COMPARATIVE CULTIVATION EXPERIMENTS OF *PLEUROTUS ERYNGII* ISOLATES

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ABSTRACT

Sixteen *Pleurotus eryngii* isolates, mostly of Hungarian origin, were cultivated on lignocellulose substrate containing soy bean based enrichment. Composition of the substrate: 65% beech sawdust; 17% bran; 9% beech woodchips; 3.5% gypsum; 5.5% soybean supplement (Promycel 480). Water content was adjusted to 60%, and then the mixture was filled into plastic bags, sterilized and spawned. The blocks were cased by peat-based commercial *Agaricus* casing soil. We determined the amount of yield, number of fruiting bodies, period of flushes, average weight of fruiting bodies, biological efficiency (BE, %) and productivity (P, %) for each strains. The amount of yield was given for 100 kg of substrate. The highest yield was produced by the Ple-4V (41.5 kg/100 kg) and Ple-5V (39.5 kg/100 kg) strains, whereas the lowest yield was found in case of the PEL (9 kg/100 kg) and PEG (11 kg/100 kg) strains. The average yield of the strains was 27,53 kg calculated for 100 kg substrate. The number of fruiting bodies was 1488 pcs, the average weight of fruiting bodies (concerning the species) was 19.95 g. Very high biological efficiency was produced by the Ple-4V (156.2%) and Ple5V (140%) strains. The lowest efficiency was found at the PEL (28.5%) and PEG (37.8%) strains. The average yield was over 25 kg/100 kg spawned substrate.

Keywords: king oyster mushroom, *Pleurotus eryngii*, cultivation experiment, strain, isolate, biological efficiency

INTRODUCTION

Pleurotus species can be found on the northern hemisphere between latitude 30 and 50, mainly in steppes, in arid grasslands or sometimes in mountainous areas, and always in association with umbelliferous plants [1, 2]. In their natural habitat, taxa of the species complex are facultative biotrophs, white rot fungi of certain plant species belonging to the families *Apiaceae (Umbelliferae)* and *Asteraceae* (*Compositae*) [1, 3-8]. In spite of the fact that fruit bodies are primarily formed in association with umbelliferous plants, in cultivation they do not need the presence of these plant species. A number of lignocellulose based substrates can be used for intensive cultivation [9-13]. Numerous varieties are listed in the *P. eryngii* species complex: var. *eryngii* (DC.: Fr) Quel.; *ferulae* Lanzi /syn.: *P. fuscus* var. *ferulae*/; *elaeoselini* Venturella *et al.*; *nebrodensis* (Inzenga) Sacc.; *tingitanus* Lewinsohn *et al.*; *tuoliensis* C. J. Mou; *P. hadamardii* Constantin; *P. fossulatus* (Cooke Sacc.) [1, 6-8, 14-16].

Nowadays *P. eryngii* is cultivated in several Asian countries and in many European mushroom farms, and it is present in the European markets as well. In the USA *P. eryngii var. eryngii* is grown since 2000 [2]. Pasteurization and sterilization are the common procedures in substrate preparation. The substrate is than filled into bags, blocks, PP bottles or boxes. For achieving higher yields it is possible to apply peat-based casing, but the mushroom is able to grow on the surface of the lignocellulose substrate itself as well [2, 17, 18].

Different international sources report a wide range of biological efficiency depending on the substrate composition, application of casing and quality and quantity of supplementation. According to Sonnenberg *et al.* [19], the biological efficiency in Germany and in the Netherlands is rather low (10-15 %), but in their experiments they could achieve 20-25 %. Kirbag and Akyuz [20] used different agricultural byproduct based substrates and reported 45 % biological efficiency. Rodriguez *et al.* [21] succeeded179 % with applying different combinations of supplementation and casing.

Nowadays spawn producing companies use wild strains selected by growers, but in the future breeding will hold the key for *P. eryngii* production. From the species complex, *P. eryngii* is an exceptional choice for cultivation, as it is considered to have the best taste amongst all oyster species [21]. It is relatively easy to cultivate, has a fairly long shelf life, it also has a reduced spore load and a quite high price on the market [22]. Our aim was to conduct a comparative growing experiment of different, mainly Hungarian isolates of *P. eryngii* to identify the growing properties and the biological efficiency of the 16 isolates under the same ambient conditions, on the same substrate.

MATERIALS AND METHODS

Strains

The majority of the strains were collected from the grasslands and hills of the eastern part of Hungary (Table 1). Following collection of tissue samples of the fruit bodies were transferred onto malt agar, and by repeated inoculations clean vegetative mycelia cultures were achieved.

Table 1. *Pleurotus eryngii* isolates and their origin used in trial

Code	Origin	Cultivated/collected
PEA	Tószeg; Hungary	collected
PEP	Eger, Pásztorvölgy; Hungary	collected
PEG	Tószeg; Hungary	collected
PEF-i	Demjén, Vas-tanya; Hungary	collected
PEF	Kecskemét; Hungary	collected
PEC	Eger, Pásztorvölgy; Hungary	collected
Ple1V	Novaj; Hungary	collected
Ple2V	Novaj; Hungary	collected
Ple3V	Bogács; Hungary	collected
Ple4V	Heves; Hungary	collected
Ple5V	Novaj; Hungary	collected
Ple6V	Novaj; Hungary	collected
PES	Netherlands	cultivated
PE-SZM	Malaysia	cultivated
PEL	Italy	cultivated
PEK	China	cultivated

Propagation material (spawn)

Rye-based spawn was made in case of all 16 strains by the prevalent standard spawn production process.

Substrate

2000 ml capacity polypropylene bags were filled with 900 g wet substrate. The substrate consisted of 65 % beech sawdust, 17% wheat bran, 9% beech chips, 3.5% gypsum and 5.5% soy supplement (Promycel 480) (values referring to dry weight). Water content of the substrate was adjusted to 60%. The bags were closed with paper plugs and covered by tinfoil before sterilization in autoclave on 121°C for 2.5 hours. Following cooling the substrate was inoculated with spawn (10 m/m% spawn rate) in a laminar flow box.

Samples of fresh and spent substrate of the 16 strains were taken and dried to constant weight on 105 °C for measuring dry matter content. The dry matter content of the fresh and spent substrate and the difference in water content of the material before and after cropping were determined. Nitrogen content was measured by Kjeldahl method using a Büchi B-324 distillation unit.

Growing conditions

The bags were placed in a room with 25 °C temperature for spawn run. Since in case of each strain spawn run was evenly completed by the 10th day following spawning, the temperature was lowered to 10 °C. On the 12th day the bags were opened and folded and 3 cm casing was applied using sterilized peat based button mushroom casing soil. Following irrigation the bags were covered by *vlies*. From this day 12-12 hours light and dark periods were set and relative humidity was adjusted to 95%. On the 15th day the temperature was raised to 17.5 °C while keeping the surface covered and the humidity set on 95%. By the 17th day the mycelium appeared on the edge of the surface of the first bags. On the 19th day the vlies was removed, which lowered the CO_2 level below 800 ppm inducing fruit body formation.

After the primordia have appeared temperature was adjusted to 19 °C for the cropping period. During cropping, the surface of the bags, the floor and the walls of the room were irrigated daily to maintain proper humidity level. Ambient conditions in the cropping cycle: 19 °C (±2 °C) growing room temperature, 95% relative humidity and CO_2 level below 800 ppm.

Determination of growing properties

The yield and number of fruit bodies were registered during the entire cropping period; the values are given in kg and pcs per 100 kg substrate. The flushes, days of picking and average fruit body weights were determined in case of each strain. BE% and P% were also measured on fresh and spent substrates [21, 22].

Correlation and cluster analysis

Based on the results of substrate analysis and yield values the following questions were formed: a) Does the dry weight loss of the substrate correlate with the yield? b) Does the weight loss of wet materials correlate with the yield? c) Is there a correspondence between the total nitrogen content and the yield? To answer these questions Pearson's correlation analysis was conducted by using SPSS 15 program. Classification of the strains based on their yields was done by Tukey's correlation analysis and SPSS 15 program. The aim of the analysis was to determine which strains belong to the same group based on yields.

RESULTS

Yields

The highest yields were registered in case of strains Ple-4V (41.5 kg/100 kg) and Ple-5V (39.5 kg/100 kg), while the lowest in case of strains PEL (9 kg/100 kg) and PEG (11 kg/100 kg). Based on the results of this experiment *P. eryngii* can be characterized with an average 27.53 kg/100 kg yield, a total number of 1488 fruit bodies and an average fruit body weight of 18.5-19.95 g (Fig. 1). Fig. 1 also shows that based on the Tukey cluster analysis only two strains (PE-SZM and PES) could be placed in the same group, the others had various yields from lower to higher values.

In our opinion high yields (besides being genetically determined) were influenced by the following factors: casing

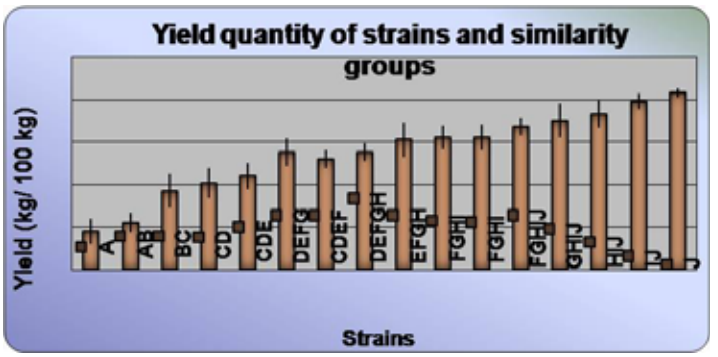

Figure 1. Yields of strains calculated for 100 kg substrate (kg), average weight of fruiting body (g), value of similarity and dissimilarity between strains. (The more identical characters in the name of the strains mean higher degree of similarity, while less identical character refers to less similarity).

prevented the drying of the lignocellulose substrate, provided equalized water supply and constant humidity for the mushrooms on the surface of the bags. The 12-16 days long spawn run described [21] was shorter (only 8-10 days) in our experiment due to the higher (10%) spawn rate. According to Stamets [22] the first flush is to be expected on the 20-29th day, but in spite of the shorter spawn run, we could not pick fruit bodies of the first flush sooner than 27-28 days. It has to be noted that others only picked on the 38th [20], or even on the 37-54th days [19] from different substrate compounds.

Biological efficiency and productivity

When taking the spent compost weight into account, relatively high biological efficiency can be reported in case of most strains. Especially high BE% was observed for instance by strains Ple4V (156%) and Ple5V (140%), while the lowest by PEL (29%) and PEG (38%). The average biological efficiency was 98% (based on spent substrate weight) and average productivity was 44%. If BE% and P% are calculated based on fresh materials, both values are lower (26% and 17%) because of the forthcoming weight changes during cultivation. In our opinion it is important to calculate these parameters in case of both the fresh and the spent substrates, since in international research papers it is not stated whether the presented BE% and P% values are calculated based on measurements before or after cropping. This fact might be the reason of the significant differences experienced in various researches. Table 2 shows the number and average weight of fruit bodies, biological efficiency and productivity (calculated for 100 kg substrate).

Table 2. Important data of comparative cultivation experiments, calculated for 100 kg substrate

Strains	Yield (kg/100 kg)	Total no. of fruit bodies (pcs/100 kg)	Av. weight of fruit bodies (g)	BE (%) (spent)	P (%) (spent)	[a]BE (%) (diff.)	[b]P (%) (diff.)
PEL	9	1050	8.6	28.52	11.11	4.8	2.11
PEG	11	1500	7.3	37.82	14.29	8.9	3.29
PEP	18.5	550	33.6	56.31	20.56	7.6	2.06
Ple6V	20.35	1050	19.4	74.35	32.82	20.8	12.47
PEFi	22	1100	20	72.29	31.65	14.4	9.65
Ple1V	26	1400	18.6	97.33	44.83	28.9	18.83
PEC	27.5	1600	17.2	100.28	45.45	27.9	17.95
Ple2V	27.6	1400	19.7	104.38	45.62	31.7	18.02
Ple3V	30.5	2450	12.4	109.43	58.65	29.1	28.15
PES	31	1500	20.7	111.2	43.06	29.6	12.06
PE-SZM	31	2150	14.4	120	55.36	38.4	24.36
PEA	33.5	1700	19.7	118.44	45.58	30.2	12.08
PEF	35	1400	25	119.7	53.44	27.5	18.44
PEK	36.5	950	38.4	128.26	65.18	32.2	28.68
Ple5V	39.5	1400	28.2	140.03	65.29	36	25.79
Ple4V	41.5	2600	16	156.18	76.85	46.9	35.35
Mean (species)	27.53	1488	19.95	98.41	44.36	25.9	16.83
Deviation	9.401	541.140	8.258	35.773	18.640	11.713	9.973

[a]BE% (diff.): BE% calculated to spent substrate – BE% calculated to fresh substrate.
[b]P% (diff.): P% calculated to spent substrate – P% calculated to fresh substrate.

Correlation analysis

Fig. 2 shows a more notable weight loss of the substrates in case of strains with higher yields. It can be concluded that productive strains have more efficient enzyme systems that are capable of degrading and utilizing the substrate more efficiently up to a certain point. The most likely explanations of the weight loss of the fresh substrate are: a) the mushroom utilizes the components of the substrate to build its own "body" and we remove this mass from the system by picking the mushrooms; b) the mushroom oxidizes the substrate and the carbon compounds (originating primarily from the substrate)

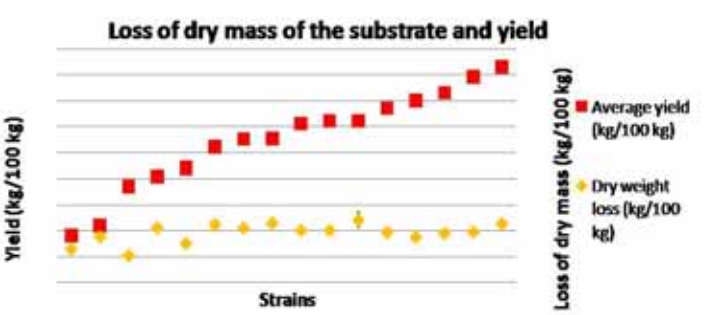

Figure 2. Differences between dry mass of substrate before and after cultivation (dry mass loss of substrate) and changes in the yield.

leave the growing material in CO_2 form which leads to the weight loss of the substrate. The average weight loss is 9.6 kg/100 kg (lowest: PEP – 5.126 kg/100 kg; highest: PE-SZM – 12.15 kg/100 kg). After a certain point the strains with higher yields are not able to a more intense degradation, regardless to the fact that the yield continued to increase. This is probably explained by other genetic differences of the strains.

The decrease in dry substrate mass and the yield was analyzed with Pearson's correlation. The Pearson's correlation coefficient of the 16 samples showed that there is a positive correlation (r = 0.542) between the weight loss and the yields by 5% significance level, thus the correlation is significant (α = 3%). There is a linear connection between weight loss and yields.

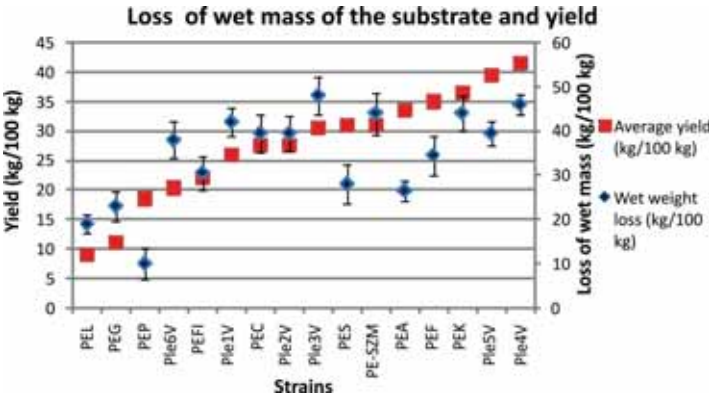

Figure 3. Differences between wet weight of substrate before and after cultivation (weight loss of wet substrate) and changes in the yield

Similar results can be seen on Fig. 3 where the connection between yields and the wet weight change of the substrate before and after cultivation is presented. Besides the picking of the mushrooms, other factors could serve as reasons for the weight loss of the wet substrate: a) intensity of evaporation, influenced by ambient conditions (primarily relative humidity and airflow); b) thickness of casing applied; c) water content of the casing soil; d) water transport from the casing soil towards the fruit bodies and evaporation intensity of the fruit bodies. For *P. eryngii* it is not yet known whether there is a water transport from the casing soil to the fruit bodies like in case of button mushrooms and if there is such, in what extent. Another question is how much does the evaporation capacity of the different strains vary?

Since *P. eryngii* has sufficient quantity and quality crop on supplemented substrate, we were looking for a connection between the total nitrogen content and yields. The results are shown on Fig. 4.

The Pearson's correlation coefficient of the strains showed that there is also a significant correlation between the nitrogen content and the yield. The correlation is negative (r = -0.593), the level of significance is at least 5 % (α = 1.5%). According to the results strains with lower yields had higher nitrogen levels in their spent substrates, while in case of the strains with higher yields the nitrogen level was lower.

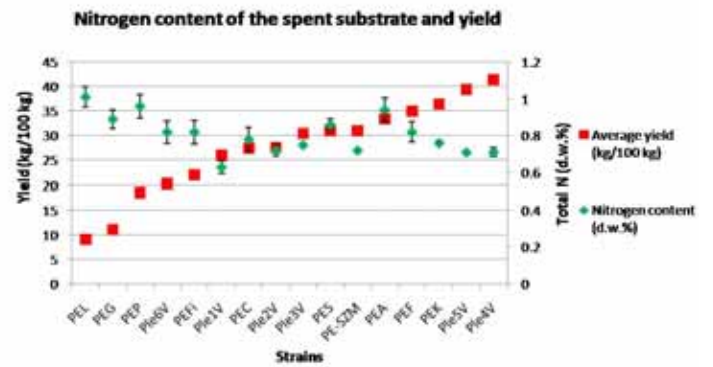

Figure 4. Correlation between total nitrogen content of substrate and amount of yield

Qualitative and quantitative elements

The results presented above mainly refer to physiological features and yields of the strains and the species. For the morphological and qualitative evaluation a photo documentation and a detailed cultivation guide was prepared, from which the following elements should be highlighted. Based on the days necessary to form the first flush and fruit bodies the quickest strains are: Ple-3V, Ple-4V, Ple-5V, PES and PEA. The first mature fruit bodies of these strains could be picked on the 27-28th day. The mushrooms reached mature state on the 30-31st day in case of strains PEP, PEC, PE-SZM, Ple-1V, Ple-2V and Ple-6V; 32-34th in case of PEK, PEF and PEF-i and 37th for strains PEL and PEG.

Strain PEC had the most intense development in the vegetative state, but its mycelium was apt to become stoma-like. Despite this phenomenon, numerous fruit bodies were formed in the stoma-like areas as well. Some distorted mushrooms appeared in case of strains Ple-1V and Ple-2V. PEL and PEG were the two strains with the lowest quality and quantity features. Strain PEL formed only a few regular but small fruit bodies, while PEG had pencil-shaped, curved fruit bodies without caps. These strains should be excluded from future breeding materials. By using sterilized substrate and providing maximal hygienic conditions no mycoparasites, competitor moulds or pests appeared in the course of the experiment. Only in one case did high relative humidity cause bacterial (*Pseudomonas* sp.) spots to form on the cap of a single fruit body of Ple-5V.

Description of strain PES

On the 23rd day, next to the edge of the bags a number of nice pea-sized, sometime walnut-sized fruit bodies developed. On the 26th and 27th day spherical, brown-greyish, marble patterned fruit bodies with curled up edges appeared. The first flush was ready to be picked on the 28th day. The *cap* of the immature fruit body is hemispherical, the edge is curled up at first, then it becomes flat and in the end funnel like, with a small lump in the middle. The color of the cap is brown-grayish with a hint of purple in it; the surface is radially filamentous, moderately felty and drily matt. By time the edge of the cap is less prone to discoloration (yellowing). The *stem* is usually centered. The *gills* are quite dense, whitish when young, then turn greyish. The gills run down on the top third of the stem and then continue as veins, fibers towards the base. The spore is white. The strain (Fig. 5) is recommended for commercial use. Picking dates: day 28, 30, 32, 37, 47, 56 and 60th. Other features: BE% = 111.2; P% = 43.06; average yield: 31 kg/100 kg; average number of fruit bodies: 1500 db/100 kg; average fruit body weight: 20.7 g.

Figure 5. Fruiting bodies of PES strain that was found to be appropriate for cultivation

Description of strain PEF

The first flush was ready to be picked on the 34th day. At first the *cap* is hemispherical, then is flattens and become funnel like. On the older fruit bodies the irregular, wavy edge is clearly apparent. The color is brownish with a slight grey shading, lighter on the edge. The surface of the cap is radially filamentous and matt.The stem is white, centered or eccentric, sometimes flattened on the sides. The gills are white when young, then become greyish. The gills are quite dense and run down deep on the stem. The spores are white. The fruit bodies grow in groups. The groups are sometimes big because of both the number and the size of the fruit bodies (Fig. 6). In spite of the few days longer growing period, this strain is highly recommended for cultivation. Picking dates: day 3, 47 and 56th. Other features: BE% = 119.7; P% = 53.44; average yield: 35 kg/100 kg; average number of fruit bodies: 1400 pcs/100 kg; average fruit body weight: 25 g.

Figure 6. Fruiting bodies of PEF strain that was found to be appropriate for cultivation

CONCLUSION

Future improvements of cultivation technologies should focus on the following problems. Besides using selected wild strains, breeders should produce hybrids for the industry. It would be advised to find the proper supplementation process and to adapt heat treatment procedures to the *P. eryngii* substrate. Another possible way of improving cultivation technology is to define the exact specific ambient conditions of the species or even the strains.

Nowadays the mushrooms in the fruit body stage ready to be picked are not evenly mature and so the picking date has to be adjusted the smaller fruit bodies would better be left on the bags to grow and most mature mushrooms can be plucked. If we just pick the big mushrooms and leave all the other on the bag, in most cases the remaining pieces of the group die. It would be necessary to find a way to synchronize fruit body development to have mushroom groups that are evenly mature.

During fruit body induction a high number of primordia appear, but only a few become full-grown mushrooms. The remaining fruit body initials die and serve as ground for bacterial and fungal diseases. An improved way of fruit body induction should be found to reduce primordia size and to promote equalized development. To prevent mushroom groups to form and to achieve uniform fruit body development, growers should try ruffling (a technique already used in button mushroom cultivation) in case of *P. eryngii* as well. Another solution is to apply a form of CACing, where kind of a 'Phase III. substrate' or casing spawn is mixed into the casing soil.

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