Evaluation of *Pleurotus eryngii* strains

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Table of contents

page

1 Introduction

One of the goals of the Hortin Mushroom project is to provide good source materials for the production of a variety of mushroom species in Indonesia. The availability of superior strains, an optimal way of maintenance of strains and the production of high quality spawn is crucial for a good production system. IVEGRI has started to build a strain collection that contains most of the cultivated species in Indonesia, a number of wild collected species (mainly from Java) and a number of strains that originate from the culture collection of PPO. All strains are also deposited in the culture collection of PPO (in liquid nitrogen) and can serve as a back-up in case strains are lost at IVEGRI.
In previous experiments done at the growing facilities of PPO a number of strains of shiitake (*Lentinula edodes*) en common oyster mushrooms (*Pleurotus ostreatus*) have been evaluated. Both experiments have identified a number of strains that are high productive and suitable for the Indonesian culture conditions, i.e. good production and quality at elevated production temperatures (22 °C in stead of the 16 °C that is used in European culture conditions). These strains were handed over to IVEGRI in the last 2 years.
The Indonesian project leader (Dr Etty Sumiati) has indicated that Indonesian growers have also interest in cultivating varieties that are not common in Indonesia. One of these species is the king oyster mushroom (*Pleurotus eryngii*). The genus *Pleurotus eryngii* consists of a number of varieties: *P. eryngii,* var *eryngii, P. eryngii* var *nebrodensis*, *P. eryngii* var. *ferulae*. PPO has a number of strains of this genus and the majority has not been evaluated before. Since there is some confusion about the species concept within the *eryngii* varieties, we have genotypes all selected strains by comparing ITS sequences (species identification) and by ISSR (strain identification). Subsequently, a selection was made representing the different varieties and as much strain variation as possible.

2 Genotyping *P. eryngii* strains of the PPO collection

All strains in the PPO collection of the species *Pleurotus eryngii* and its varieties were cultivated on MMP agar plates and used to isolate DNA. Five *P. osteatus* strains were included and 2 additional *Pleurotus* strains that were not identified.
The genotyping using the ITS regions were done as described previously (Evaluation of shiitake (*Lentinula edodes*) strains of the culture collection of Applied Plant Research, report 2005-1). Three different groups could be identified (Table 1, column 1). This grouping does not coincide with the putative variety indication at the moment these strains were obtained for the collection. This emphasizes the present obscurity within the *P. eryngii* complex. The variation within variety grouping is considerably smaller than between the groups which support the grouping by ITS (Table 1, column 2). Sixteen strains were chosen for cultivation trials. These strains represent all genotypes found in the PPO collection (Table 1, indicated in bold face in column 3). One strain was added to this selection, i.e. Le Lion PE. This strain is now used by a number of Dutch growers.

3 Cultivation Trials

The substrate used has been described previously (Evaluation of shiitake (*Lentinula edodes*) strains of the culture collection of Applied Plant Research, report 2005-1) except for the nitrogen content. Previous experiments with the *P.* eryngii strains indicated that the nitrogen content of the substrate was suboptimal. We have, therefore, included 3 different N-concentration in the substrate (1.4, 1.8 and 2.0%; Table 2) in order to find an optimum. Substrate was incubated for 70 days at 24 °C in a dark room and subsequently transferred to a production room. Of each tray the top layer of substrate was removed and water was placed on top of the substrate and incubated overnight. The water was drained the next day. Previous experiments have shown that this treatment improves the yield.

ITS-RFLP	ISSR	Collection name	Strain designation	Genus	Species	Possible variety
Pleurotus ostreatus	1	MES 01475	pn 001	Pleurotus	ostreatus	
	1	MES 11825	B2	Pleurotus	ostreatus	
	1	MES 11826	B3	Pleurotus	ostreatus	
	1	MES 11827	B4	Pleurotus	ostreatus	
	11	MES 11824	B1	Pleurotus	ostreatus	
Group 1	2	MES 11884	Eryngii 1	Pleurotus	eryngii	ferulae
	2	MES 11885	Eryngii 2	Pleurotus	eryngii	ferulae
	2	MES 11886	Eryngii 3	Pleurotus	eryngii	ferulae
	2	MES 11889	Eryngii 6	Pleurotus	eryngii	ferulae
	6a	MES 02001	pl 03/01	Pleurotus	eryngii	
	6a	MES 03467	Somycel 3065	Pleurotus	eryngii	
	6b	MES 00693	K203	Pleurotus	eryngii	
	6c	MES 03412	Somycel 3060	Pleurotus	eryngii	
Group 2	5a	MES 01996	pl 02/09	Pleurotus	eryngii	
	5a	MES 03462	wilde nebrodensis	Pleurotus	eryngii	nebrodensis
	5a	MES 10620	MES 10620	Pleurotus	eryngii	
	5b	MES 02073	Mycelia 2600	Pleurotus	eryngii	
	5b	MES 11565	Mycelia 2600	Pleurotus	eryngii	
	5b	MES 11806	Mycelia 2600	Pleurotus	eryngii	
	5c	MES 03757	pl 02/08	Pleurotus	eryngii	
Group 3	3	MES 11883	Eryngii 0	Pleurotus	eryngii	ferulae
	3	MES 11887	Eryngii 4	Pleurotus	eryngii	ferulae
	7	MES 03387	Somycel 3058	Pleurotus	eryngii	
	7	MES 03411	Somycel 3058	Pleurotus	eryngii	
	8	MES 03314	Les Miz 1735	Pleurotus	eryngii	
	9	MES 03319	84/02	Pleurotus	flabellatus	
	10	MES 03329	Royal P510	Pleurotus	eryngii	
	12	MES 03313	Les Miz 1336	Pleurotus	eryngii	
	4a	MES 11772	Nebrodensis-2	Pleurotus	nebrodensis	
	4a	MES 11774	Nebrodensis-4	Pleurotus	nebrodensis	
	4b	MES 11773	Nebrodensis-3	Pleurotus	nebrodensis	
	4c	MES 11771	Nebrodensis-1	Pleurotus	nebrodensis	
Not Pleurotus	13	MES 03445	99/04	Pleurotus		
	14	MES 03756	Ph 3-2	Pleurotus		sajor-caju

Tabel 1. The banding patterns generated with RFLP- ITS were used to type strains on species levels. Based on these data strains were divided in groups (column 1). To identify the genetic variety with strains of identical species, ISSR was used. Column 2 indicate strain variety. Identical numbers with different letter additions are strains that are different but closely related.

N	Saw dust	corn meal	linseed	Water	Total kg
0.8% N	44.11	8.30	4.34	43.25	100.00
1.4% N	26.69	15.89	8.21	49.21	100.00
2.0% N	10.21	22.97	11.86	54.88	99.92

Table 2. Substrate composition at different N-percentages.

	Nitrogen content substrate			Species	Strain
Strain	0.8 % N	1.4 % N	2.0 % N	ITS	ISSR
84/02	0.0%	10.9%	8.2%	3	9
Eryngii 0	10.4%	14.3%	8.6%	3	3
Eryngii 1	8.6%	21.3%	10.2%	1	2
K203	9.5%	22.8%	12.4%	1	6b
Le Lion PE	11.3%	24.6%	8.6%	n.d.	n.d.
Les Miz 1336	7.8%	0.0%	0.0%	3	12
Les Miz 1735	0.0%	0.0%	0.0%	3	8
Mycelia 2600	20.5%	25.0%	12.5%	2	5b
Nebrodensis-1	0.0%	0.0%	0.0%	3	4c
Nebrodensis-2	0.0%	0.0%	3.6%	3	4a
Nebrodensis-3	0.0%	0.0%	0.0%	3	4b
pl 02/08	17.5%	35.4%	12.8%	2	5c
pl 03/01	13.5%	22.7%	11.5%	1	6a
Royal P510	10.9%	0.0%	0.0%	3	10
Somycel 3058	10.6%	15.8%	0.0%	3	7
Somycel 3060	18.0%	25.6%	12.2%	1	6c
wild nebrodensis	20.4%	26.5%	15.0%	2	5a

Production of *Pleurotus eryngii* types at **16 °C**.

	Nitrogen content substrate			Species	Strain
	0.8 % N	1.4 % N	2.0 % N	ITS	ISSR
84/02	4.4%	19.0%	24.6%	3	9
Eryngii 0	11.5%	24.2%	17.8%	3	3
Eryngii 1	7.8%	9.4%	4.9%	1	2
K203	9.2%	20.2%	0.0%	1	6b
Le Lion PE	7.9%	24.6%	0.0%	n.d.	n.d.
Les Miz 1336	0.0%	0.0%	0.0%	3	12
Les Miz 1735	0.0%	0.0%	0.0%	3	8
Mycelia 2600	10.3%	0.0%	0.0%	2	5b
Nebrodensis-1	0.0%	0.0%	0.0%	3	4c
Nebrodensis-2	0.0%	0.0%	0.0%	3	4a
Nebrodensis-3	0.0%	0.0%	0.0%	3	4b
pl 02/08	20.7%	35.9%	4.1%	2	5c
pl 03/01	12.2%	22.7%	9.9%	1	6a
Royal P510	0.0%	0.0%	0.0%	3	10
Somycel 3058	11.3%	15.1%	0.0%	3	7
Somycel 3060	11.3%	23.1%	10.0%	1	6c
wild nebrodensis	20.6%	32.6%	5.6%	2	5a

Production of *Pleurotus eryngii* types at **22 °C**.

Tabel 3. Yield of different *P. eryngii* strains at 2 temperatures and 3 different concentration of nitrogen in the substrate. Yield is expressed as % BE (biological efficiency, i.e. ratio fresh weight fruitbody and fresh weight substrate).

As in previous cultivation trials of the common oyster mushroom and shiitake, the cultivation trials were carried out at two different temperatures, i.e. 16 °C and 22 °C. The lower temperature is used in European countries. A test at higher temperature will reveal what strains are suited for Indonesian conditions were cooling is mostly impossible.

3.1 Yield

Five of 17 strains at 16 °C and six of 17 strains at 22 °C did not show any (or hardly any) production (Table 3; see appendix for a graphical presentation). These strains are all members of the Group 3 variety. Growers of the variety *nebrodensis* claim that a cold shock is needed to induce fruit body formation. This might be the reason that these strains did not or hardly fruit here. One of these strains (Nebrodensis 2) showed some initial fruiting when transferred from the incubation room to the production room. As stated before, the upper layer of the substrate is removed, water place on top of the substrate and drained after one night. It might be that this strain is not able to reform initials for fruiting.
The producing strains acted differently on the 2 temperatures during fruit body production. This difference was influenced by the nitrogen concentration of the substrate. The strains Eryngii 1, Mycelia 2600, Royal P510 and Somycel 3060 had considerably better (or slightly better) production at a lower temperature. For strains K203, Le Lion PE, Somycel 3058, pl 02/08, pl 03/01 and the wild nebrodensis, production were comparable at the 2 cultivation temperatures. The strains 84/02 and Eryngii 0 had a higher production at 22 °C.
In general, productions were optimal at a nitrogen concentration of 1.4% N.
Some strains showed productions as high as 35% biological efficiency (BE: ratio of fresh weight fruit bodies

and fresh weight substrate). The general production levels in the Netherlands and Germany are usually between 10 and 15% BE. For these productions, strains Le Lion PE and Mycelia 2600 are used. These strains produced in our trials between 20% BE (on 0.8% N) and 25% (on 1.4% N). The difference in production level is very likely due to a difference cultivation system used here compared to what is used by commercial growers. It is likely that 2 items have an influence on production levels, i.e.
the size of the production unit (substrate volume) and the N-content of the substrate. In our experiments we use 1 kg containers whereas the commercial growers use 5 kg bags. Smaller production units usually lead to a better production. Our experiments have also shown that the N-content of the substrate has a prominent effect on production levels. In our experiments we obtained high productions on 1.5% nitrogen. Commercial growers in Holland use substrate with ca 0.8% N.
'Summing the differences of Dutch growing conditions and what we have tested here are:

Dutch	PPO
5 kg substrate/production unit	1 kg substrate/production unit
Pasteurized substrate (95 °C)	sterilized substrate (121 °C)
0.8% N in substrate	1.4% N in substrate
Low bulk density in substrate	high bulk density in substrate

Reasons why Dutch growers produce under suboptimal conditions are that small production unit (substrate bags) and sterilization of substrate are more expensive than large production units and pasteurization of substrate. High N contents of the substrate also form risks because of overheating of the substrate during colonization (low surface/volume ratio). In addition, infections on pasteurization of substrate occur more often than on sterilized substrate. If the nitrogen content is high, infections will cause more damage. Small production units are common use in Indonesia where labor is cheap. There are also enough high nitrogen waste materials that can be used for the preparation of substrate. Large autoclaves, imported from China, are also not too expensive for farms with a certain production volume.

4 Quality

Some quality characters of strains were measured in the 16 °C production room: time needed to colonize substrate, time between transfer to production room and the first day of picking and the general quality (shapes and remarks).
In general, the colonization time of the substrate took 13 to 21 days for most of the strains in substrates with 0.8 or 1.4% nitrogen. Most strains showed identical figures for these two N concentrations. An exception was the commercial strain Mycelia 2600 that used more time for colonizing 0.8% N substrate (21 days) than 1.4% N substrate (13 days). This is useful information for Dutch growers that might consider trying different N-concentrations in their substrates. The highest N-concentrations lead in most cases to a very dense colonization and a colonization of only part of the substrate. Some strains failed to colonize the substrate to a substantially part at this high concentration. The days needed between transfer to the production room and the first day of picking varied between 11 and 21 days. The commercially used strains needed 18-21 days. Some of the new strains only needed 11 days and are clearly faster.

Figure 2. Examples of the two types of fruit bodies produced by the selected P. eryngii strains. Left pl 03/01 and right pl 02/08

Most strains showed an acceptable to good quality (regular shaped fruit bodies; see appendix for an impression). Only strain 84/02 showed many

deformations and is not suited for commercial production.
Based on the general appearance, strains can be distributed between 2 types:

- Light brown to brown caps with smooth edge
- Grey-brown caps with spots and indented edges

Strain	N% substrate	Mushroom shape	Remarks	Substrate colonization (days)	Picking (days after transfer to growing room)
Eryngii 1	0.80%	Regular		13	14
Eryngii 1	1.40%	Regular		20	16
Eryngii 1	2.00%	Regular	Caps a bit funnel shape	>55	19
pl 03/01	0.80%	Regular and good shape		13	14
pl 03/01	1.40%	Regular and good shape		20	14
pl 03/01	2.00%	Regular and good shape		Not fully colonized	15
K203	0.80%	Regular		13	15
K203	1.40%	Regular		20	14
K203	2.00%	Regular		>100	15
Somycel 3060	0.80%	Regular		16	15
Somycel 3060	1.40%	Regular	Caps a bit funnel shape	20	15
Somycel 3060	2.00%	Regular		Not fully colonized	15
wilde nebrodensis	0.80%	Regular	Spotted caps; indentated cap edges	20	14
wilde nebrodensis	1.40%	Regular	Spotted caps; indentated cap edges	20	14
wilde nebrodensis	2.00%	Regular	Spotted caps; indentated cap edges	>55	18
Mycelia 2600	0.80%	Regular		20	20
Mycelia 2600	1.40%	Regular		13	18
Mycelia 2600	2.00%	Regular	Some deformations	47-55	21
pl 02/08	0.80%	Regular	Spotted caps; indentated cap edges	21	11
pl 02/08	1.40%	Regular	Spotted caps; indentated cap edges	21	11
pl 02/08	2.00%	Regular	Spotted caps; indentated cap edges	56	19
Eryngii 0	0.80%	Regular		21	11
Eryngii 0	1.40%	Regular		21	11
Eryngii 0	2.00%	Regular	Pale fruit bodies	Not fully colonized	11
Somycel 3058	0.80%		Funnel shape caps	21	14
Somycel 3058	1.40%	Many mushrooms	Funnel shape caps	21	16
Somycel 3058	2.00%		Funnel shape caps	Not fully colonized	
Les Miz 1735	0.80%			21	
Les Miz 1735	1.40%			21	
Les Miz 1735	2.00%			Not fully colonized	
84/02	0.80%	Irregular	Deformations	21	
84/02	1.40%	Irregular	Deformations	21	14
84/02	2.00%	Irregular	Deformations/Bacterial spots	poorly colonized	11
Royal P510	0.80%	Regular		21	18
Royal P510	1.40%	Regular		21	
Royal P510	2.00%	Regular		Not fully colonized	
Les Miz 1336	0.80%	Medium quality	Funnel shape caps	21	14
Les Miz 1336	1.40%			34	
Les Miz 1336	2.00%			poorly colonized	
Nebrodensis-2	0.80%			21	
Nebrodensis-2	1.40%			27	
Nebrodensis-2	2.00%			poorly colonized	19
Nebrodensis-3	0.80%			21	
Nebrodensis-3	1.40%			21	
Nebrodensis-3	2.00%			poorly colonized	
Nebrodensis-1	0.80%			21	
Nebrodensis-1	1.40%			21	
Nebrodensis-1	2.00%			poorly colonized	
Le Lion PE	0.80%	Regular		21	21
Le Lion PE	1.40%	Regular		21	19
Le Lion PE	2.00%	Regular		poorly colonized	21

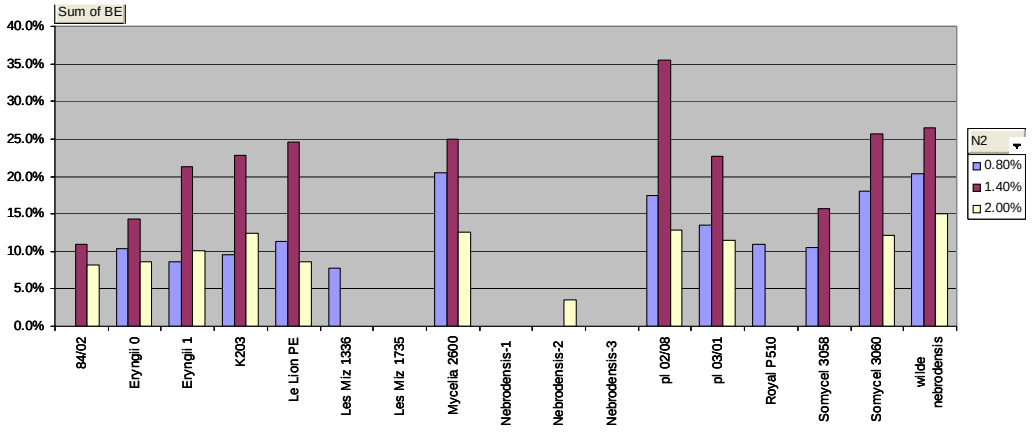
Tabel 4. Quality aspects of strains were measured in the16 °C production room: Time needed to colonize the substrate; time between transfere to the production room and picking, and general quality (shapes and general remarks).

5 Summary

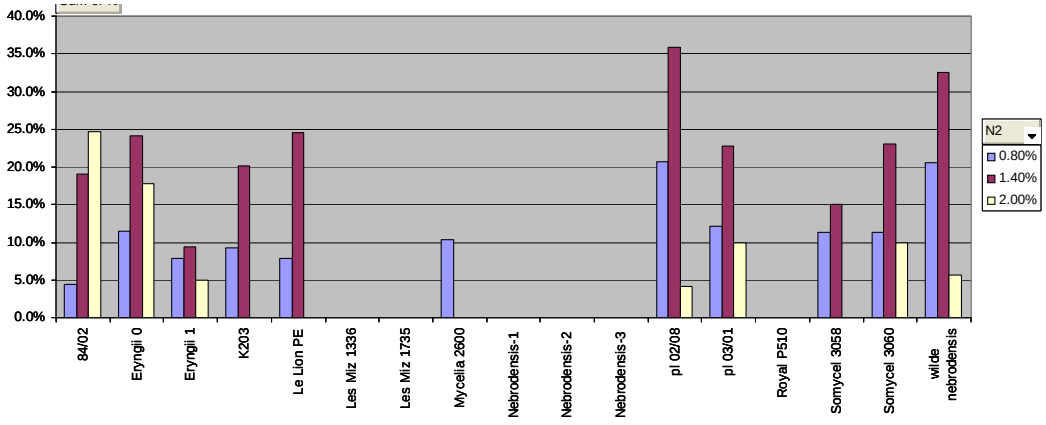
We have evaluated at PPO 17 genetically different *Pleurotus eryngii* strains. This selection represents 5 present-day strains, 4 old commercial lines and 8 strains of wild or unknown origin.
This evaluation revealed some strains that show a higher production than the present day or old commercial

lines. A number of these strains have a higher production at both low and high temperatures than present-day commercial lines and are thus suited for Indonesian production systems (good production at elevated temperatures).
The evaluation showed also that cultivation on small production units, a substrate nitrogen percentage of 1.4% and sterilization of substrate improves the production level considerately.
This project has thus identified a number of *Pleurotus eryngii* strains that are suited for Indonesian production conditions. Those strains will be handed over to IVEGRI.

Appendix 1



Production temperature: 16 °C



Production temperature: 22 °C

Figure 1. Biological efficiency of *Pleurotus eryngii* strains tested at PPO. Biological effciency is expressed as the ratio of fruit body weight and substrate weight (both fresh).


Pl 02/08; 1.4% N; 16 ºC


Eryngii 0; 1.4% N; 16 ºC


Pl 03/01; 1.4% N; 16 ºC

Eryngii 0; 1.4% N; 22 ºC

Pl 02/08; 1.4% N; 22 ºC

Pl 03/01; 1.4% N; 22 ºC

Wilde nebrodensis; 1.4% N; 22 ºC

K203; 1.4% N; 16 ºC

Somycel 3060; 1.4% N; 16 ºC

N% compared

Somycel 3060; 22 oC; 0.8, 1., and 2.0% N

N% compared

K203; 22 oC; 0.8, 1.4 and 2.0 % N

N% compared

Wild nebrodensis; 22 oC; 0.8, 1.4 and 2.0 % N

N% compared

Wild nebrodensis; 16 °C; 0.8, 1.4 and 2.0 % N

N% compared

Pl 02/08; 16 °C; 0.8, 1.4 and .0 % N

N% compared

Pl 03/01; 16 °C; 0.8, 1.4 and 2.0 % N