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Comparative studies on the cultivation and phylogenetics of King Oyster Mushroom (*Pleurotus eryngii* (DC.: Fr.) Quél.) strains

József SZARVAS[1]
email: szarvasj@ektf.hu

Károly PÁL[2]

András GEÖSEL[3]

Júlia GYŐRFI[3]

[1]Department of Food Chemistry and Biochemistry,
Eszterházy Károly College, Eger, Hungary

[2]Department of Microbiology and Food Technology,
Eger, Hungary

[3]Department of Vegetable and Mushroom Growing,
Faculty of Horticultural Science,
Corvinus University of Budapest, Hungary

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Abstract. The king oyster mushroom (*Pleurotus eryngii*) is still not a well known species amongst producers, despite its excellent taste and relatively easy cultivation technologies. Nevertheless, European growers show growing interest towards it. We collected king oyster mushroom strains from various habitats to get a better view about their vegetative growth, molecular based taxonomic relationship and cultivation parameters. In the *in vitro* experiments we investigated the growth of vegetative mycelia at various temperatures and pH. All strains were cultivated on lignocellulose substrate for 60 days under adequate climatic conditions and yield quantity, flushes, number and average weight of fruiting bodies

were determined. Biological Efficiency (BE%) and Productivity (P%) were calculated for each strains. We investigated taxonomic relationships among *P. eryngii* isolates by means of RAPD-PCR method. Twenty-five random primers were tested and six of them supplied us with sufficient data for generation of a neighbour-joining tree.

Results of the cultivation experiments showed that environmental conditions resulted in a very different growth rate of the various P. eryngii strains on different temperature and pH. Calculated for 100 kg substrate the highest yield was produced by the Ple-4V strain (41.5 kg), whereas the lowest yield was found at the PEL isolate (9 kg). The average yield of the investigated strains was 27.53 kg. Average weight per fruiting body was 19.95 g. We found the highest BE% at the Ple-4V and the lowest BE% at the PEL strains, 156.18% and 28.52%, respectively. These results showed that cultivation properties of the isolates are very different. The neighbour-joining tree revealed the taxonomic relations amongst the Hungarian, Malaysian, Italian and Dutch isolates. No correlation was found between the taxonomical position and growth rate of the investigated isolates. The average yield showed that the *P. eryngii* might be a suitable alternative of the popular tree oyster mushroom (*P. ostreatus*). Our results can be useful for subsequent cross breeding experiments and the dataset might support further investigations.

Keywords: vegetative mycelium, RAPD-PCR, neighbour-joining tree, biological efficiency

1 Introduction

Edible mushrooms are low energy foods with high nutritional value. Though their role in the healthy, reform and dietetic nourishment is significant, mushroom consumption in Hungary is still not remarkable. Amongst the traditionally cultivated and potentially new mushroom species, the king oyster mushroom (*Pleurotus eryngii*) bears extraordinary opportunities for cultivation and consumption.

Pleurotus species belong to the *Pleurotaceae* family, a member of the *Basidiomycota* phylum. There are white rot saprobe and facultative parasitic species in the genus that degrade polymers of lignin and cellulose, as well. *Pleurotus eryngii* varieties can be found in pastures, meadows, gardens and seldom in grassy forest clearings and hilly areas. This species is mainly associated with members of the *Apiaceae* (*Umbelliferae*) plant family in its natural habitats [1, 2, 3, 4].

Taxonomic relationships of the host-specialized *P. eryngii* species complex have not obviously defined yet. According to the latest phylogenetic research,

the following taxons are suggested: *P. eryngii* var. *eryngii*, *P. eryngii* var. *ferulae*, *P. eryngii* var. *elaeoselini*, *P. eryngii* var. *nebrodensis*, P. *hadamardii*, *P. fossulatus* [5, 6] and the newly described variety *P. eryngii* var. *tingitanus* [7, 8]. The taxonomy debates could be ended by comprehensive molecular biology, speciation and co-evolutionary researches.

Intensive production of the species started in Hungary in the 1950's, on mixture of composted hay and sawdust [9, 10, 11, 12]. In the 1960s Véssey tried to produce the mushroom on sterile substrate [13]. Cailleux & Diop used sterile medium consisting of wheat straw and oat for the production [14]. There are much more raw materials that can be used for cultivation, than those mentioned in the literature. Most of these materials originate from agriculture and forestry, such as straw of different grains, sawdust, cotton-straw, soybean straw, corn stalk, *Cyperus alternifolius* stalk, corn flour, cooked grain seeds, malt, bamboo powder, cornmeal, ricebran etc.[15, 16, 17, 18, 19, 20]. The substrate can be produced either by wet heat treatment and dry heat treatment (xerotherm method) or by sterile technology [8, 21]. A critical point in the preparation of the substrate is the adjustment of nitrogen content, since both the too low or too high level of nitrogen results a decrease in the amount of mushroom [22]. The danger of supplementation is the emergence of various moulds (e.g. *Trichoderma* spp., *Penicillium* spp. etc.) on the blocks and later on the fruiting bodies.

Generally, cultivation is carried out on blocks, in bottles or in plastic bags. Casing material can be applied that might be the same as which used for the cultivation of white button mushroom (*Agaricus bisporus*). Fruiting bodies appear on both of the non-cased and cased substrate, as well. Győrfi & Hajdú [20] found significant yield increase compared to the non-cased cultures, when casing soil mixtures were used in different thickness. Another possible cultivation method is when the spawn run blocks are sunken 25-30 cm deep into the soil and then covered by 3 cm of casing soil.

Mushroom strains vary in their ability to convert substrate materials into mushrooms as measured by a simple formula known as the "Biological Efficiency (BE) Formula". BE = weight of fresh mushroom fruiting bodies / weight of dry substrate × 100 [12, 23]. Sonnenberg et al. [22] published that BE is often very low (10-15%) in Germany and the Netherlands, but it could be increased 20-25% in cultivation experiments. Kirbag & Akyüz [12] reported 48-85% BE when agricultural waste was used as substrate. Rodriguez Estrada et al. [24] varied supplementation and casing and reached 179%. Productivity (P) is used sometimes by producers as an indicator of yield quantity calculated for wet substrate weight. Productivity (P) = (fresh mushroom weight / fresh

substrate weight) × 100 [25].

The aim of this study was to determine the yield of wild *P. eryngii* isolates and their taxonomical relations on the basis of RAPD-PCR fingerprints.

2 Materials and methods

Isolation of strains: isolates were collected from various grassy areas in Hungary. Inoculation onto malt agar plates was made by the use of plectenchyma originated from the broken pileus, and then the plates were incubated on 25°C. After incubation samples were taken from the regular sectors of the vegetative mycelia and inoculated three more times onto new agar plates. Strains were maintained on slant agar in test tubes and replicas were stored in liquid nitrogen.

Three strains (PE-SZM, PEL and PES) originated from the Strain Research Laboratory of the Hungarian Mushroom Growers' Association in vegetative mycelial form. Originally, the PE-SZM originated from Malaysia, whereas the PEL and PES were collected in Italy and in the Netherlands, respectively.

Investigation of the effect of temperature: 10 days old vegetative mycelial mats of the 15 strains were inoculated into the centre of malt agar plates, one strain on each plate. Mycelial mats were collected by 0.8 cm diameter cork borer. Incubations were performed in the following temperatures: 5°C, 10°C, 15°C, 20°C, 25°C, 30°C and 35°C. Incubations on each temperature were repeated three times with each strain, the control strains were grown on 25°C. In addition to the collected strains in this experiment the Korona 357 (K 357) *P. ostreatus* industrial hybrid strain, originated from the crossing of the popular Gyurkó HK 35 hybrid, was investigated, as well.

Investigation of the effect of pH: pH of the malt agar plates was set between 4 and 9 in 0.5 steps by 1 M HCl and 1 M NaOH. Inoculations were made the same way as in the temperature experiments in three replicates and the plates were incubated on 24°C. The pH of the control malt agar plates was set to 6, and the K 357 industrial hybrid was used as a control strain.

Spawn making: rye based spawn was produced from each isolates. 5.5 l rye was cooked then thinly covered with gypsum. 200 g rye was filled into a 500 ml Erlenmeyer flask and sterilized on 121°C for 2 hours. After cooling, the rye was inoculated by 1.5-2 cm diameter agar discs covered with 7 days old mycelia and then incubated on 25°C for 10 days.

Substrate and cultivation: 900 g wet substrate was filled into 2,000 ml polypropylene bags. Composition of substrate was the following (calculated

for air dry material): beech sawdust 65%, wheat bran 17%, beech chips 9%, gypsum 3.5%, soy supplement (Promycel 480) 5.5%. Moisture of substrate was set to 60%. The bags were closed by paper plugs, then covered by aluminium foil and autoclaved on 121°C for 2.5 hours. After cooling, the substrate was inoculated by 10 m/m% spawn.

Dry weight content was calculated after drying the substrate at 105°C until constant mass. Dry weight of initial and spent substrates was determined. In order to calculate productivity, initial wet mass and spent wet mass of the substrate were measured.

Spawn running was carried out on 25°C. Due to the 10 m/m% rate used for spawning, all the substrates were evenly colonized in 10 days from the inoculation, then the bags were cooled onto 10°C. On the 12th day the bags were opened and the substrate within was cased with peat based casing soil. It was followed by spraying with water in order to lightly moisturize the soil then the bags were covered with veil foil. From this point 12 hours of light and 12 hours of darkness by turns and 95% relative humidity was adjusted. On the 15th day air temperature was raised to 17.5°C. On the 17th day the mycelia reached the surface of the casing soil at the edges of the bag. On the 19th day the veil foil was removed, the level of CO_2 dropped below 800 ppm and initiation of fruiting was facilitated.

After the appearance of primordia the temperature was raised to 19°C (+/- 2°C) and the casing soil, the floor and walls of the growing house were sprayed with water.

Quantity of yield and number of fruiting bodies were registered and calculated for 100 kg of substrate. Average weight of fruiting bodies was determined. BE and P values and their differences were calculated for initial and spent substrates. Based on the average results of the strains, growing characteristics of the species were deduced.

Grouping of the strains on the basis of yield was made by Tukey correlation analysis tool of the SPSS 15 software.

DNA-extraction: a modified protocol of Shure [26] was used for DNA extraction. Thirty mg of freeze-dried mycelium powder was placed into a microcentrifuge tube, then 325 μl DNA isolation buffer (0.6 M NaCl, 0.1 M Tris (pH 7), 40 mM EDTA, 4% Sarcosyl, 1% SDS), 325 μl urea (60 g urea dissolved in 100 ml distilled water) and 6.5 μl 1 M $Na_2O_5S_2$ was pipetted onto it and the mix was vortexed for 1 min. The tubes were then incubated on 37°C for 45 min and vortexed for 15 s after 15 and 30 min of incubation. The proteins were precipitated by 650 μl phenol:chloroform:isoamyl alcohol 25:24:1 solution, then the samples were centrifuged at 9,500 g for 7 min. The

supernatant was pipetted into a new tube. The phenol-chloroform treatment was repeated twice, then the water phase was pipetted into a new Eppendorf tube and the DNA was precipitated with 70% (v/v) cold isopropanol. The tubes were incubated for 5 min then centrifuged at 9,500 g for 7 min. The isopropanol was removed from the tube and the pellet was washed three times with 1 ml 70% ethanol. The DNA was dried in vacuum concentrator, and then dissolved in 80 μl 1×TE buffer. The DNA solution was treated with 2 μl RNase on 37°C for 45 min, and then stored on -20°C. Quality and quantity of the DNA was measured with a NanoDrop 1000 (Thermo Fisher Scientific, USA) spectrophotometer.

RAPD-PCR: after the RAPD-PCR conditions were optimized, DNA extracts of the 15 P. eryngii isolates were tested with a P. ostreatus strain, which was chosen as outgroup. For RAPD the OpA and OpB random decamers (Operon Technologies) were used. The PCR reaction volume was 25 μl that contained 0.5 μl $MgCl_2$ (25 mM), 2.5 μl Taq buffer, 0.5 μl dNTP (10 mM), 1 μl primer (10 pM), 0.125 μl Taq polymerase (5 U μl^{-1}; Fermentas, Canada), 2 μl template DNA (200 ng μl^{-1}) and 18.375 μl distilled water. PCR amplifications were performed in a Corbett Research PCR Thermal Cycler (Corbett Life Science, Australia) using the following cycling parameters: initial denaturation at 95°C for 2 min, followed by 35 cycles of amplification comprising a denaturation step for 1 min at 95°C, annealing at 34°C for 1 min, and extension at 72°C for 1 min and a 5 min final extension step at 72°C.

Eight μl of the PCR product was electrophoresed at 110 V for 1 h on a 1% agarose gel (SeaKem LE Agarose, Lonza) stained with GelRed (Biotium, USA) in 1×Tris Borate EDTA buffer. A 100 bp BenchTop ladder (Promega, USA) was used as a molecular size marker.

Construction of phylogenetic tree: RAPD-PCR experiments were repeated three times and the resulted band patterns were scored visually. Presence or absence of bands was recorded in a binary matrix, separately for each primer. Genetic distance matrices were calculated using PHYLTOOLS software package and Nei-Li coefficient. The neighbour-joining tree was generated by the NEIGHBOR program of the PHYLIP software package [27, 28].

3 Results and discussion

Strain collection: in the period of 2006-2008 12 strains from different areas of Hungary were isolated (Figure 1) and deposited in the Laboratory of Microbiology of the Eszterházy Károly College (Eger).

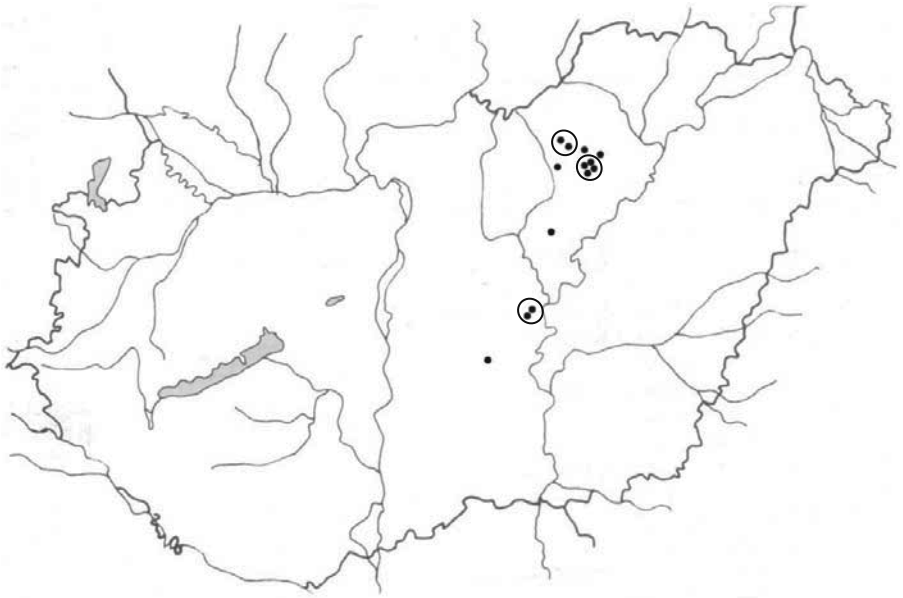

Figure 1: Location of Hungarian strains. 1: Kecskemét; 2: Tószeg (two isolates); 3: Hevesi Füves Puszta (Heves Grass Plains); 4: Demjén; 5: Novaj (four isolates); 6: Bogács; 7: Síkfőkút; 8: Eger, Pásztorvölgy (two isolates)

Growth rate of vegetative mycelia on different temperatures: mycelia of the various strains showed very similar growth rate on minimum and maximum temperatures. In contrast to this, significant differences of the growth rate were noticed on optimal temperature, which was between 25-30°C for most of the strains. In average, the isolates showed 6.6-8 mm daily growth and the PEC and PEFi strains produced the highest growth rate. Since the optimal temperature range is used for spawn run in the course of cultivation, it is worth determining the growth rate of strains before mass production, because significant differences can be found between them. Table 1 shows the time required by the strains to reach the maximum colony diameter (8 cm) on a given temperature. Grey fields show the shortest time the strains needed to reach the maximum colony diameter and the lowermost line gives a summary about the number of strains that reached the maximum diameter on the given temperatures.

pH tolerance: *Pleurotus* species have high pH tolerance, but significant differences in the optimum pH value were found among the strains. A strongly limiting value for some strains was pH 4, whereas others grew almost at the same rate as at higher pH. It was surprising that some isolates showed a rela-

tively fast rate of growth on alkaline pH (8-9). This feature may be used for protection against competitive organisms and microparasites in order to avoid the use of pesticides.

Table 1: Time (days) required by the different strains to reach maximum colony diameter (8 cm) on certain temperatures. C: K 357 P. ostreatus hybrid control.

Strain/Temp.	5 °C	10 °C	15 °C	20 °C	25 °C	30 °C	35 °C
PEP	41	32	24	**20**	**20**	23	-
PEC	41	32	24	10	**7**	10	-
PEF	41	32	24	12	**10**	**10**	-
PE-SZM	-	32	19	**12**	**12**	**12**	-
Ple-5V	41	32	19	**12**	**12**	**12**	-
Ple-4V	41	32	14	**10**	12	12	-
Ple-3V	41	32	19	17	12	**10**	-
Ple-2V	41	32	14	**10**	**10**	**10**	-
Ple-1V	41	32	14	**10**	**10**	**10**	-
Ple-6V	41	32	19	**12**	**12**	**12**	-
PEFi	41	32	14	12	**7**	10	-
PES	41	32	14	12	**10**	**10**	-
PEA	41	32	19	12	**10**	**10**	-
PEL	-	32	24	**12**	**12**	17	-
PEG	41	32	19	**12**	14	14	-
C (*P.o.*)	41	32	10	7	10	10	-
Mean	41.00	32.00	18.67	12.33	11.47	12.13	-
Deviation	0.000	0.000	3.994	2.717	3.137	3.603	-
Prevalence	0	0	0	9	12	9	0

Table 2 shows the connection between pH values and the time required for the strains to reach the maximum colony diameter. We found that the isolates grew faster on two pH values: one optimum was at the acidic pH 4.5, the other one was in the alkaline pH 7.5-8.5 range. Since the result was obtained from the mean growth rate of 15 strains and the strains did not show significant differences, it is more characteristic for the species than for its different strains.

Yield of various isolates: the average yield was calculated for 100 kg substrate. The highest yield was produced by the Ple-4V and Ple-5V strains, 41.5 kg and 39.5 kg, respectively. The lowest yield was found at the PEL and PEG strains, 9 kg and 11 kg, respectively. The average yield calculated for 100 kg substrate was 27.53 kg. Average weight of fruiting bodies was 19.95 g. Average yield of strains and weight of fruiting bodies is shown in Figure 2. We

performed Tukey cluster analysis and found that only two strains (PE-SZM and PES) can be grouped together, the remaining isolates show continuous transition from the lower towards the higher yield.

Table 2: Time (days) required by the different strains to reach maximum colony diameter (8 cm) depending on the pH of agar plates. C: K 357 P. ostreatus hybrid control.

Strain/pH	**4**	**4.5**	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**
PEA	14	14	14	13	13	13	11	**8**	11	10	11
PEC	17	11	9	10	9	**8**	**8**	**8**	**8**	9	9
PEG	14	**13**	14	14	14	17	14	14	14	14	14
PEF	17	14	14	14	13	13	12	**11**	14	**11**	**11**
PEFi	11	11	10	10	10	10	**8**	**8**	**8**	9	9
PEL	14	14	14	14	14	**13**	14	14	14	17	16
PEP	**17**	**17**	19	22	22	22	22	22	22	22	22
PES	18	17	17	17	15	14	13	13	**11**	**11**	**11**
PE-SZM	14	14	**11**	14	13	13	13	13	13	13	14
Ple-1V	14	11	10	10	10	10	10	**9**	11	10	10
Ple-2V	11	**9**	**9**	10	11	13	10	10	**9**	**9**	11
Ple-3V	14	**11**	**11**	13	13	14	**11**	**11**	**11**	**11**	**11**
Ple-4V	14	11	11	13	11	13	11	11	11	**10**	11
Ple-5V	14	14	14	13	13	13	13	16	16	**11**	17
Ple-6V	14	14	14	13	14	13	13	**11**	**11**	13	14
C (P.o.)	9	9	9	9	9	9	8	8	8	8	9
Mean	14.47	13.00	12.73	13.33	13.00	13.27	12.40	11.93	12.27	12.00	12.73
Deviation	2.031	2.299	2.915	3.109	3.047	3.173	3.397	3.693	3.535	3.525	3.515
Prevalence	1	4	3	0	0	2	3	7	6	6	3

The dataset suggests that not only the genetic background had an effect on the relatively high average yield, but other factors were also responsible. Casing inhibited desiccation of lignocellulose substrate, ensured even moisture content and water uptake for the mushroom and attenuated errors of climatization. Changing of climatic conditions (temperature, CO_2 level, RH, air flow) played an important role, as well. An additional key factor was the raise of nitrogen level, what was assured by use of nitrogen containing soy supplement (Promycel 480). Sterile cultivation technology, low substrate quantity and 60 days long cultivation (with more flushes depending on the strain) were also important factors.

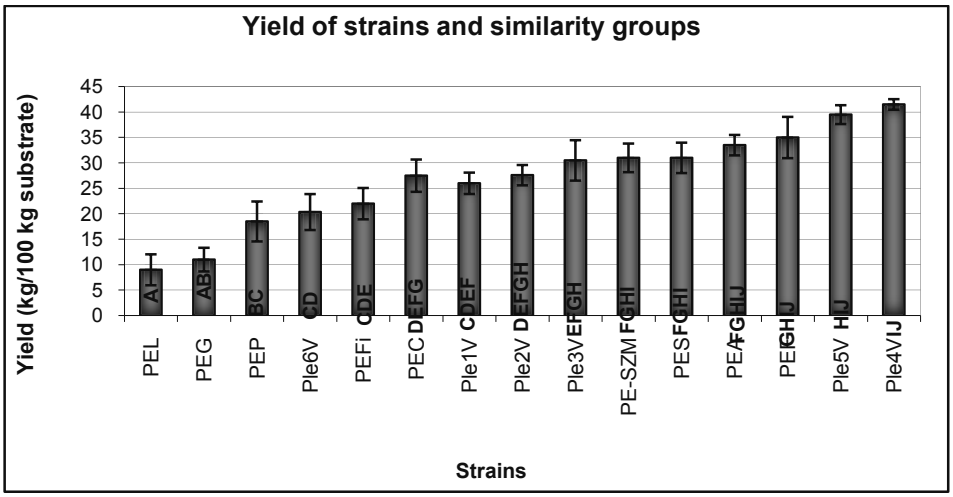

Figure 2: Yield amount of strains calculated for 100 kg substrate (kg), average weight of fruiting body (g), value of similarity and dissimilarity between strains. The more identical characters in the bars mean higher degree of similarity; while less identical character refer to less similarity.

The 12-6 days spawn running period given by Stamets [23] had been shortened to 8-0 days in our case, since we used high (10%) spawn rate. Even so, we could harvest the first flush only at the 27-28th day, which was very close to the 20-29th day described earlier [23]. However, other authors stated that they could harvest the first flush only at the 38th day [24] or in a 37-54 day period [12], depending on the composition of the substrate.

Biological efficiency and productivity: a relatively high BE value was resulted for most of the strains when the weight of the spent substrate was used in the calculations. Especially high BE value was found at the Ple-4V (156.18%) and Ple-5V (140.03%) strains. The lowest BE was found at the PEL (28.52%) and PEG (37.82%) strains. BE value of the species, calculated as average BE of the strains, was 98.41%, whereas the productivity was 44.36%. As long as the calculations were made with the initial substrates instead of the spent substrates, both the BE and P values were lower, since the weight of the substrate changed during cultivation. Average BE calculated for the initial substrate was 25.9%, the productivity was 16.83%. We think it is important to calculate these two parameters (BE and P) for both the initial and spent substrate, because in the literature it is not stated which version was used for calculations, though, it might be the reason of the serious differences of BE values published by different authors.

Table 3 summarizes our results: quantity of yield calculated for 100 kg substrate, number of fruiting bodies, mean weight of fruiting bodies, biological efficiency and productivity, for each investigated strains.

Table 3: Summary of the main results calculated for 100 kg substrate. Mean weight values were rounded to the first decimal.

Strain	Quantity of yield (kg/100 kg)	Total nr. of FBs (pcs/100 kg)	[a]Mean weight of FB (g)	BE % spent substrate	P % spent substrate	[b]BE % (D)	[c]P % (D)
PEL	9	1.050	8.6	28.52	11.11	4.8	2.11
PEG	11	1.500	7.3	37.82	14.29	8.9	3.29
PEP	18.5	550	33.6	56.31	20.56	7.6	2.06
Ple-6V	20.35	1.050	19.4	74.35	32.82	20.8	12.47
PEFi	22	1.100	20	72.29	31.65	14.4	9.65
Ple-1V	26	1.400	18.6	97.33	44.83	28.9	18.83
PEC	27.5	1.600	17.2	100.28	45.45	27.9	17.95
Ple-2V	27.6	1.400	19.7	104.38	45.62	31.7	18.02
Ple-3V	30.5	2.450	12.4	109.43	58.65	29.1	28.15
PES	31	1.500	20.7	111.2	43.06	29.6	12.06
PE-SZM	31	2.150	14.4	120	55.36	38.4	24.36
PEA	33.5	1.700	19.7	118.44	45.58	30.2	12.08
PEF	35	1.400	25	119.7	53.44	27.5	18.44
Ple-5V	39.5	1.400	28.2	140.03	65.29	36	25.79
Ple-4V	41.5	2.600	16	156.18	76.85	46.9	35.35
Mean (species)	27.53	1.488	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]FB: fruiting body
[b]BE % (D): BE % calculated with spent substrate – BE % calculated with initial substrate
[c]P % (D): P % calculated with spent substrate – P % calculated with initial substrate
BE% and P% values calculated with the initial substrate are not shown in the table

Molecular biology examinations: as a result of the preliminary RAPD-PCR experiments, six primers were chosen that produced differentiating bands for each strain. These decamers were the following: OPA 05, OPA 07, OPA 10, OPA 13, OPA 18 and OPB 10.

Most of the strains could be differentiated with the OPA 05 and OPA 13 decamers, but high degree of similarity was found between the Ple-1V/Ple2V and Ple3V/Ple-4V isolates, respectively. Ple-1V and Ple-2V strains were isolated from the same habitat, but the Ple-3V and Ple-4V isolates originated from different locations. Another interesting result is that the OPA 05 could not differentiate the Ple-5V and Ple-6V isolates, but OPA 13 patterns of the two isolates were not identical, though these two strains were collected from the same place in two successive years. RAPD-PCR reactions were repeated three times under previously optimized circumstances and the differentiating bands were used for calculation of distance matrices and generation of the neighbour-joining tree. Some primers resulted RAPD fingerprints that were unique for certain strains, so these primers might be suitable for characterization of these strains in the future. By cloning and sequencing of differentiating bands specific primers can be designed for a given isolate. Therefore, these primers might be valuable tools for breeders and spawn makers for origin protection and differentiation of isolates.

The neighbour-joining tree illustrates the taxonomical relations of the collected *P. eryngii* strains (Figure 3). There are two large groups on the tree: four strains were found to be slightly related to the Malaysian strain, while nine isolates were closer related to the Italian strain. It is remarkable that in one case we could isolate the same strain in both years (Ple-5V and Ple-6V in 2007 and 2008) from the same area. It is also interesting that samples from Kecskemét and Demjén show close relation, though the areas are 140 km apart from each other. Furthermore, no significant differences were found amongst the samples collected in the mountains and around Kecskemét's low landscape, which exhibit different climatic and environmental conditions.

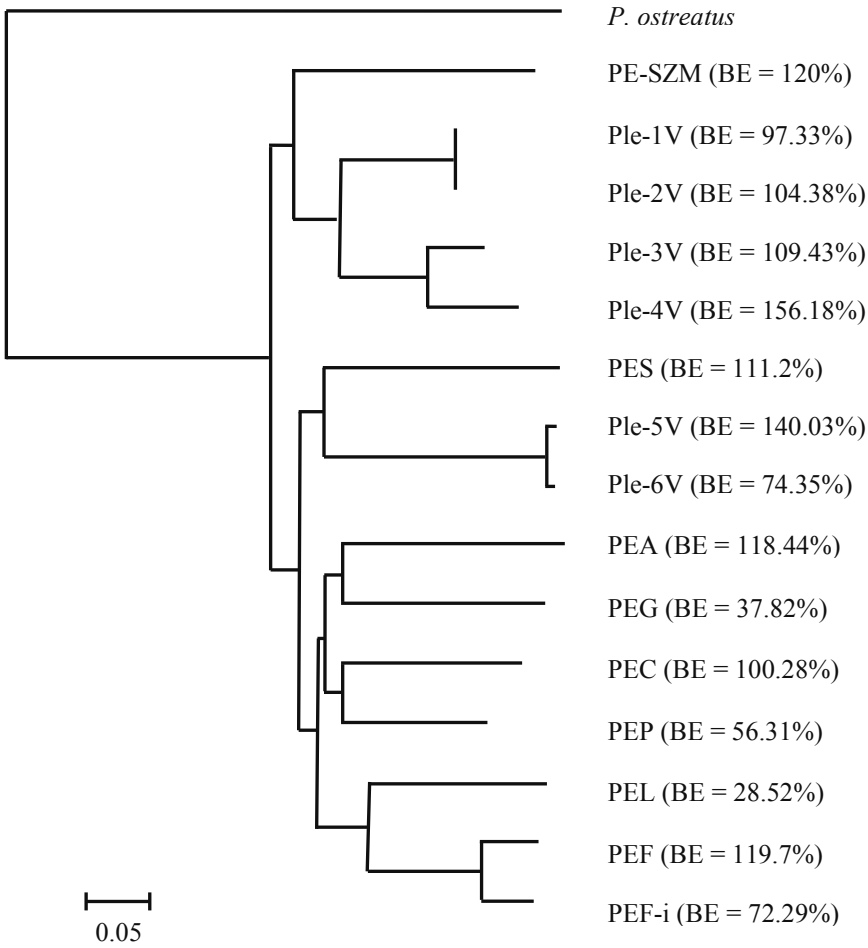

Figure 3: Dendrogram based on the RAPD analyses, created by Neighbor-Joining program [27]. The scale bar represents genetic distance. Biological efficiency (BE) is shown in brackets. Origin of strains: PE-SZM: Malaysia; Ple-1V: Hungary (Novaj 2007); Ple-2V: Hungary (Novaj 2007); Ple-3V: Hungary (Bogács); Ple-4V: Hungary (Hevesi Füves P.); Ple-5V: Hungary (Novaj 2007); Ple-6V: Hungary (Novaj 2007); PEA: Hungary (Tószeg); PEG: Hungary (Tószeg); PEC: Hungary (Pásztor-völgy); PEP: Hungary (Pásztor-völgy); PEL: Italy; PES: the Netherlands; PEF: Hungary (Kecskemét); PEF-i: Hungary (Demjén).

4 Summary

In the present research 13 wild *P. eryngii* strains of Hungarian origin were used in order to determine in vitro the growth rate of the vegetative mycelia of the species and the different strains, under various environmental conditions. Growth rate of various *P. eryngii* strains was very diverse, according to the well defined values of the environmental factors. On the basis of the average growth rate of the strains, we could conclude what are the optimum ecological values of the species, though these conclusions did not always coincide with the optimum values of the strains.

RAPD-PCR analysis was applied to examine the genetic diversity of the *P. eryngii* strains. Twenty-five primers were tested and six were chosen for further analysis and calculation of binary matrices on the basis of RAPD fingerprints. A neighbour-joining tree was generated from the matrices by the NEIGHBOR program of the PHYLIP software package. The tree reflects taxonomic relations amongst the Hungarian isolates and shows that some of them are related to a strain of Malaysian origin, whereas others to an Italian strain.

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References

[1] Hilber, O. (1982), Die Gattung Pleurotus (Fr.) Kummer unter besonderer Berücksichtigung des *Pleurotus eryngii* Formen-komplexes, Bibliotheca Mycologica, 87.

[2] Joly, P., Cailleux, R., Cerceau, M. (1990), La stérilité mâle pathologique, élément de la co-adaptation entre populations de champignons et de plantes-hôtes: modèle des *Pleurotes* des Ombellifères, *Bull. Soc. Bot. Fr.*, 137, pp. 71-85.

[3] Zervakis, G., Balis, C. (1996), A pluralistic approach in the study of *Pleurotus* species with emphasis on compatibility and physiology of the European morphotaxa, *Mycol. Res.* 100, pp. 717-731.

[4] Ewald, G. (2007), Gombászok kézikönyve. Pozkal-Inowroclaw, Lengyelország (Hungarian translation by Cs. Locsmándi) M-érték Kiadó Kft., 218.

[5] Venturella, G. (2000), Typification of *Pleurotus nebrodensis, Mycotaxon* 75, pp. 229-231.

[6] Zervakis, G., Venturella, G., Papadopoulou, K. (2001), Genetic polymorphism and taxonomic infrastructure of the *Pleurotus eryngii* species-complex as determined by RAPD analysis, izoenzyme profiles and ecomorphological characters, *Microbiology* 147, pp. 3183-3194.

[7] Lewinsohn, D., Wasser, S. P., Reshetnikov, S. V., Hadar, Y., Nevo, E. (2002), The *Pleurotus eryngii* species-complex in Israel: distribution and morphological description of a new taxon, Mycotaxon, 81, pp. 51-67.

[8] Rodriguez Estrada, A. E. (2008), Molecular phylogeny and increases of yield the antioxidants selenium and ergothioneine in *Basidiomata Pleurotus eryngii*, PhD Dissertation, Pennsylvania State University, Department of Plant Pathology.

[9] Kalmár, Z. (1960), Termesztési kísérletek ördögszekér laskagombával, Kísérletügyi Közlemények, Kertészet 52/c, 4, pp. 119-125.

[10] Szili, I., Véssey, E. (1980), A csiperke és más gombák háztáji termesztése, Mezőgazdasági Kiadó, Budapest, p. 113.

[11] Szili, I. (1994), Gombatermesztés, Mezőgazda Kiadó, Budapest, pp. 143-144.

[12] Kirbag, S., Akyüz, M. (2008), Effect of various agro-residues on growing periods, yield and biological efficiency of *Pleurotus eryngii, Journal of Food, Agriculture and Environ.*, 6, pp. 402-405.

[13] Véssey, E. (1971), Adatok az ördögszekér laskagomba termesztéséhez, *Mikológiai Közlemények*, 3, pp. 121-131.

[14] Cailleux, R., Diop, A. (1974): Recherches expérimentales sur les conditions d'ambiance requises pour la fructification du *Pleurotus eryngii* et de l'Agrocybe aegerita. *Mushroom Sci.* IX (Part I.), pp. 607-619.

[15] Sawa, S. (1996), Cultivation characteristic of *Pleurotus eryngii* (in Japanese). *Bull. Aichi. For. Res. Cent.*, 33, pp. 41-46.

[16] Ohga, S. (1999), Suitability of bamboo powder for the sawdust-based cultivation of edible mushroom. *Mushroom Sci. Biotechnol.*, 7, pp. 19-22.

[17] Ohga, S. (2000), Influence of wood species on the sawdust base cultivation of Pleurotus abalonusa and Pleurotus eryngii. *J. Wood Sci.*, 46, pp. 175-179.

[18] Szarvas, J., Szarvas, G. (2002), Az ördögszekér laskagomba (*Pleurotus eryngii*) termesztése. *Magyar Gombahíradó*, 10 (36), pp. 6-7.

[19] Ohga, S., Royse, D. J. (2004), Cultivation of *Pleurotus eryngii* on umbrella plant (*Cyperus alternifolius*) substrate, *J. Wood Sci.*, 50, pp. 466-469.

[20] Győrfi, J., Hajdú, Cs. (2007), Casing-material experiments with *Pleurotus eryngii*, *International Journal of Horticultural Science*, 2, pp. 33-36.

[21] Kószó, S. (1997), Az ördögszekér laskagomba (*Pleurotus eryngii*) termesztése, *Magyar Gomba*, 2, pp. 12-13.

[22] Sonnenberg, A.S.M., Patrick, M.H. & Etty S. (2006), Evaluation of Pleurotus eryngii strains, Research report, Wageningen University, Applied Plant Research Institute, Mushroom Research Unit.

[23] Stamets, P. (2000), Growing gourmet and medicinal mushrooms, Ten Speed Press, Berkeley, pp. 55-57.

[24] Rodriguez Estrada, A.E., Mar Jimenez-Gasco, M., Royse, D.J. (2009), Improvement of yield of *Pleurotus eryngii var. eryngii* by substrate supplementation and use of a casing overlay, Bioresource Technol., 100, pp. 5270-5276.

[25] Andrade, M.C.M., Kopytowski, F.J., Minhoni, M.T.A., Coutinho, L.N., Figueiredo, M.B. (2007), Productivity, biological efficiency, and number of *Agaricus blazei* mushrooms grown in compost in the presence of *Trichoderma* sp. and *Chaetomium olivacearum* contaminants, Braz. J. Microbiol., 38, pp. 243-247.

[26] Shure, M., Wessler, S., Fedoroff, N. (1983), Molecular identification and isolation of the Waxy locus of maize, Cell, 35, pp. 225-233.

[27] Nei, M., W.-H. Li. (1979), Mathematical model for studying genetic variation in terms of restriction endonucleases, Proc. Natl. Acad. Sci. 76, pp. 5269-5273.

[28] Felsenstein, J. (1995), PHYLIP (Phylogeny Inference Package) Version 3.57c. Department of Genetics, University of Washington, Seattle.