, Sajben E, Vágvölgyi C, Kredics L. (2010): Biological control of oyster mushroom green mould disease by antagonistic *Bacillus*

species. IOBC WPRS Bulletin, in press

Kredics, L., Körmöczi, P., Cseh, T., Hatvani, L., Manczinger, L., **Nagy, A.**, Vágvölgyi, C. (2009): Green mould disease of oyster mushroom in Hungary and Romania: ecophysiology of the causative agents. In: Kiss, I (ed): Proceedings of the 10th INTERNATIONAL SYMPOSIUM "INTERDISCIPLINARY REGIONAL RESEARCH", Hunedoara, Romania, 23-24 April, 2009, CD-ROM, paper S3-10, 1-6.p.

Hatvani, L., Kocsubé, S., Manczinger, L., Antal, Zs., Szekeres, A., Druzhinina,S. I., Komon-Zelazowska, M; Kubicek P.C., **Nagy, A.,** Vágvölgyi, Cs., Kredics, L. (2008): The green mould disease global threat to the cultivation of oyster mushroom (*Pleurotus ostreatus*): a review. In: Science and cultivation of edible and medicinal fungi: Mushroom Science XVII: Proceedings of the 17th Congress of the International Society for Mushroom Science, Cape Town, South Africa, 20–24 May 2008. 485-495.p.

Nagy, A. Sajben, E., Antal, Z., Manczinger, L., Vágvölgyi, C. (2008): Extracellular enzyme producing abilities of *Pseudomonas* isolates pathogenic to oyster mushroom. In: Science and cultivation of edible and medicinal fungi: Mushroom Science XVII: Proceedings of the 17th Congress of the International Society for Mushroom Science, Cape Town, South Africa, 20–24 May 2008. 628-637.p.

Nagy, A. (2001): Prosperous cultivated mushroom industry in Hungary. 1st International Conference for Young Researchers, Szent István Egyetem, Gödöllő. Konferencia Kiadvány 254-260.p.

INTERNATIONAL CONFERENCE PUBLICATIONS (ABSTRACTS IN ENGLISH)

Hatvani, L., Kocsubé, S., Nagy, L., Komoń-Zelazowska, M., Manczinger, L., Cseh, T., Körmöczi, P., **Nagy, A**., Druzhinina, I.S., Kubicek, C.P., Vágvölgyi, Cs., Kredics, L. (2009) Epidemiological questions of the green mould disease of cultivated oyster mushroom. In: CD-ROM, Abstracts of the 3rd Congress of European Microbiologists, FEMS 2009, Gothenburg, Sweden, June 28-July 2, 2009.

Nagy, A., Manczinger, L., Kredics, L., Hatvani, L., Győrfi, J., Turóczi, Gy; Antal, Zs., Sajben, E., Vágvölgyi, Cs. (2008): Green mold disease of *Pleurotus ostreatus* in Hungary and advances in its biocontrol. The 6th International Conference on Mushroom Biology and Mushroom Products, September 29. - October 3. 2008, Bonn, Germany, Book of Abstracts, 60.p.

Sajben, E., Antal, Z., Manczinger, L.**, Nagy, A.**, Vágvölgyi, C. (2007): Taxonomical and physiological investigation of *Bacillus* strains antagonistic to oyster mushroom pathogenic pseudomonads. 15th International Congress of the Hungarian Society for Microbiology, Budapest, 2007. Acta Microbiol. Immunol. Hung. 54(S): 111.p.