Genotyping and Evaluation of *Pleurotus ostreatus* (oyster mushroom) strains

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1 Summary

Oyster mushrooms (*Pleurotus ostreatus*) represent one of the most popular mushroom species grown in Indonesia. There is a need for strains that are better adapted to the climate conditions at Java, where most mushrooms in Indonesia are produced. Strains that can produce mushrooms at 22 to 28 °C and have a good yield and quality would improve the profit of mushroom growers substantially. In order to find strains that can be used for Indonesian growing conditions a large number of available strains were genetically examined. Two sources were used in this study:

- A collection of strains commercially used in Indonesia. These strains are collected by Dr Etty Sumiati from farmers and institutes at Java.
- The collection of fungal strains present at PPO, Horst, The Netherlands. This collection contains a substantial number of strains representing the genus *Pleurotus.* Most of these strains have not been genotyped or evaluated for the production of fruiting bodies.

Unfortunately, no strains were obtained from natural environment in Indonesia. Most strains sent by Etty Sumiati appeared to be infected at arrival at PPO. We have identified the species type of the strains representing the genus *Pleurotus* by ITS-RFLP in order to identify unambiguously the species *P. ostreatus* (common oyster mushroom). Next, all *P. ostreatus* strains were genotyped by ISSR to examine the genetic variation between strains. It appeared that the PPO collection contains 167 *P. ostreatus* strains representing 68 different genotypes. The 24 strains collected from Indonesia represent 7 different genotypes but more than 50% is represented by one variety. This variety, i.e. type *florida*, originate from wild (Florida, USA) and can be grown at higher temperatures. It lacks, however, the yield and quality present in most strains used in Europe and the USA.
Based on the genetic data and data on the origin of strains a selection of 40 strains was made that has been tested in 2 parallel cells (16 and 22 °C production temperature). ………..

2 Introduction

The oyster mushroom (*Pleurotus*) is one of the most cultivated mushroom types worldwide. The genus consists of a number of species. However, more than 90% of the *Pleurotus* crops grown world wide concerns the common oyster mushroom, *P. ostreatus*. Its ease of cultivation on different substrates makes it a popular species for small farmers. No expensive climate controlled growing houses are needed because of the availability of varieties that can complete their life cycle under different climatic conditions. The common oyster mushroom is also one of the most cultivated mushroom species in Indonesia, especially on Java. It is grown inland on elevated regions (1000 or more meters above sea level) where temperatures do not exceed 22-28 ^{0}C.
In Europe and the USA, oyster mushroom varieties are grown at low temperatures (fruiting bodies are produced at temperatures of 15 to 18 °C). Mushroom farmers obtain high quality strains from companies specialized in the production of spawn (pure fungal inoculum for substrate). A large number of strains are available but previous research has shown that they represent only a limited number of genotypes. There are no spawn companies in Indonesia and import of spawn is too expensive for most growers. Strains are usually obtained by taking along strains from contacts in Western countries (or recently China and Taiwan) and they are stored on slant tubes in a refrigerator or even at room temperature. This is not an optimal way of maintaining strains often resulting in decrease of quality after some time. Next to strain qualities, also a lack of varieties

specially adapted to the Indonesian growing/climate conditions is a problem. There is only one commercial variety available ("*florida*" variety) that is adapted to cultivation at higher temperatures.
The goal of this research is to see if strains can be found that are more suitable for Indonesian cultivation conditions, i.e. can produce fruiting bodies above 22 °C and have a good yield and quality. Two types of sources for strains are used in this study: the collection of PPO and the collection of commercial strains used in Indonesia (collected by IVEGRI at Lembang). Since space and money is limited in this project, we first genotypes the available *Pleurotus* species and identified all *P. ostreatus* strain. Subsequently, the genetically variation within the species *P. ostreatus* was determined. A selection of 40 genetically different strains were tested on fruiting in two parallel cell test at 16 and 22 °C respectively.

3 Genotyping Collections

The collection of PPO contains ca. 5000 strains representing more than 100 species. Approximately 270 strains are *Pleurotus* species (including 24 strains sent by Etty Sumiati/IVEGRI). Most of these are the widely cultivated common oyster mushroom *P. ostreatus*. The collection *P.ostreatus* of PPO consists of old and present-day commercial lines, gifts from other collections and private persons and strains collected from natural environment in different parts in the world. Especially in the last years, a number of strains have been obtained from China and Korea. Most strains of this collection have not been genotyped and not evaluated in a crop. Dr Etty Sumiati has collected 31 fungal strains from different sources in Indonesia of species that are grown commercially. Most of these are *P. ostreatus* species. She also has collected a number of strains from the wild, mainly in Java. Unfortunately, most of these strains appeared to be infected after arrival at PPO (those that were purified were not *Pleurotus* species).
Since this subproject focus now on *P. ostreatus* we have first checked on the correct species name. All *Pleurotus* spp. were genotyped using ITS-RFLP as described earlier (Report: "Evaluation of shiitake strains of the PPO collection"; Hortin Mushroom 2005-1). Four different restriction enzymes were used to digest the amplified ribosomal DNA region. In this way all species could be discriminated into the four groups in accordance with intersterility groups (Appendix: Table 1; Figure 1). The latter name is derived from the compatibility test between strains. Strains from identical species are compatible (fertile) whereas strains from different species can not be crossed (intersterile).

In table 2 (Appendix) a list is presented of commercial strains collected by Dr Etty Sumiati from different farms and institutes in Java. All these strains were identified as *P. ostreatus* by ITS-RFLP.

In order to evaluate what strains have potentials for the Indonesian cultivation conditions we tested strains at 2 different fruiting temperatures, 16 and 22 °C. In this way we could also see what strains have potentials for the European and USA market or what strains could be a base for a breeding program for both climatic conditions. Since we have a limited space in our experimental farm and the budget is also limited we had to make a selection of the 168 identified as *P. ostreatus* species. For this, all strains were first analyzed on genetic relatedness using the ISSR technique as described earlier (Report: "Evaluation of shiitake strains of the PPO collection"; Hortin Mushroom 2005-1). In this way we have made a selection representing a broad genetic variation. 68 Different genotypes were identified in 168 strains indicating that the collection *P. ostreatus* strains represent indeed a broad variation (figure 2; table 3). Most European (and USA)

commercial lines are represented by 3 genotypes: varieties with large fleshly fruiting bodies (HK 35 type), varieties with smaller fruiting bodies (P24 types) and varieties representing hybrids between the HK35 types and the *florida* type. The *florida* type has been isolated 50 years ago in Florida (USA) and can be grown at high temperatures. The hybrids can be grown at a broader range of temperatures. The fourth large group (genotype group 4) represents the original *florida* isolate. These 4 groups represent almost 50% of the number of *P.ostreatus* strains present in the collection. Most of the other strains represent wild isolates or are derived from collections of institutes where no detailed information is available on the origin of the respective strain. These lines show a much higher genetic variation than the commercial lines.
Etty Sumiati has collected 23 strains from farms and institutes on Java. The ISSR genotyping showed that more than 50% (13 out of 24) of these strains are identical to the original *florida* type. This indicates that this variety, capable of producing at higher temperatures, is the most popular/suitable strain among farmers in Indonesia. The *florida* variety, however, is not a strain that has the same yield and quality as the commercial strains used in Europe and the USA. The intension of the cell tests was thus to find a strain that has an improved quality and yield when producing mushrooms at 22 °C.

4 Fruiting tests

Based on the genotypes and the information we had on the origin of strains a selection was made of 40 strains (Table 4). They represent available strains from Java (4 strains; one of the *florida* type), wild strains originating from different countries, and commercial lines used in European and USA crops. Spawn has been prepared form each strain using sorghum as carrier and pasteurized straw was used as substrate. This is a different substrate than used in Indonesia (saw dust with nitrogen rich additives). It is possible that the test results might not be completely comparable to Indonesian crop systems but it is likely that the performance of the strains (yield and preferred temperature) are comparable in the two systems, i.e. those producing best on straw at 22 °C will also be the best producer on saw dust with additives in Indonesian crop systems.

4.1 Yield

A number of strains did not fruit at all (Table 5). Most of these were strains collected from wild and had not been tested before. This is not an unusual observation for mushrooms collected from the wild. When comparing strains that did produce mushrooms, a considerable variation in yield is seen (Figure 3). A number of strains did produce only at 16 °C. This is also not unexpected as a number of strains are used for production in European and US crops and might have been selected for optimal growth at low temperatures. Most strains producing at both temperatures had a higher yield at 22 °C. At least 9 strains had a higher yield than the best producing Indonesian strain (boxed blue in figure 3).

4.2 Colour

The colour of the fruiting body is affected by the environmental temperature. At low temperatures,

mushrooms intend to have a darker colour than at higher temperature. The markets in Europe and the USA prefer dark mushrooms whereas the Indonesian people prefer light (even white) mushrooms. It might be that this preference is stimulated by the market as convenience because it coincides with the environmental temperatures used in both production systems. We have measured here the lightness of the mushrooms with a Minolta Chromometer.
As expected, most strains have a lighter color at 22 °C than at 16 °C (Table 6). It is interesting to see, however, that some strains do have a darker color at the highest temperature. Such strains (as HK35) might be useful in Western countries in summer periods.
The strains with a higher production as the Indonesian strains have a similar lightness as the Indonesian strains or are darker. Tests at IVEGRI will show what the color of these strains will be under Indonesian conditions.

4.3 Quality

Important quality characters of strains are the form and brim of the cap. In Europe and the USA oyster mushroom caps should look like shells and have a smooth brim. Mushrooms should also be large, have a regular look and a good shelf life. Whether all these characters are also important for the Indonesian market is not known yet. From what I have seen at farms and on the local markets on Java, size and shapes of the caps are characteristics less important than in Europe and the USA (figure 5). For super markets and export, however, these characters might be more important. Shelf life is important for all markets world wide.
We have examined in this projects characters as size of caps, shape of caps and cap brims Table 7). In addition, in some cases a general impression of the mushrooms are given. From the assessments of traits at both production temperatures it appeared that more strains tend to grown in a funnel like shape at higher temperatures. Nine strains had a more funnel like shape at 22 °C than at 16 °C, 9 strains showed no difference at both temperatures and only 3 strains tend to a more shell like shape at 22 °C. The Indonesian strains vary in form from pure shell to funnel like.
Cap sizes vary from small (8 cm diameter), medium (between 8 and 12 cm) and large (>12 cm diameter). The Indonesian strains represent all sizes at both temperatures. Hood brims of the Indonesian strains also vary from smooth to fraying. One Indonesian strain (P. osteatus-7) showed a quality usually seen with the best European strains (large caps, smooth brims, shell shape). This strain also was designated as “good looking” in the assessment of general quality and had a reasonable yield.

5 Conclusions

From the two cell tests done we can conclude that nine strains had a slightly or considerable higher yield than the 4 Indonesian control strains (Table . The colour and quality of these 9 strains vary from good to reasonable based on standards for the Western markets. It is wise to evaluate these strains under Indonesian conditions. A first test at the facilities at IVEGRI should indicate what strains can be an improvement for the Indonesian growers. The selected strains should then be grown by a limited number of growers near Lembang so that the project leader can evaluate the performance of the selected strains. IVEGRI can maintain these strains for companies so that fresh inoculates or mother spawn can be obtained on a regular base. IVEGRI can set up a business for this and include new varieties for shiitake strains (*Lentinula edodes*). PPO can serve as a back-up for mother cultures.

Species	Varieties/subspecies	Intersterility group	# strains	Indonesian origin
P. ostreatus		I	168	24
P. pulmonarius	P.sapidus, P.sajor-caju	II	59	3
P.cornucopiea		IV	5	
P. eryngii	P. nebrodensis	VI	27	
P.cystidiosys	P. abalonus, P. flabellatus, P. eous	VII	15	
total			274	26

Table 1. Species of the PPO collection and commercially available strains of Indonesia identified by ITS-RFLP

Designation by Dr Sumiati	ISSR genotype	ITS-RFLP	Origin
Grey P. ostreatus	0	P. ostreatus	Originate from Germany and used in commercial crops
P. ostreatus -3	4	P. ostreatus	Micrology LabFMNS, bogor Agnic. University
Pleurotus s.c.	4	P. ostreatus	Micrology LabFMNS, bogor Agnic. University
P. ostreatus -5	4	P. ostreatus	P4S Melati, Pasir Gaok Village, Kemang-Bogor (Maryam)
P. ostreatus -6	4	P. ostreatus	P4S Kaliurang, Kalimuncar Village, Tugu Utara- Cisarua, Bogor (Badri)
P. ostreatus -9	4	P. ostreatus	P4S Nusa Indah, Ciomas Village, Bogor (Cucu- Saraglh)
P. ostreatus -10	4	P. ostreatus	Wisma Jamur - Cimacan, Cianjur (Yufan Oscar)
P. ostreatus -11	4	P. ostreatus	PT. Cibodas Mas Biotek,Jl. Raya Cibodas Cianjur
P. ostreatus -15	4	P. ostreatus	Rahmat, Cisarua Lambang
P. ostreatus -16	4	P. ostreatus	Badri, Tugu Cisarua Bogor
P. ostreatus -21	4	P. ostreatus	Forestry Research and Development/ Enjah, Ciapus- Bogor
P. ostreatus -25	4	P. ostreatus	Bandung Institute of Technology, Bandung
P. ostreatus -27	4	P. ostreatus	Bandung Institute of Technology(from Germany)
P. ostreatus -28	4	P. ostreatus	Cita Lestari (Rahmat), Cisarua Lembang (from Cananada)
P. ostreatus -7	8	P. ostreatus	P4S Kaliurang, Kalimuncar Village, Tugu Utara- Cisarua, Bogor (Badri)
P. ostreatus -24	9	P. ostreatus	BP2AHP - Ngipiksari-Kaliuranf, Yogyakarta
P. ostreatus -26	9	P. ostreatus	Kurdrat-Sukabumi
P. ostreatus -29	9	P. ostreatus	Badri, Sukabumi
P. ostreatus -4	10	P. ostreatus	Microbiology Lab-Indonesian Center for Science, Bogor
P. ostreatus -8	11	P. ostreatus	P4S Nusa Indah, Ciomas Village, Bogor (Cucu- Enjah)
P. ostreatus -14	11	P. ostreatus	Cucu - Sahati, Bogor
P. ostreatus -13	12	P. ostreatus	Cucu - Sahati, Bogor
P. ostreatus -17	12	P. ostreatus	Alang, Cisarua Lembang
P. ostreatus -12		P. ostreatus	Beny Cibodas - Lembang

Table 2. Strains of P. ostreatus collected by Etty Sumiati from farms and institutes on Java. All strains represent commercial varieties. Most strains appeared to represent the florida variety that can be grown at higher temperatures.

ISSR	# strain in group	Indonesian	Type strain
0	28	0	European commercial strain ostreatus x florida
1	14	0	European commercial strains like HK35/Somycel 3015
2	8	0	European commercial strains like P24
3	3	0	European commercial hybride like Italspawn P70
4	20	13	P.ostreatus var. Florida
6	1	0	P.ostreatus var. Florida
8	1	1	Strain from Indonesia
9	3	3	Strains from Indonesia
10	1	1	Strain from Indonesia
11	4	2	Strains from China and Indonesia
12	3	2	Indonesian strains P.ostreatus var. florida
13	3	0	Strains from China
14	1	0	Strain from Korea
15	1	0	Strain from Korea
16	1	0	Strain from China
17	2	0	Strains from China
18	3	0	Strains from China and Korea
19	2	0	Strains from China and Korea
22	7	0	European commercial strains reduced spore production
23	5	0	European "winter"strain produces at low temperatures
24	5	0	European "winter"strain produces at low temperatures
25	1	0	Dutch Wild Isolate
26	1	0	Dutch Wild Isolate
27	1	0	European commercial strain
28	1	0	European commercial strain
29	1	0	Dutch Wild Isolate
30	1	0	Dutch Wild Isolate
32	3	0	European commercial strains like Somycel 3035
33	1	0	Strain from France
34	1	0	No Information
35	1	0	Commercial strain from Slovenia
36	1	0	Dutch Wild Isolate
37	1	0	Dutch Wild Isolate
38	1	0	No Information
39	1	0	Strain from Korea
40	1	0	Wild strain form China
41	2	0	Wild strain form China
42	2	0	European commercial strain and Russian wild strain
43	3	0	Strain from Russia
44	2	0	European commercial strains
45	1	0	Strain from Brasil
46	1	0	Strain from Hungary
47	2	0	European commercial strains (ostreatus x florida)
48	1	0	Strain from Russia
49	1	0	Strain from Russia
50	2	0	European commercial strain Amycel 3000 (P.columbinus??)
51	1	0	Strain from Germany
52	1	0	Strain from Hungary
53	1	0	European commercial strain
54	1	0	Strain from Japan
55	1	0	No Information
56	1	0	Wild strain form China
57	1	0	European commercial strain
58	1	0	European commercial strain
59	2	0	Strains from Hungary and Russia
60	1	0	Strain from Russia
61	1	0	Strain from France
62	1	0	Wild strain form China
63	1	0	Wild strain form China
64	1	0	Wild strain form China
65	1	0	Wild strain form China
66	1	0	Strain from Korea
67	1	0	Strain from Russia

Table 3. List of genotypes of P. ostreatus species present in the PPO collection including strains collected in Indonesia by Etty Sumiati from framers and institutes.

Strain	Origin	Comm/Wild
HK35	Hungary	commercial
32 (GH)	Hungary	Wild
Pleurotus ostreatus -5	Indonesia	commercial
ATCC38538(Fungi)	China	?
Pleurotus ostreatus -7	Indonesia	commercial
Pleurotus ostreatus -29 var. Canada	Indonesia	commercial
pl 02/27	China	commercial
Pleurotus ostreatus -13	Indonesia	commercial
pl 02/28	China	commercial
ASI 2327	Korea	commercial
ASI 2163	Korea	commercial
pl 02/24	Italy	commercial
ASI 2024	Korea	commercial
FV001	Netherlands	wild
MES 02004	Netherlands	wild
3029	?	commercial
IVK001	Netherlands	Wild
JF001	Netherlands	Wild
49 (BHP-c)	Hungary	?
G24	Slovenia	commercial
RS001, Pleurotus spp.	Netherlands	Wild
RS002, Pleurotus spp.	Netherlands	Wild
ASI 2253	Korea	commercial
pl 02/26	China	commercial
pl 02/25	China	commercial
ostreatus 53 (T-1)	Rusia	?
Mycelia 2120	Europe	commercial
pc 8-2-02 p2	Brazil	commercial
Amycel A 3000	Europe	commercial
ATCC 66376	USA	wild
MES 02000	?	commercial
pn 001	China	commercial
pl 02/14	China	commercial
pl 02/15	China	commercial
pl 02/17	China	commercial
pl 02/32	China	commercial
ASI 2066	Korea	commercial
K12	Europe	commercial
PLX195	Netherlands	Breeding line
Ital spawn P24	Europe	commercial

Table 4. List of strains used in cell tests at 2 production temperatures (16 and 22 ^{o}C).

Strain	Production Temp. 16 oC	22 oC	Strain	Production Temp. 16 oC	22 oC
3029	780	1037	ostreatus 53 (T-1)	356	443
32 (GH)	865	1725	P24	943	1001
49 (BHP-c)	1143	861	pc 8-2-02 p2	543	716
Amycel A 3000	830	961	pl 02/14	358	0
ASI 2024	866	0	pl 02/15	600	0
ASI 2066	556	550	pl 02/17	804	0
ASI 2163	560	206	pl 02/24	0	0
ASI 2253	690	0	pl 02/25	754	0
ASI 2327	561	0	pl 02/26	644	782
ATCC 66376	507	1168	pl 02/27	819	1005
ATCC38538	303	0	pl 02/28	806	293
FV001	0	0	pl 02/32	382	0
G24	291	484	Pl ostr 5	469	179
HK-35	1040	873	PL ostr 7	821	839
IVK001	0	0	Pleurotus ostreatus -13	549	436
JF001	488	0	Pleurotus ostreatus -29 var. Canada	648	655
K12	1172	1722	PLX195	981	700
MES 02000	658	0	pn 001	0	0
MES 02004	0	0	RS001	533	523
Mycelia 2120	615	640	RS002	0	0

Table 5. Yield (grams per package straw; ca. 18 kg) of different *P. ostreatus* strains grown at two different temperatures (16 and 22 ^{o}C).

Strain	16 ^{o}C	22 ^{o}C
HK35	73.30	67.28
32 (GH)	70.19	76.19
Pleurotus ostreatus -5	76.78	82.92
Pleurotus ostreatus -7	79.16	83.20
Pleurotus ostreatus -29 var. Canada	80.55	80.54
pl 02/27	73.34	72.16
Pleurotus ostreatus -13	79.47	77.99
pl 02/28	50.12	50.06
3029	59.27	60.45
49 (BHP-c)	76.65	80.04
G24	83.93	76.79
RS001, Pleurotus spp.	66.92	79.62
pl 02/26	66.31	72.98
ostreatus 53 (T-1)	62.27	71.12
Mycelia 2120	70.30	77.04
pc 8-2-02 p2	67.24	71.77
Amycel A 3000	71.02	79.04
ATCC 66376	75.59	73.39
ASI 2066	82.09	84.93
K12	67.96	70.49
PLX195	64.58	72.03
Ital spawn P24	68.63	76.24

Table 6. Colour of mushrooms caps (L: Lightness of fruit bodies measured with a Minolta chromometer). Most strains show a lighter colour at 22 than at 16 ^{o}C.

Production at 16 °C				
Strain	**Size cap**	**Shape of cap**	**brim of cap**	**Visual assessment**
HK35	Large	shell	smooth	good shape
32 (GH)	Small	shell-like	smooth	
Pleurotus ostreatus -5	Middle sized	funnel-like	fray-like	weak flesh
ATCC38538(Fungi)	middle-small	shell-funnel	fray-like	
Pleurotus ostreatus -7	middle	shell-funnel	fray-like	easily injured
Pleurotus ostreatus -29 var. Canada	middel	funnel-like	medium fraying	
pl 02/27	Large-middel	shell-like	smooth	
Pleurotus ostreatus -13	small	funnel-like	fray-like	good looking but easily injured
pl 02/28	middle	shell	fray-like	no stipe, dark
ASI 2327	middle	shell-like	smooth	good shape
ASI 2163	small	shell	smooth	spoon shape
ASI 2024	middle	shell	fray-like	thick stipe
3029	large	shell	fray-like	dark color
JF001	middle	shell-funnel	smooth	thick stipe and good shape
49 (BHP-c)	middle	shell-like	smooth	good shape
G24	small	shell	smooth	
RS001, Pleurotus spp.	small	shell-like	smooth	long stipes
ASI 2253	middle	shell	smooth	good shape
pl 02/26	small-medium	funnel-like	fray-like	
pl 02/25	middle	shell-like	smooth	good shape
ostreatus 53 (T-1)	middle	funnel-like	fray-like	
Mycelia 2120	small	funnel-like	smooth-fraying	
pc 8-2-02 p2	small	shell	smooth	good shape, fan shaped
Amycel A 3000	large	shell	smooth	good shape
ATCC 66376	mdium	shell	smooth-farying	
MES 02000	middle	funnel-like	smooth	
pl 02/14	small	shell-like	fraying	
pl 02/15	small	shell	fray-like	
pl 02/17	small	shell	smooth-farying	undulant brim
pl 02/32	small	funnel-like	smooth-like	undulant brim but good shape
ASI 2066	middle	funnel-like	fray-like	thick stipe , bublbing but otherwise good shape
K12	large	shell	smooth	good shape
PLX195	large	shell	smooth	dark color
Ital spawn P24	small	shell	smooth-like	

Production at 16 °C				
Ras	**grootte hoed**	**vorm hoed**	**hoedrand**	**Opmerkingen**
HK35	large	shell-like	smooth	
32 (GH)	small-medium	funnel-like	smooth-fraying	
Pleurotus ostreatus -5	small	shell	fraying	
Pleurotus ostreatus -7	large	shell	smooth	good shape
Pleurotus ostreatus -29 var. Canada	middle	funnel	smooth-fraying	
pl 02/27	small-medium	shell-like	frays	
Pleurotus ostreatus -13	small	funnel-like	fraying	
pl 02/28	large	shell	fraying	dark color
3029	large	shell-like	frays	Flowering, thick stipe, good shape
49 (BHP-c)	middle	shell-like	smooth	spoon like, firm
G24	small	shell	frays	weak, thin
RS001, Pleurotus spp.	small	funnel	smooth	funnel-like
pl 02/26	small	funnel-like	fraying	
ostreatus 53 (T-1)	small	funnel-like	smooth-fraying	
Mycelia 2120	small	funnel-like	smooth-fraying	
pc 8-2-02 p2	small	shell-like	smooth	
Amycel A 3000	middle	funnel-like	smooth-fraying	
ATCC 66376	middle	funnel-like	fraying	
ASI 2066	large	funnel-like	fraying	thick stipe, good shape
K12	large	funnel-like	frays	dark color
PLX195	middle	shell	smooth	
Ital spawn P24	small-middle	funnel-like	smooth	good shape

Table 7. Quality assessment of mushrooms produced by strains at 16 and 22 °C.

	Lihtness		Yield (grams per package)		
Strain	16 °C	22 °C	16 °C	22 °C	Origin
HK35	73.30	67.28	1040	873	European commercial
32 (GH)	70.19	76.19	865	1725	Wild/Hongarian
Pleurotus ostreatus -7	*79.16*	*83.20*	*821*	*839*	*Indonesia*
pl 02/27	73.34	72.16	819	1005	China
3029	59.27	60.45	780	1037	European commercial?
49 (BHP-c)	76.65	80.04	1143	861	Wild/Hongarian
Amycel A 3000	71.02	79.04	830	961	European commercial
ATCC 66376	75.59	73.39	507	1168	Wild/USA
K12	67.96	70.49	1172	1722	European commercial
Ital spawn P24	68.63	76.24	943	1001	European commercial

Table 8. Strains selected for IVEGRI based on the resulsts of the assessments of traits. The strains and data in ital is the best performing Indonesian strains in this evaluation.

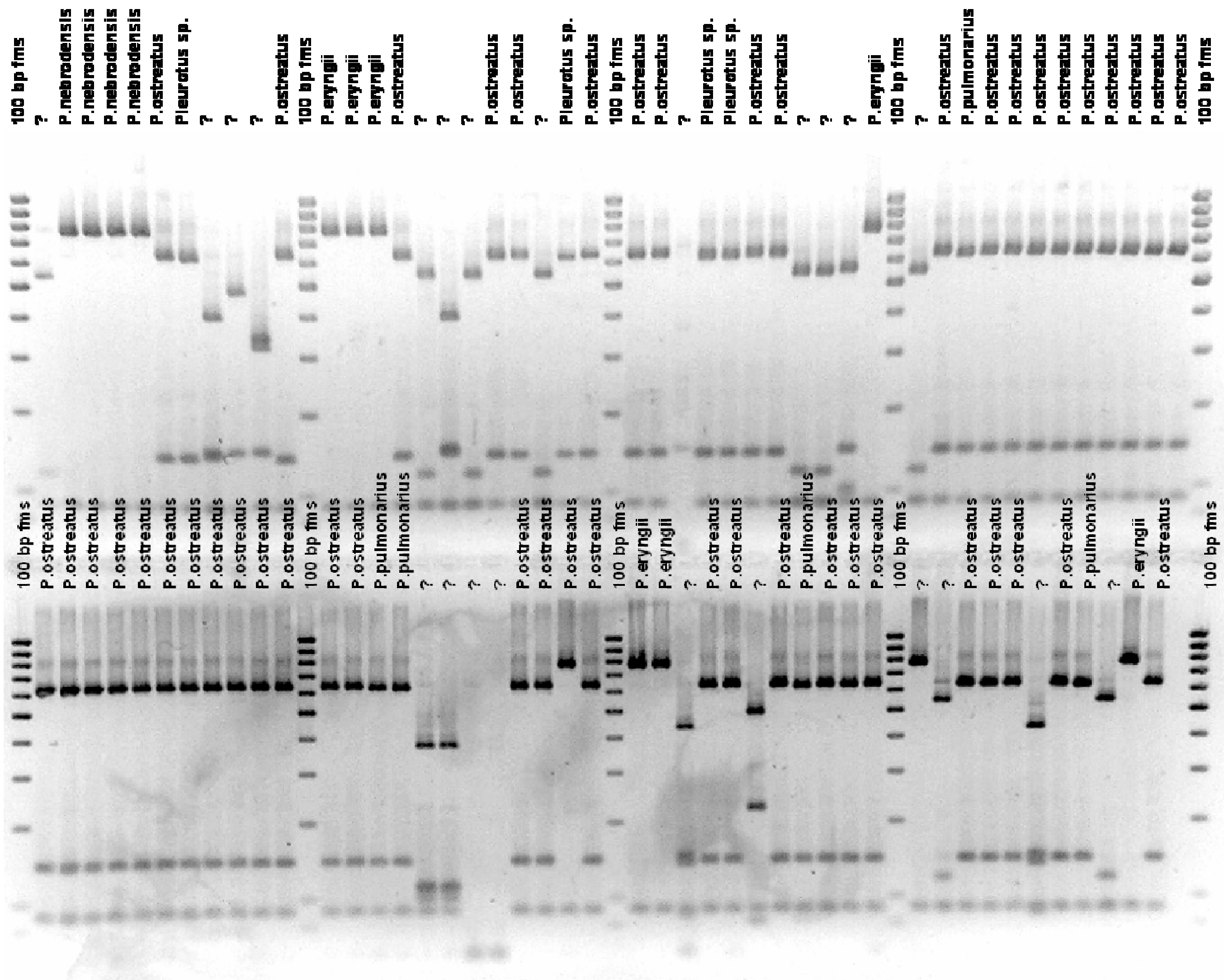


Figure 1. Identification of species within the genus *Pleurotus* using ITS-RFLP. Primers were used to amplify the ITS region of ribosomal DNA. This DNA was digested with Alu-I and resulting fragments separated on agarose gel and dyed with Ethidium Bromide. Next to Alu_I, 3 other restriction enzymes were used on separate samples (data not shown). In this way all *P. ostreatus* species could be identified within the collection and used for genotyping by ISSR.

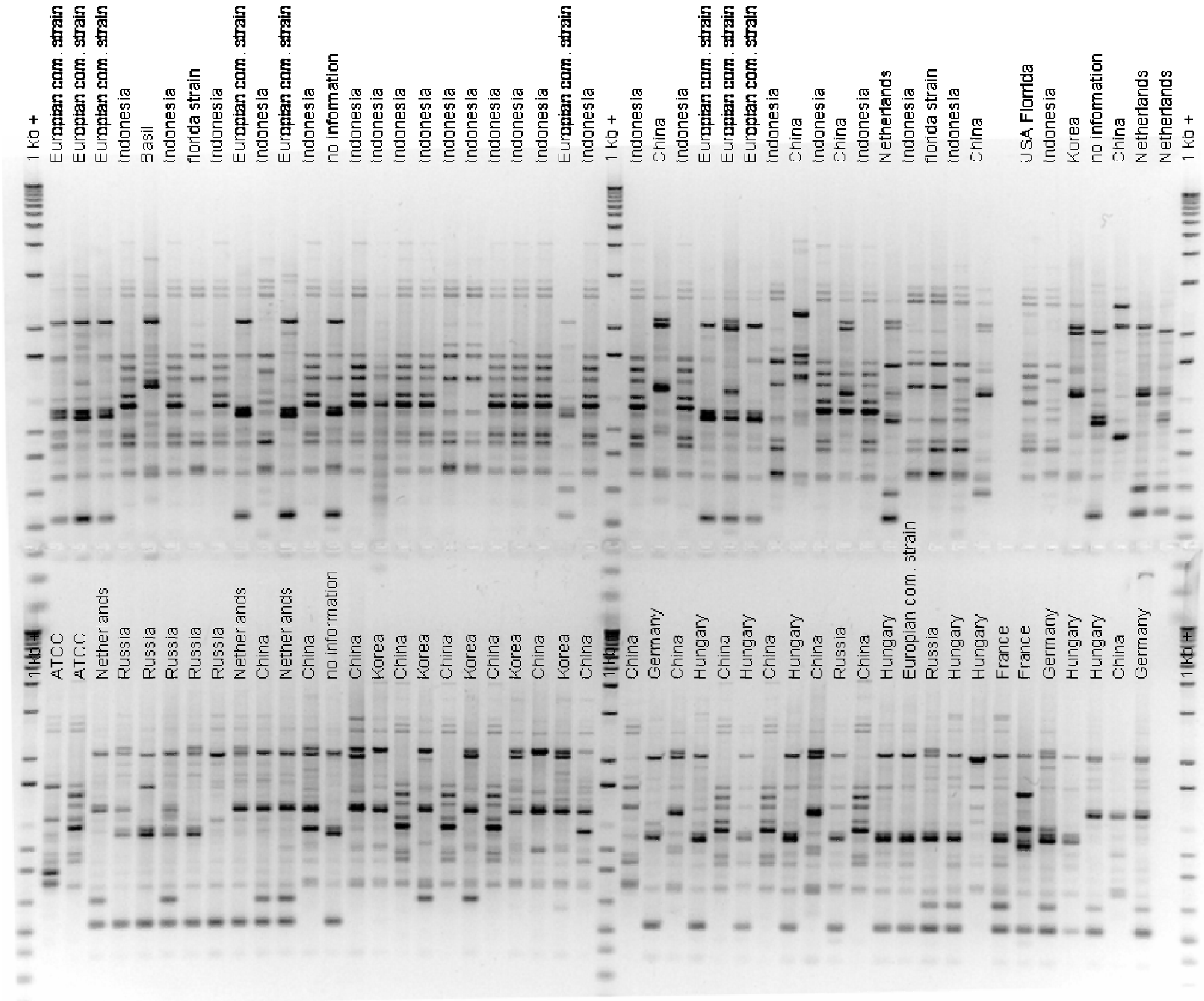


Figure 2. ISSR patterns of a selection of *P. ostreatus* strains originated from Indonesia and the collection of PPO. The picture shows that especially the wild collected strains show a high genetic variability. Based on these genetic fingerprints a selection 40 genotypes was made that will be tested in 2 parallel growing cells (16 and 22 ºC fruiting temperatures).

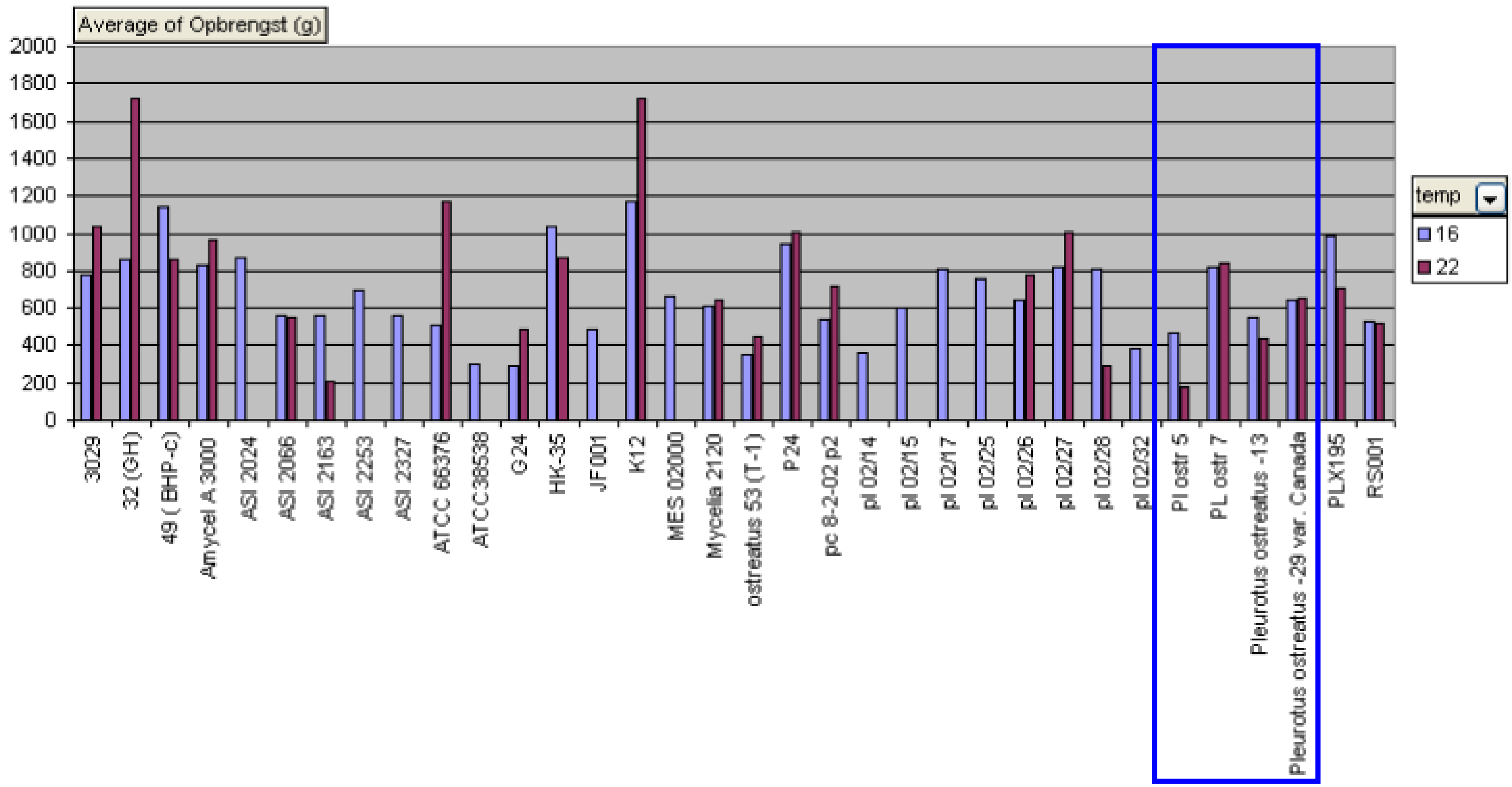


Figure 3. Yields of 40 *P. ostreatus* strains tested in the growing facilities of PPO. Strains were grown on pasteurized straw and incubated at 24 ºC for 14 days during the colonization of the substrate. This was done in two parallel cells. After this vegetative growth, both cells were vented (supply of fresh air with low CO_2 content) and temperature lowered to 16 ºC (first cell; yields in blue bars) or to 22 ºC (second cell; yields in red-brown bars). It can be seen that some strains do not produce at both temperatures. A relatively large number of strains do produce at 16 ºC but not al 22 ºC. Strains used in Indonesia are boxed blue.

Figure 4. Funnel (left) and shell (right) shape of oyster mushrooms. Most markets prefer the shell like shapes. More strains produced funnel like mushrooms at 22 oC than at 16 oC. Whether this is an unwanted character for the Indonesian market is not

Figure 5. Examples of oyster mushrooms produced in Indonesia. Mushrooms are white and shape and color deviate from those in Western countries.

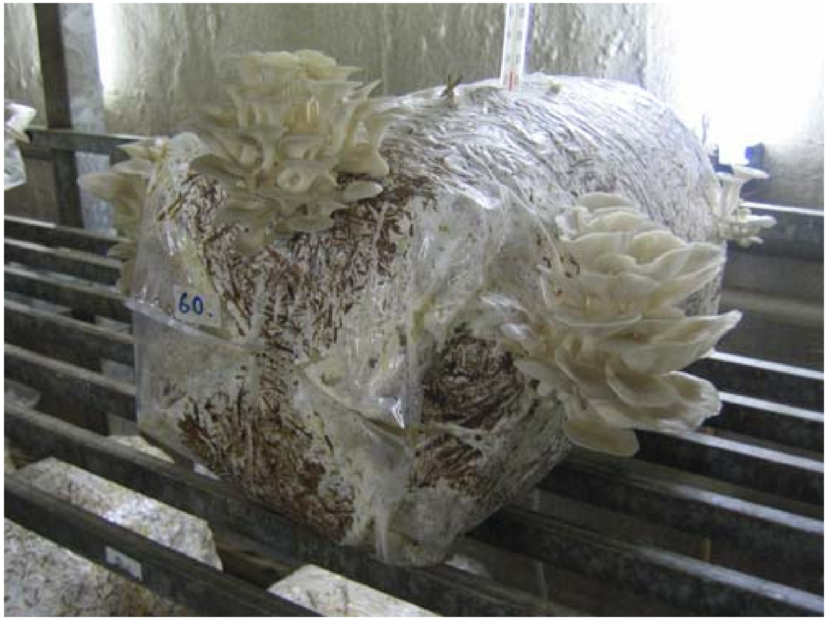
HK35 European commercial

16 ^{o}C

22 ^{o}C

P24 European commercial

$16\ ^{o}C$



$22\ ^{o}C$

K12 European commercial

16 °C

22 °C

32 (GH) Wild Hungarian

16 ^{o}C

22 ^{o}C

P-ostreatus-7 (Indonesia)

16 ^{o}C

22 ^{o}C

49 (BHP-c) Wild/Hungarian

16 ^{o}C

22 ^{o}C

Amycel 3000 (Commercial)

$16\ ^{o}C$

$22\ ^{o}C$

Pl 02/27; China

16 ^{o}C

22 ^{o}C

3020; European/commercial?

16 ^{o}C

22 ^{o}C

ATCC 66376; Wild/USA

16 ^{o}C

22 ^{o}C