Evaluation of shiitake (*Lentinula edodes*) strains of the culture collection of Applied Plant Research

Hortin/CNC-Exotics

Anton S.M. Sonnenberg & Jos G.M. Amsing

Applied Plant Research
Research Unit Mushrooms
Januari 2005

PPO no. 2005 - 1

Hortin (LNV) en CNC-Exotics

Project no. PPO: 620154

Applied Plant Research (Praktijkonderzoek Plant & Omgeving B.V.)
Mushroom Research Unit
Address : Peelheideweg 1, The Netherlands
: P.O. Box 6042, 5960 AA Horst, The Netherlands
Tel. : +31 77 4647575
Fax : + 31 77 4641567
E-mail : info.ppo@wur.nl
Internet : www.ppo.wur.nl

Table of contents

1 Introduction

Applied Plant Research (PPO), Mushroom Research Unit, has a unique collection of fungi that is used for research in edible mushrooms. The collection contains approximately 6600 strains representing more than 100 species. Most of the species are represented by *Agaricus bisporus* (button mushroom) and *Pleurotus ostreatus* (oyster mushroom). The high number of strains for *A. bisporus* in the collection is due to the focus of research on this species. That is because the Dutch mushroom industry produces almost exclusively button mushrooms (99% of all mushrooms produced in the Netherlands). The Dutch mushroom industry, however, becomes more and more interested in other mushroom species more commonly produced in other countries, especially in the Far East. Next to China, Japan and Korea, also Indonesia is producing several mushroom species not, or hardly produced in the Netherlands. One of these species is shiitake (*Lentinula edodes*, also designated as *Lentinus edodes*). Especially in the last few years PPO has collected a number of strains of these species from China and Japan. The collection contains now ca. 60 different strains of shiitake.
The purpose of this project was to evaluate these strains on characters that are important for the cultivation in the Netherlands and in Indonesia. The project is financed by HORTIN and by CNC Exotics b.v (formerly Fungi 2000), The Netherlands. Since there is no budget to test a high number of strains, all strains were first screened for genetic differences and a selection was made to represent most genotypes found in the collection.

1.1 Genotypic variation of shiitake strains

The genetic fingerprinting of strains was done in two steps. First, all strains were genotypes with a species-specific method. This was done because most strains have been obtained as vegetative mycelium and it is impossible to identify fungi on hyphal morphology only. We used the ITS region of ribosomal DNA to identify all strains and confirm that they are indeed *Lentinula edodes*. The ITS DNA sequence is highly conserved within a species. Primers were used to amplify this region and subsequently digested with restriction enzymes to check for sequence differences. Usually, if strains are members of one species, fragments of identical length will be amplified and no polymorphism will be seen after digestion with restriction enzymes. Except for two lines all showed fragments of the same length (figure 1). One of the deviant lines was included unintentionally and appeared to be *Lentinus lepideus*. The other aberrant line is compatible with other shiitake strains and must, therefore, be a real *Lentinula edodes*. What causes this difference is not known but it is not uncommon that also with the ITS region some variation occur within one species that results in bands with different lengths after enzymic digestion. An absolute certainty on the identity of this strain can be obtained by sequencing the fragment but that was not necessary since this strain is compatible with other shiitake strains, hence it must be a *L. edodes*. We have confirmed in this way that all lines are indeed *Lentinula edodes* strains and can thus be used in this trial.
A second screening was used to examine genetic differences between the lines. For this we have used primers that amplify regions between short repeated sequences, the so called microsatellite DNAs. Length and genomic positions of these repetitive elements are not well conserved within a species and can thus be used to examine genetic relationships. Figure 2 shows an impression of the kind of patterns generated by this type of fingerprinting on a number of shiitake strains. It shows that a large number of lines have identical or similar banding patterns. These are lines used for commercial growth in different countries. It is not surprising that these lines are similar since it is common use in the commercial production of mushroom that growers use identical strains all over the world. It is also common practice for suppliers of spawn (the inoculum for substrate) to copy strains and sell these under different names. The screening indicated, however, that we had enough genetic differences to allow a growing test. Eighteen lines were chosen and complemented with an additional four commercial lines used in Europe (Table 1). An overview of the genetic variation in the shiitake lines of the collection based on the ISSR screening is given in the table of Appendix A.

2 Experimental Setup

From all strains (table 1) spawn was prepared by inoculating pure cultures on sterilized sorghum grain. Spawn was prepared in 5 litre bags and, after a colonisation period of two weeks, used to inoculate 5 kg bags of substrate. Each substrate bag was inoculated with ca. 66 ml spawn. The substrate composition is:

- 5 m^3 Sawdust (Beech; moister content 30-40%)
- 20 kg Chopped straw
- 150 kg cornmeal
- 75 kg linseed (rich in oil)

The moisture content of the substrate is 58%, pH 5.5.

The purpose of the strain testing was to evaluate agronomical characters that are important for Dutch and Indonesian growers. Among these are appearances of synthetic blocks during colonization of substrate (white versus brown mycelium; formation of "bubbles" on surface of substrate, also called "bumping"), the influence of time of substrate colonisation on yield and appearance of mushrooms, mushroom morphology and color and the influence of temperature during fruiting on yield and mushroom quality. The experimental variables applied here were the length of the vegetative growth and the temperature during mushroom production (Figure 3).

2.1 Vegetative growth

Bags were filled with pasteurised (95 °C) substrate and inoculated by CNC Exotics (formerly Fungi 2000). Bags were incubated in a growing room of CNC Exotics for ca. 20 days and inspected for infections. Infected bags were removed and the remaining bags were transported to the experimental farm of Applied Plant Research in America (Horst). Approximately 700 bags of 5 kg colonised substrate were placed in a growing room for further development of the vegetative mycelium (figure 3). Climate parameters were: 20 °C (air temperature), 1500 ppm CO_2, 90% RH. After two days 51 bags had to be removed because of infections with green moulds.
During the first three weeks the room was illuminated 4 hours a day. After this period the illumination has been reduced to 1 hour a day and the temperature increased to 24 °C. This has been done to inhibit pin formation. A number of bags had already started to form fruit bodies underneath the plastic.
All bags showed some browning after 6 weeks. The extent of browning differed considerably between treatments and also, the further development of the browning differed highly between strains. Browning is considered as an important process of maturation of the substrate that has a great impact on the production. Table 2 shows an examination of the browning and bumping of the bags 7 weeks after spawning where it can be seen that there is a considerable differences between the strains.
After 5 weeks a number of strains started to form so many fruiting bodies that the plastic bags were removed. These strains (3, 6 and 21) were incubated for another week in the vegetative growing room. Half of the bags of each of these three treatments were transferred to a production room with 16 °C air temp. and the other half to a room with 24 °C air temp. Especially treatment 6 seems to be an interesting strain since this one is producing after a relatively short vegetative growth period and the production level is good.

2.2 Fruiting

Normally the vegetative period for Dutch shiitake cultivation is 16 to 18 weeks and the temperature during mushroom production is 16 °C. Here, we wanted to test a variation in the period of vegetative growth on the production. In addition, we wanted to test production at temperature of 16 and 24 °C in order to see what strains are suited for the Dutch growers and what strains can perform well under Indonesian

conditions were temperatures are higher. The conditions in the production room were:
1000 ppm CO_2, 90% rh and 12 hours light a day. Except for strains 3, 6 and 21, 5 bags of each were transported to each production room (16 and 24 °C) after 12 and after 16 weeks of vegetative growth. We have, therefore, three different length of the vegetative growth, i.e. 7, 12 and 16 weeks, where for 7 weeks only 3 treatments have been examined. Plastic was removed from all bags for all strains after transfer to the production rooms. From now on the experimental units are designated as (synthetic) blocks.
One week after the transfer of bags to the production room all blocks were examined on pinning, bumbing and the presence of infections (For detailed description see Appendix B). Most blocks showed pins or had already started to produce mushrooms. Blocks that were placed in the 16 °C growing room and had a vegetative period of 16 weeks were allowed to produce a second flush. For this, a week "rest" was incorporated where blocks were dipped in water and incubated for one week at a climate optimal for vegetative growth. Especially in the second flush most blocks had green mould. There was a correlation between the presence of green moulds and the production level. Blocks that produced no or hardly mushrooms had less green moulds than blocks that had a substantial production.

2.2.1 First Flush

The production levels for the first flush are listed in tables 3 and 4. The standard deviations are given for the average of each group and are just an indication of the variations in this test. The variations are quite large and we consider this test, therefore, as an estimation of the potentials of these strains and not as an accurate estimate of production under different conditions. The second flush (only for the production cell at 16 °C) will be discussed separately. A photographic impression of the mushrooms is given in Appendix C.
Seven strains had production efficiencies lower than 10% at 16 °C with both lengths of vegetative growth periods. Some had hardly any production. The rest of the strains varied in production efficiencies from 10 to 29%. At 24 °C most strains had a low production or did not produce at all (compare figure 5 and 6). Only three strains had production efficiencies higher than 10% at 12 weeks vegetative growth. With 16 weeks vegetative growth, however, 10 strains showed a production efficiency higher than 10. Mushrooms tent to ripen quicker when produced at 24 °C.
Of the three strains for which the plastic bag was removed after 7 weeks, number 6 (sh02/05 from China) showed a good production of approximately 19% efficiency. The two other strains had a considerable lower production. The conditions for vegetative growth were not optimal as was described previous. We had to increase the temperature during the vegetative growth and reduce the daily light periods to inhibit too early fruiting. Even before 7 weeks of vegetative growth this strain started to form fruit bodies. Due to the presence of plastic these mushrooms were malformed. Those mushrooms that were formed later had a large shape, dark cap and white stipe. The morphology was quite normal and mushrooms tent to produce in clusters. Strain number 3 (sh02/02) showed many abnormal fruit bodies due to production under plastic. Strain number 21 (Mycelia 3782; semi commercial strain) produced hairy mushrooms due to mycelial fragments on stipes and caps. The mushrooms had a lighter color than strain 3 and 6.
Only for strain number 3 (sh02/02) we had enough bags to have also production after 12 and 16 weeks of vegetative growth. This strain showed a good production after 16 weeks of vegetative growth (25% efficiency). The mushrooms have a relatively large size cap and are dark colored. They have a regular morphology after 16 weeks of vegetative growth whereas this is less regular after 12 weeks of vegetative growth. Strain 3 had a reasonable good production at a temperature of 24 °C (18% efficiency) and a vegetative growth period of 16 weeks. It might, therefore, also be useful for Indonesian farmers.
The most productive strain at 16 °C was strain number 1 (4B Su Xiang) from China. This strain showed a biological efficiency of 29 and 28% at 16 °C after a 12 week and 16 week period of vegetative growth, respectively. Many fruit bodies are produced per log and the cap size is medium. Cap color is brown with a light margin. The fruit body morphology is regular. The mushroom morphology is also good at fruiting temperature of 24 °C. This strain is, therefore, very suitable for Indonesian producers.
Next to this line, strain 20 (Somycel 4087; a commercially available strain but spawn prepared from the strain maintained in our collection) was also a good producer. This strain is especially suited for a 12 weeks vegetative growth period. Its production efficiency is 25 and 27% at 16 and 24 °C, respectively. This strain is, therefore, also suited for production at high temperatures. The caps are dark brown with a lighter margin. Caps are also a bit hairy. Morphology of the mushrooms is similar at both production

temperatures.
Strain 5 (sh02/04 from China) has a good production at 16 ^{0}C at both lengths of vegetative growth. Production is, however, low at 24 ^{o}C. It produces mushrooms of middle size, brown to light brown caps and reasonable morphology.
A number of other strains had a good production at 16 ^{0}C (low at 24 ^{0}C) and produced better after 16 weeks than 12 weeks of vegetative growth (Table 4). In appendix B images are shown that give an impression of the morphology of the fruit bodies. The color of the images, however, does not always correlate well with the actual color of the mushrooms.
A number of strains showed a better morphology after 16 weeks than 12 weeks vegetative growth (strain 4, 7, 14, 19, 20 and 22). Strain 17 seems to have a better morphology after 12 weeks of vegetative growth.

2.2.2 Second flush

The productivity in a second flush was tested for 19 strains. This was only tested for strains that had a 16 weeks vegetative growth and production at 16 ^{o}C. Usually, the production levels in second flushes are considerably smaller than first flushes. It is, nevertheless, worthwhile to produce a second flush since it takes a lot of time and effort for a new production. A second flush can be produced after soaking the blocks in water for several hours and incubating the blocks for one week at higher temperatures. This “rest period” restores vegetative growth. After cooling to 16 ^{0}C a second flush will appear within one week. Some strains had a substantial production in the second flush. Especially strains 5, 8, 16 and 20 had a good production (Table 6). Six strains showed a production efficiency above 30% when productions from flush 1 and 2 are added (figure 5).

3 Summary

This cell test has been carried out to get an impression of the potentials of the strain collection of the Applied Plant Research, Mushroom Research Group. The interest was directed to shiitake strains that could be produced in a shorter cultivation cycle in a system that is used in The Netherlands and in Indonesia. In both countries substrate colonisation and browning is done in plastic bags. Production is done after either by opening the bag at the top site (Indonesia) or completely removing the bag (Indonesia and The Netherlands).
This test has shown that the collection contains at least one strain that will produce after 7 weeks of vegetative growth. Since mushroom production for this strain was seen even before this period it might be that the vegetative growth period can be shortened further. At least 5 strains had a good production at 16 ^{o}C after 12 weeks of vegetative growth. This period is shorter than the period used by most Dutch growers. There are 5 strains that showed a good production at 24 ^{0}C either after 12 or 16 weeks of vegetative growth. These strains will be useful for Indonesian growers. In March 2005 all 5 strains will be handed over to IVEGRI where further tests can be done either by IVEGRI alone or by growers.
CNC Exotics can make experimental batches of strains that can be further tested in Dutch cultivation system.

PPO will maintain all strains in its collection as mother cultures to ensure that all characters will be preserved. These cultures can be obtained on request by IVEGRI and by CNC Exotics but will remain property of PPO. Negotiations will start with IVEGRI and CNC Exotics under what conditions licences will be given for the commercial production.

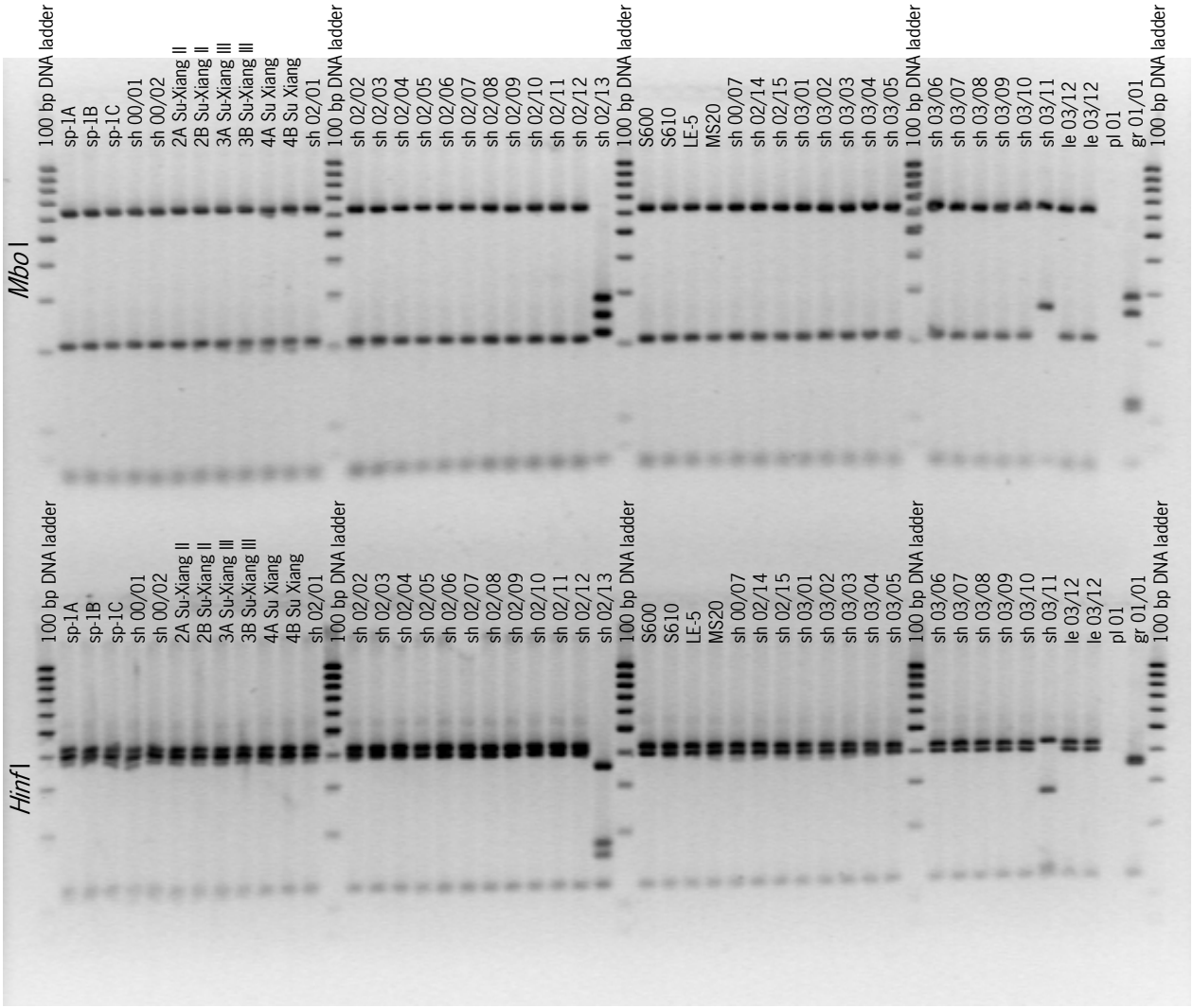


Figure 1. Screening shiitake lines for species identity. The ITS ribosomal DNA region was amplified and digested with either *MboI* (upper panel) or *HinfI* (lower panel) and examined on agarose gel. As a control two pleurotus lines were included (pl01 and pl 01/01, indicated above the relevant lanes). As can be seen, all lines show identical patterns, except for two strains, i.e. sh02/13 and sh03/11. Strain sh02/13 appeared to be a *Lentinus lepideus* and indeed a different species. Line sh03/11, however, is a *Letinula edodes* since this line is compatible with other shiitake strains.

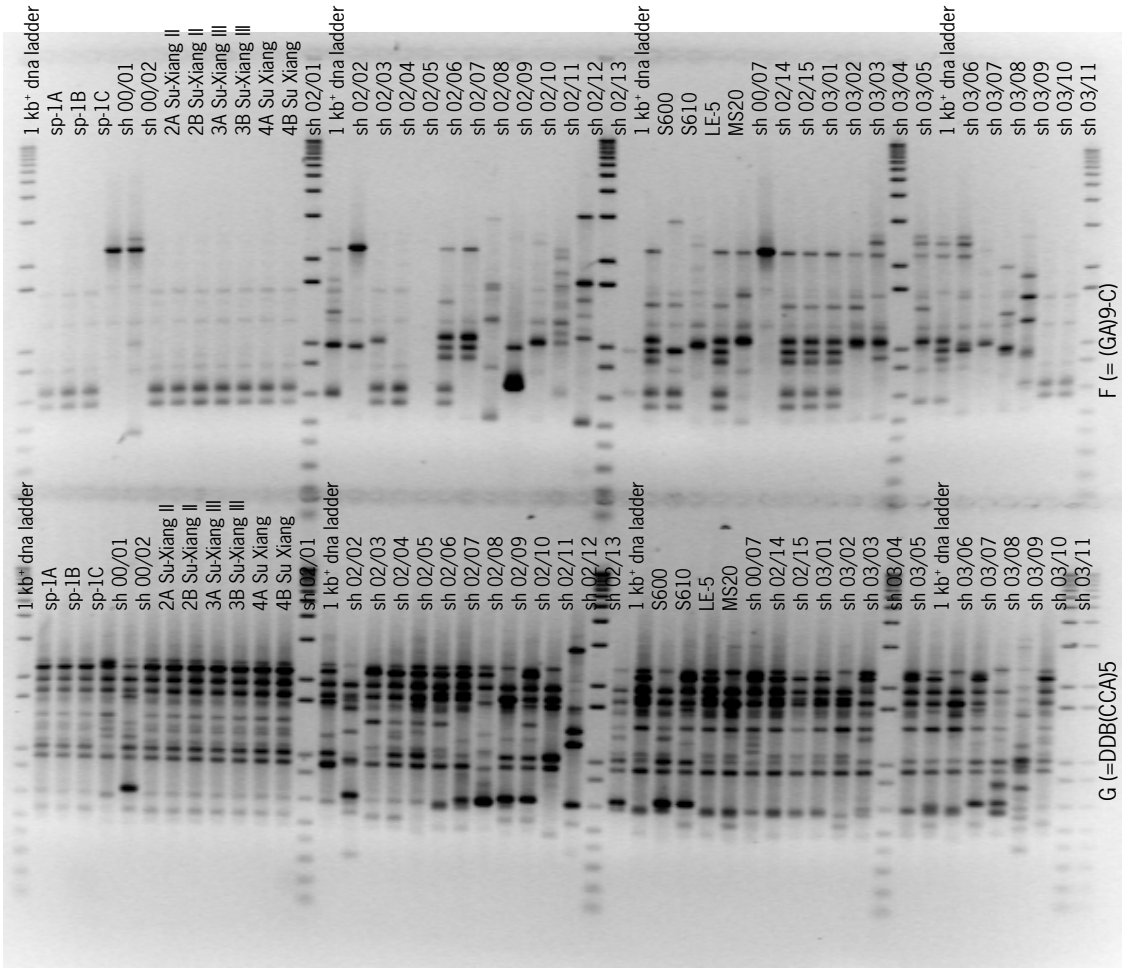


Figure 2. Example of genotypically differences between shiitake strains in the PPO collection.The upper and lower panel were screened with different primers. The grouping in different genotypes has been used to make a selection for a growing test.

Figure 4. Left an impression of the incubation room for the vegetative growth phase. Bags of approximately 5 kg substrate were inoculated with pure cultures of different shiitake lines and incubated for different periods. After vegetative growth plastic was removed and the blocks were transported to a production room. Above a block from which plastic was removed after only a short period of vegetative growth. This was done because mushrooms started to form under need the plastic. As can be seen, browning of this block is incomplete (only the top part shows some browning.

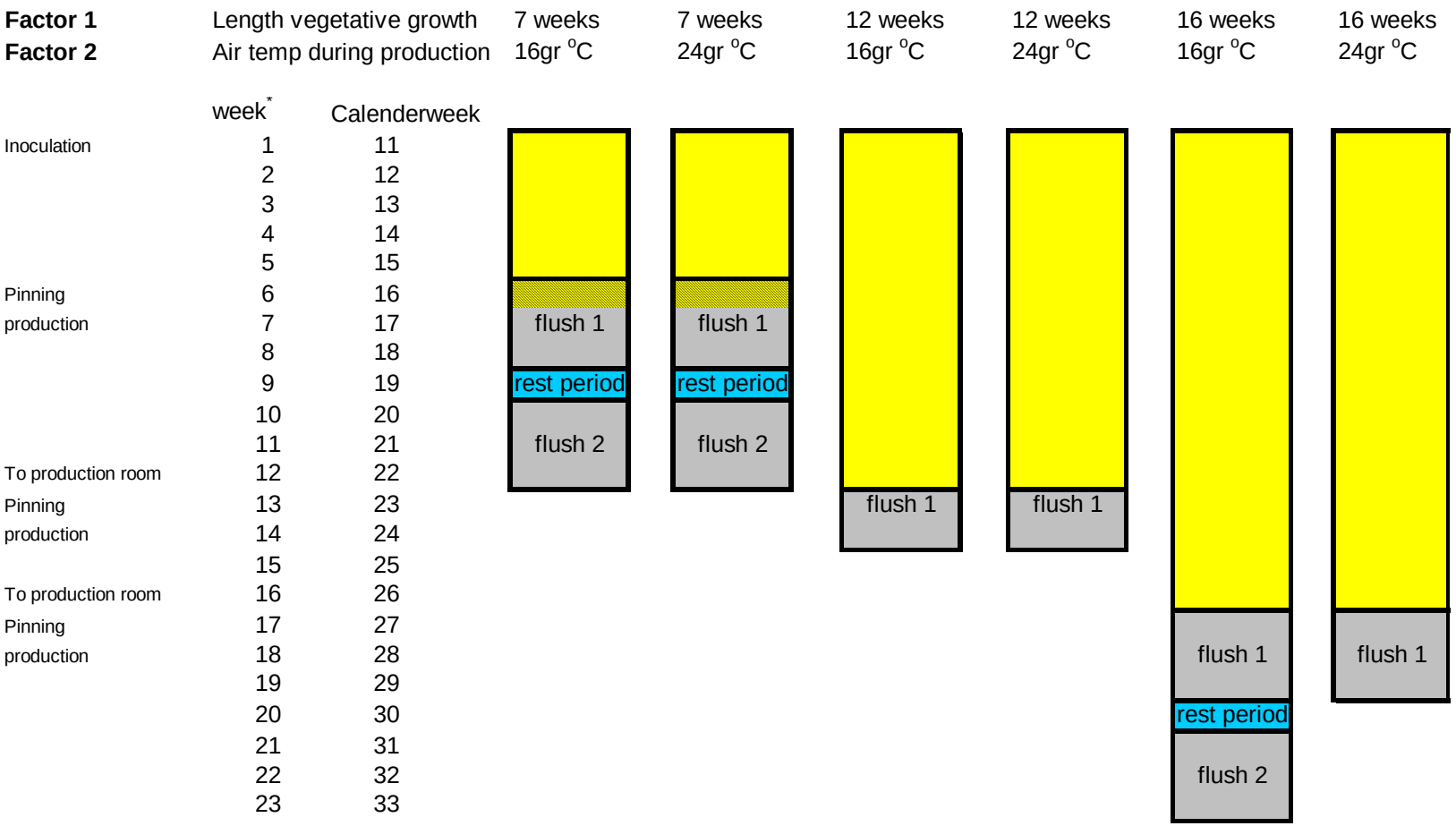


Figure 3. Schematic presentation of growing trial of shiitake strains. Time schedule is indicated in th left two columns. The vegetative growth periods are indicated in yellow blocks; the production periods in gray blocks and the rest period between flushes in blue blocks.

Treatment nr.	*strain name APR*	*Origin of strain*
1	4B Su Xiang	China
2	00/02 Quingyan 3	China
3	sh 02/02 shiitake 2477	China
4	sh 02/03 shiitake	China
5	sh 02/04 shiiteke 626	China
6	sh 02/05 shiitake 867	China
7	sh 02/06	Japan
8	sh 02/07	China
9	sh 02/08	China
10	sh 02/09	China
11	sh 02/10	China
12	sh 02/12	China
13	sh 03/04 H600	Japan
14	sh 03/05 KV92	Japan
15	sh 03/06 ML 8	Japan
16	sh 03/07 ML 12	Japan
17	sh 03/08 A567	Japan
18	sh 03/09 MM 1	Japan
19	S600	European commercial
20	le 03/13 som 4087, edodes	European commercial
21	Mycelia 3782	European commercial
22	Mycelia 3715	European commercial

Table 1. Strains tested in the trial.

Treatment	bag removed	Examination vegetative growth
	in week nr	in week 7 after inoculation
1		in 8 of 42 bags mushrooms observed
2		substrate starts browning
3	6	mushroom production
4		" dark
5		" white to dark
6	5	" fruit bodies observed
7		" bumping seen
8		" white to brown; bumping
9		" white
10		" browning starts
11		" white
12		" browning starts
13		" browning starts
14		" white
15		" browning starts
16		" white; bumping
17		" white
18		" white
19		" white to brown; bumping
20		" bumping seen
21	5	mushroom production
22		" browning starts

Table 2. Examination of vegetative growth, 7 weeks after inoculation.

	Temperature at production	16 °C		
	# weeks of vegetative growth	7*	12	16
	Strain			
1	4B Su Xiang		29	28
2	00/02 Quingyan 3		9	5
3	sh 02/02 Shiitake 2477	5	12	25
4	sh 02/03 shiitake		7	11
5	sh 02/04 shiitake 626		23	21
6	sh 02/05 shiitake 867	19		
7	sh 02/06		18	21
8	sh 02/07		7	26
9	sh 02/08		3	0
10	sh 02/09		1	0
11	sh 02/10		0	0
12	sh 02/12		14	20
13	sh 03/04 H600		19	26
14	sh 03/05 KV92		7	5
15	sh 03/06 ML 8		13	6
16	sh 03/07 ML 12		5	1
17	sh 03/08 A567		21	24
18	sh 03/09 MM 1		4	8
19	S600		13	16
20	le 03/13 Som 4087		25	16
21	Mycelia 3782	9		
22	Mycelia 3715		19	26
	lsd (p 0.05)		5	8
	lsd (p 0.01)		7	10

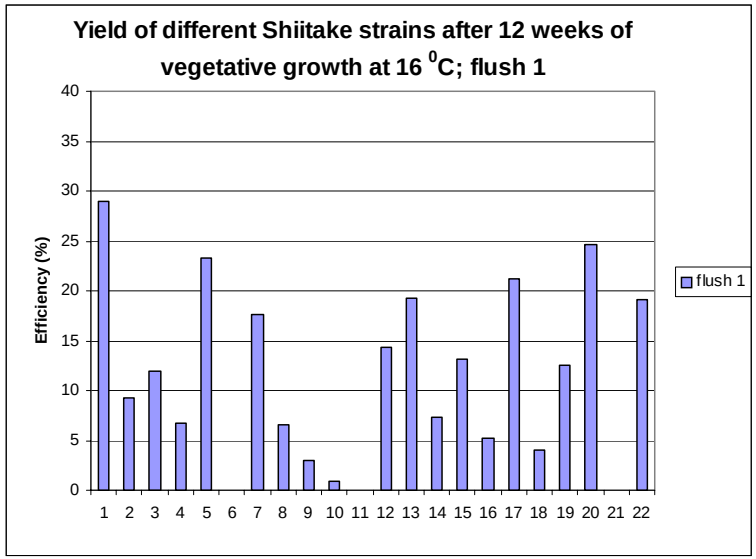
Table 3. Yield of strains grown with different periods of vegetative growth periods under fruiting temperature of 16 oC. Yield expressed in % biological efficiency (fresh weight fruit bodies/fresh weight substrate). *: Yield after 7 weeks vegetative growth estimated because substrate was not weighted.

	Temperature at production	24 °C		
	# weeks of vegetative growth	7*	12	16
	Strain			
1	4B Su Xiang		22	28
2	00/02 Quingyan 3		5	0
3	sh 02/02 Shiitake 2477	3	4	18
4	sh 02/03 shiitake		2	10
5	sh 02/04 shiitake 626		11	14
6	sh 02/05 shiitake 867	16		
7	sh 02/06		3	13
8	sh 02/07		2	20
9	sh 02/08		0	0
10	sh 02/09		0	3
11	sh 02/10		0	0
12	sh 02/12		4	4
13	sh 03/04 H600		0	4
14	sh 03/05 KV92		0	9
15	sh 03/06 ML 8		0	0
16	sh 03/07 ML 12		0	4
17	sh 03/08 A567		4	10
18	sh 03/09 MM 1		0	3
19	S600		1	17
20	le 03/13 Som 4087		27	18
21	Mycelia 3782	5		
22	Mycelia 3715		2	14
	lsd (p 0.05)		4.6	5
	lsd (p 0.01)		6.2	6

Table 4. Yield of strains grown with different periods of vegetative growth periods under fruiting temperature of 24 oC. Yield expressed in % biological efficiency (fresh weight fruit bodies/fresh weight substrate). *: Yield after 7 weeks vegetative growth estimated because substrate was not weighted.

	Temperature at production	16 °C
	# weeks of vegetative growth	16
	Strain	
1	4B Su Xiang	5
2	00/02 Quingyan 3	1
3	sh 02/02 Shiitake 2477	6
4	sh 02/03 shiitake	6
5	sh 02/04 shiitake 626	12
6	sh 02/05 shiitake 867	
7	sh 02/06	6
8	sh 02/07	11
9	sh 02/08	0
10	sh 02/09	5
11	sh 02/10	0
12	sh 02/12	8
13	sh 03/04 H600	6
14	sh 03/05 KV92	8
15	sh 03/06 ML 8	5
16	sh 03/07 ML 12	14
17	sh 03/08 A567	5
18	sh 03/09 MM 1	2
19	S600	8
20	le 03/13 Som 4087	14
21	Mycelia 3782	
22	Mycelia 3715	5

Table 5. Yield in second flush of strains after vegetative growth at 16 °C for 16 weeks. Yield expressed in % biological efficiency (fresh weight fruit bodies/fresh weight substrate).



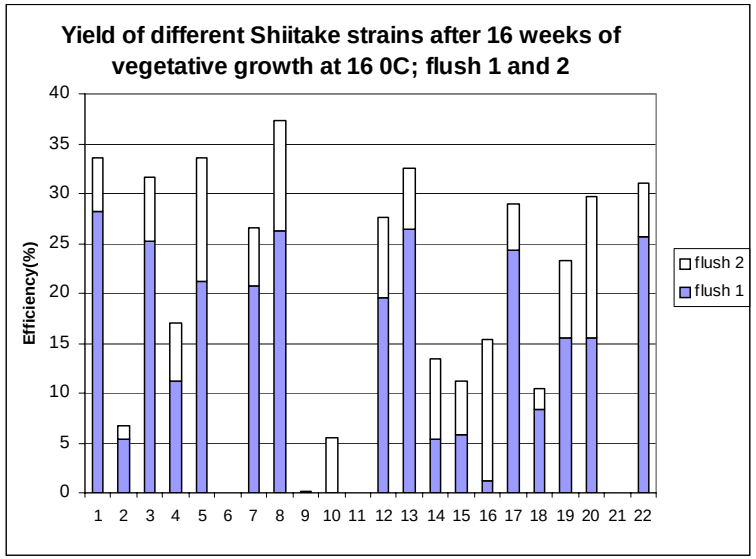
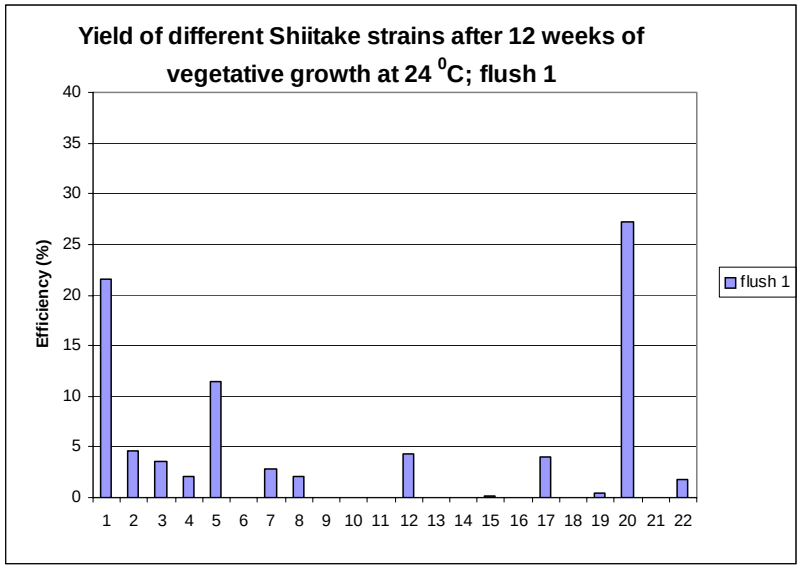


Figure 5. Graphical interpretation of production at 16 ^{0}C.



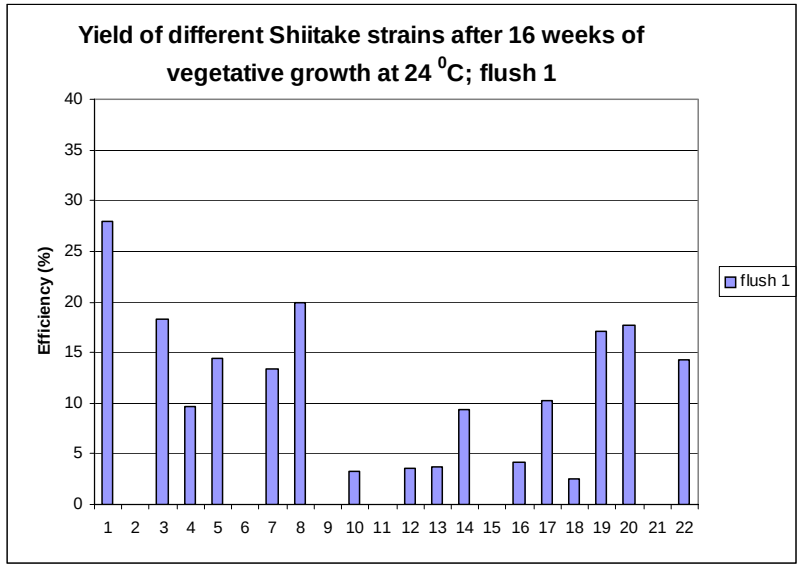


Figure 6. Graphical interpretation of production at 24 ^{0}C.

Appendix A

	Genotype			Selected for growing test		Genotype			Selected for growing test
strain nr.	primer A	primer F	Primer G		strain nr.	primer A	primer F	Primer G	
1	1	1	1		23	3	4	12	
2	1	1	1		24	3	4	12	
3	1	1	1		25	3	4	1B	
4	1	1	1		26	3	4	1B	
5	1	1	1		27	3	4	1B	x
6	1	1	1	x	28	3	4	1B	
7	1	1	1		29	10	4A	5	x
8	1	1	1		30	14-A	4B	1E	x
9	1	1	1		31	4	11	10	x
10	1	1	1		32	4	5A	10	
11	1	1	1	x	33	5	2A	5	x
12	1	1	1	x	34	6	3	4	x
13	1	7	1C		35	11	5	6	x
14	8	1A	1	x	36	12	6	7	x
15	9	-	1A	x	37	13	8	7A	x
16	1-A	2	2		38	14	13	1D	x
17	7	2A	3	x	39	15	14	15	x
18	1-A	10	11		40	4-A	13A	9A	x
19	1-A	7A	14	x	41	16	9	8	
20	1-B	12	13	x	42	17	15	16	
21	2	16	9		43	-	17	1C	
22	2	16A	9						

Shiitake lines tested for genotypes. Each line was screened for ISSR pattern with three different primers. The arrangments in genotypes was done for each primer and designated by numbers. Identical numbers indicate indiscriminable genotypes. Numbers combined with letters are genotypes that are similar but not identical. The last column indicates the selection that was made for the growing test.

4 Appendix B

first flush **12 weeks vegetative growth; fruiting at 16 °C** Examination 1 week after transfer to production room

Designation APR	Strain number	Bag numbers	bumping	remarks
4B Su Xiang	1	1-2-3-4-5	fine bumps	All bags many fruit bodies
00/02 Quingyan 3	2	55-61-62-63-64	lightly bumping	All bags only a few pinns. All bags show light infection with green molds.
sh 02/02 Shiitake 2477	3	165-171-172-173-174	coarse bumps	All bags have many pinns. All bags show light infection with green molds. Only bag 171 has a few pinns.
sh 02/03 shiitake	4	91-92-93-94-95	coarse bumps	All bags have several pinns.
sh 02/04 shiitake 626	5	143-144-145-151-152	lightly bumping	All bags have many pinns. All bags show light infection with green molds. Only bag 171 has a few pinns.
sh 02/05 shiitake 867	6			all bags in production after 7 weeks; not present in this room
sh 02/06	7	211-212-213-214-215	coarse bumps	All bags form pinns and fruit bodies in bunches. Mushrooms dark color and brown stipe
sh 02/07	8	81-82-83-84-85	coarse bumps	All bags show several pinns.
sh 02/08	9	121-122-123-124-125	coarse bumps	Bags 124 and 125 show some pinns.
sh 02/09	10	11-12-13-14-15	no bumping	No pinns. All bags show white dried mycelial spots.
sh 02/10	11	33-34-35-41-42	hardly any bumping	No pinns. All bags show white dried mycelial spots.
sh 02/12	12	71-72-73-74-75	coarse bumps	All bags show several fruiting bodies. Fruit bodies have dark cap and brown stipe.
sh 03/04 H600	13			All bags show pinns.
sh 03/05 KV92	14	111-112-113-114-115	no bumping	All bags show few pinns. Many white dried mycelial spots.
sh 03/06 ML 8	15	201-202-203-204-205	hardly any bumping	All bags show whiet dried mycelial spots.
sh 03/07 ML 12	16	21-22-43-44	lightly bumping	All bags show pinns.
sh 03/08 A567	17	191-192-193-194-195	coarse bumps	All bags show several pinns, especially on top of bags.
sh 03/09 MM 1	18	24-25-31-32	coarse bumps	Lower part of bags shows dried mycelium. No pinns.
S600	19	45-51-52-53-54	coarse bumps	All bags have several pinns.
le 03/13 Som 4087	20	161-162-163-164	lightly bumping	All bags shown mnay pinns - fruit bodies.
Mycelia 3782	21			all bags in production after 7 weeks; not present in this room
Mycelia 3715	22	181-182-183-184-185	hardly any bumping	No pinns. Bag 182 shows one big green spot (mold).

first flush **16 weeks vegetative growth; fruiting at 16 °C** Examination 1 week after transfer to production room

Designation APR	Strain number	Bag numbers	bumping	remarks
4B Su Xiang	1	6-7-8-9-10	fine bumps	Many pinns on all bags.
00/02 Quingyan 3	2	56-57-58-59-60	fine bumps and not many	Many pinns on bag 56. Other bags no pinns.
sh 02/02 Shiitake 2477	3	166-167-168	coarse bumps	All bags show pinns.
sh 02/03 shiitake	4	89-90-96-97-98	coarse bumps	All bags show many pinns. Bag 96 no pinns.
sh 02/04 shiitake 626	5	146-147-148-149-150	fine bumps	Only bags 147 and 148 show pinns.
sh 02/05 shiitake 867	6			all bags in production after 7 weeks; not present in this room
sh 02/06	7	209-210-216-217-218	fine bumps	All bags show pinns.
sh 02/07	8	77-78-79-80-86	lightly bumping	All bags show pinns.
sh 02/08	9	126-127-128-129-130	coarse bumps	no pinns
sh 02/09	10	16-17-18-19-20	lightly bumping	no pinns
sh 02/10	11	36-37-38-39-40	fine bumps and not many	No pinns. All bags show white myclial spots.
sh 02/12	12	67-68-69-70-76	coarse bumps	All bags show pinns. Bag 67 shows green mold
sh 03/04 H600	13	99-100-106-107-108	coarse bumps	All bags show pinns.
sh 03/05 KV92	14	111-112-113-114-115		
		116-117-118-119-120	lightly bumping	All bags show white mycelium; no pinns.
sh 03/06 ML 8	15	199-200-206-207	lightly bumping	white dry mycelium, no pinns
sh 03/07 ML 12	16	66-87-88	fine bumps	No pinns.
sh 03/08 A567	17	187-188-189-190	observation missing	observation missing
sh 03/09 MM 1	18	26-27-28-29-30	coarse bumps	no pinns
S600	19	46-47-48-49-50	fine bumps	All bags show some pinns, except bag 48 (white mycelium)
le 03/13 Som 4087	20	156-157-158	fine bumps	All bags show some pinns.
Mycelia 3782	21			all bags in production after 7 weeks; not present in this room
Mycelia 3715	22	177-178-179-180-186	coarse bumps	bag 177 shows green mold, bags 178-179-180-186 show pinns

Appendix C

12 weeks vegetative growth; production at 16 oC

Strain 1 (4B Su Xiang, China), 6 days after removal of the bag.

16 weeks vegetative growth; production at 16 oC

Strain 1 (4B Su Xiang, China), 7 days after removal of the bag.

Strain 1 (4B Su Xiang, China), 9 days after removal of the bag.

16 weeks vegetative growth; production at 16 oC

Strain 1 (4B Su Xiang, China), 7 days after removal of the bag.

Strain 1 (4B Su Xiang, China), 9 days after removal of the bag.

12 weeks vegetative growth production at 16 oC

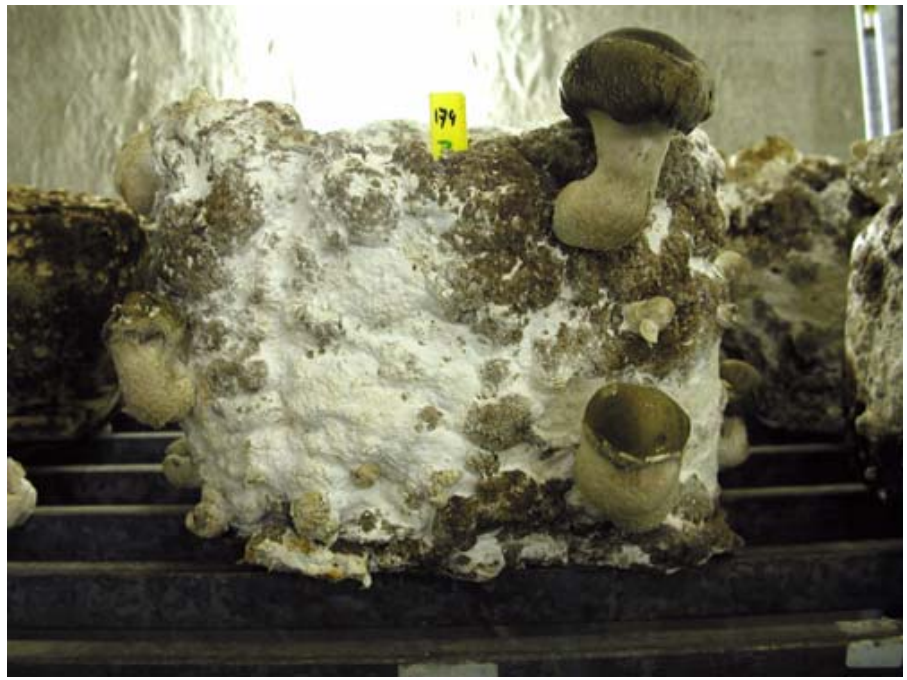
Strain 2 (sh 00/02 Quingyan, China) 4 days after removal of the plastic bag.

16 weeks vegetative growth production at 16 oC

Strain 2 (sh 00/02 Quingyan, China) 11 days after removal of the plastic bag.

7* weeks of vegetative growth; production at 16 °C

Strain 3 (sh 02/02; 2477; China) 4 days after removal of the bag.



Strain 3 (sh 02/02; 2477; China) 7 days after removal of the bag.

*: The logs were transferred after 7 weeks to the production room. The strain started to produce 6 weeks after spawning.

12 weeks of vegetative growth; production at 16 ^{o}C

Strain 3 (sh 02/02; 2477; China) 10 days after removal of the bag.

16 weeks of vegetative growth; production at 16 ^{o}C

Strain 3 (sh 02/02, 2477, China) 7 days after removal of the bag.

Strain 3 (sh 02/02, 2477, China) 9 days after removal of the bag.

12 weeks of vegetative growth; production at 16 ^{o}C

Strain 4 (sh 02/03, China) 10 days after removal of bag.

Strain 4 (sh 02/03, China) 10 days after removal of bag.

16 weeks of vegetative growth; production at 16 ^{o}C

Strain 4 (sh 02/03, China) 7 days after removal of bag.

Strain 4 (sh 02/03, China) 9 days after removal of bag.

12 weeks vegetative growth; production at 16 ^{0}C

Strain 5 (sh 02/04; 626, China) 10 days after removal of the bag.

16 weeks vegetative growth; production at 16 ^{0}C

Strain 5 (sh 02/04, 626; China) 7 days after removal bag.

Strain 5 (sh 02/04, 626; China) 10 days after removal bag.

7* weeks vegetative growth; production at 16 ^{0}C.

Strain 6 (sh 02/05, 867, China). 5 Weeks after spawning the first mushrooms were produced. Plastic was removed. Bags transferred to production room 7 weeks after vegetative growth and picture is taken 18 days after transfer to production room.

Strain 6 (sh 02/05, 867, China). 5 Weeks after spawning the first mushrooms were produced. Plastic was removed. Bags transferred to production room 7 weeks after vegetative growth and picture is taken 21 days after transfer to production room.

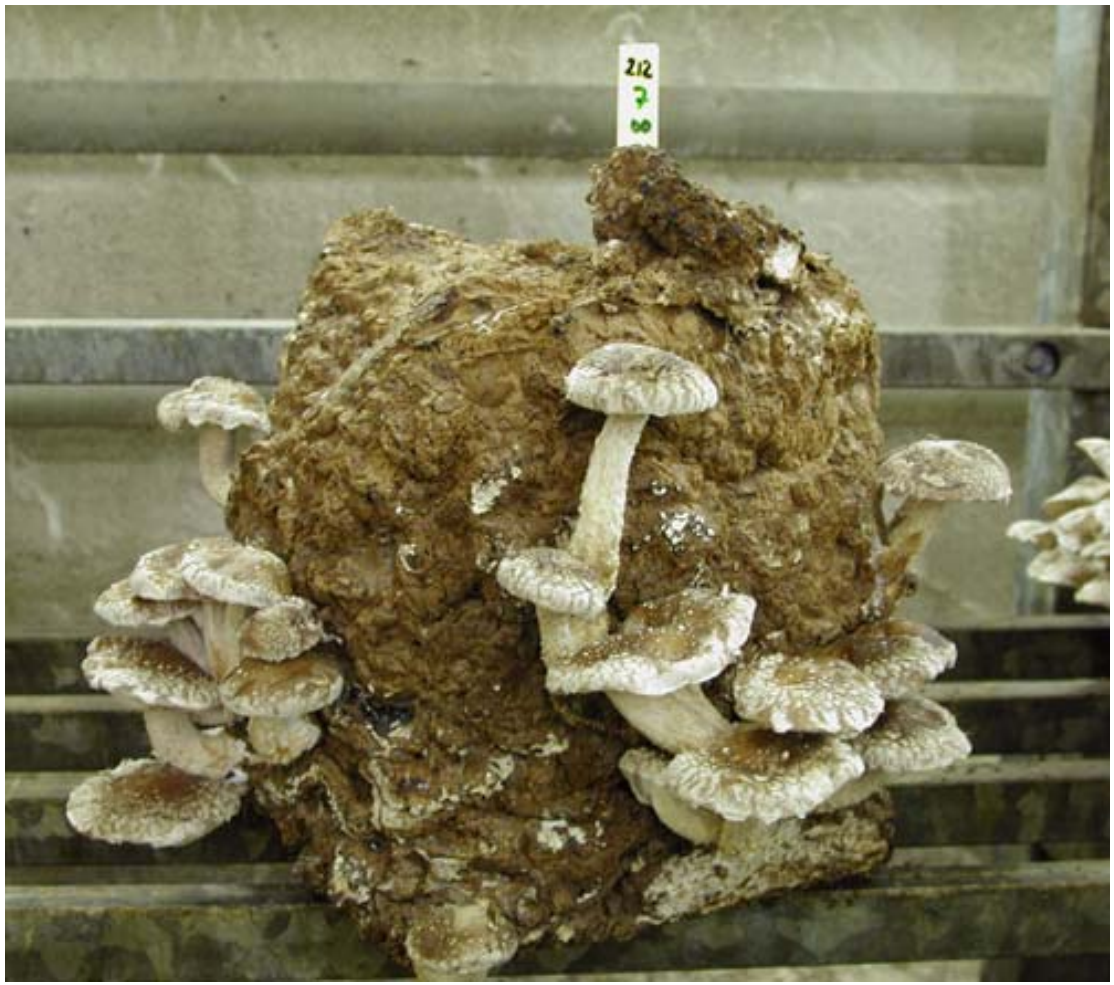
*: The logs were transferred after 7 weeks to the production room. The strain started to produce 5 weeks after spawning.

7* weeks vegetative growth; production at 16 ^{0}C.

Strain 6 (sh 02/05, 867, China). 5 Weeks after spawning the first mushrooms were produced. Plastic was removed. Bags transferred to production room 7 weeks after vegetative growth and picture is taken 32 days after transfer to production room.

*: The logs were transferred after 7 weeks to the production room. The strain started to produce 5 weeks after spawning.

12 Weeks vegetative growth; production at 16 ^{o}C



Strain 7 (sh 02/06, Japan), 10 days after removal of plastic bag.

16 Weeks vegetative growth; production at 16 ^{o}C

Strain 7 (sh 02/06, Japan), 7 days after removal of bag.

Strain 7 (sh 02/06, Japan), 9 days after removal of bag.

16 weeks vegetative growth; production at 16 ^{0}C

Strain 8 (sh 02/07, China), 7 days after removal plastig bag.

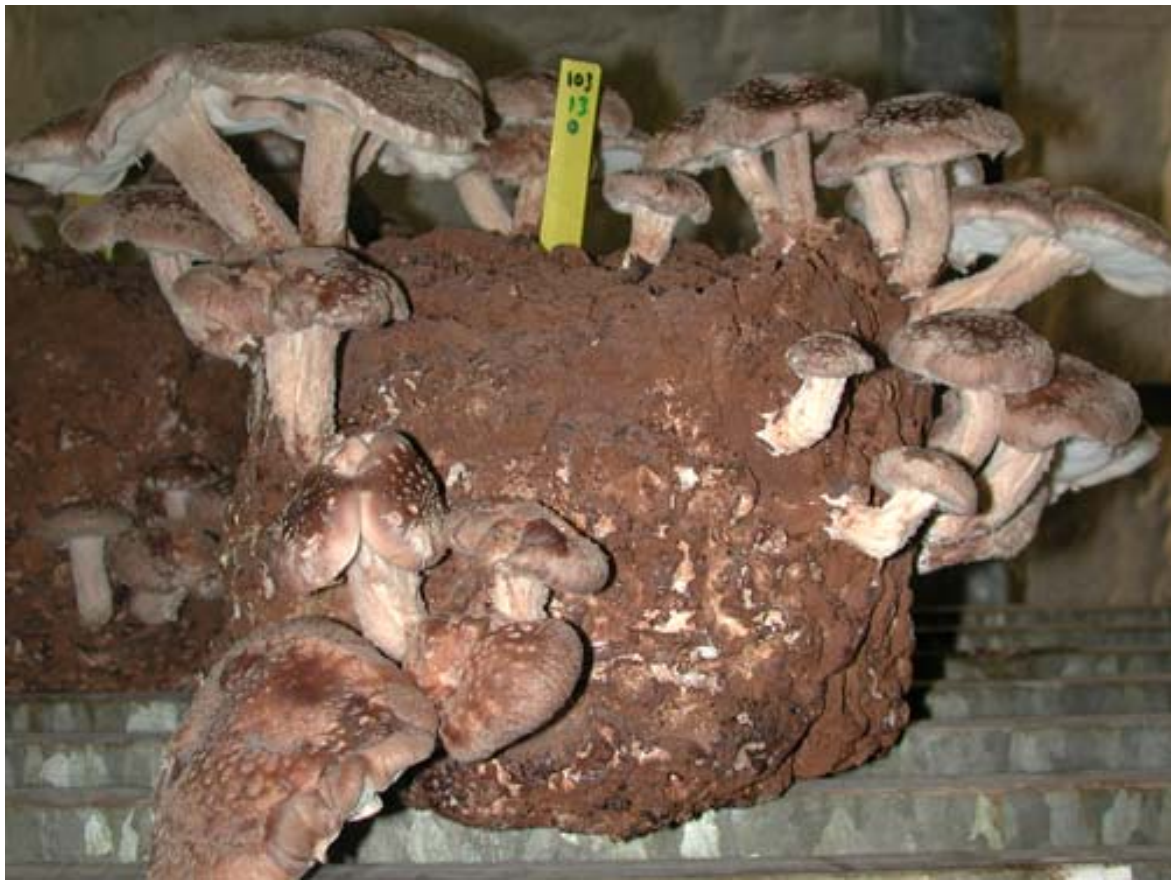
Strain 8 (sh 02/07, China), 9 days after removal plastig bag.

16 Weeks vegetative growth; production at 16 ^{0}C.

Strain 12 (sh 02/12, China) 7 days after removal plastic bag.

Strain 12 (sh 02/12, China) 9 days after removal plastic bag.

12 Weeks vegetative growth; production st 16 ^{0}C.



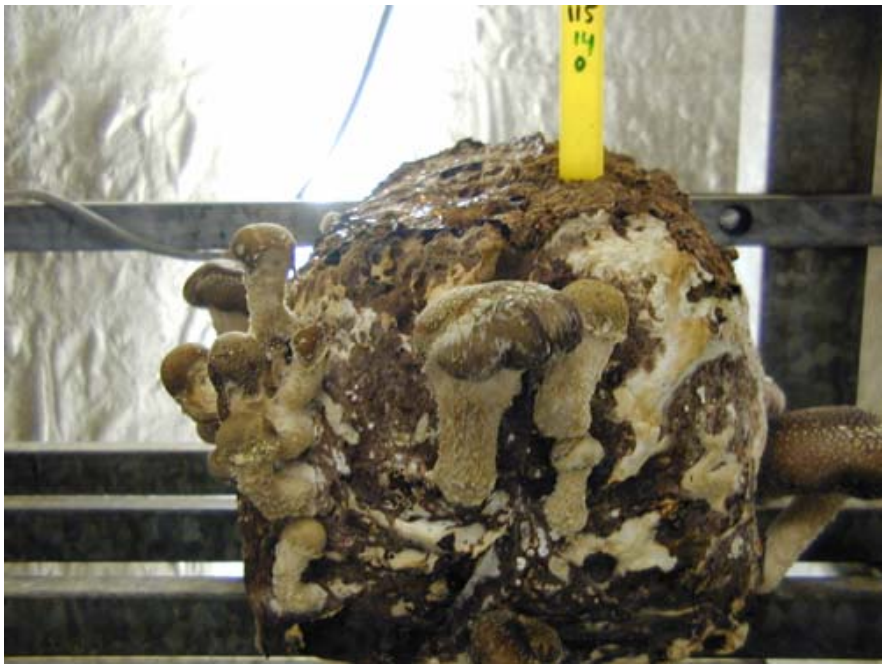
Strain 13 (sh 03/04 H600, Japan) 10 days after removalm plastic bag.

16 Weeks vegetative growth; production at 16 ^{0}C.

Strain 13 (sh 03/04 H600, Japan) 7 days after removal plastig bag.

Strain 13 (sh 03/04 H600, Japan) 9 days after removal plastig bag.

12 Weeks vegetative growth; production at 16 ^{0}C.



Strain 14 (sh 03/05 KV92, Japan) 14 days after removal plastic bag.

Strain 14 (sh 03/05 KV92, Japan) 17 days after removal plastic bag.

16 Weeks vegetative growth; production at 16 ^{0}C.

Strain 14 (sh 03/05 KV92, Japan) 10 days after removal plastic bag.

Strain 14 (sh 03/05 KV92, Japan) 15 days after removal plastic bag.

12 Weeks vegetative growth; production at 16 ^{0}C.

Strain 15 (sh 03/06, ML 8, Japan) 10 days after removal bag.

Strain 15 (sh 03/06, ML 8, Japan) 13 days after removal bag.

16 Weeks vegetative growth; production at 16 ^{0}C.

Strain 15 (sh 03/06, ML 8, Japan) 15 days after removal bag.

12 Weeks vegetative growth; production at 16 ^{0}C.

Strain 16 (sh 03/07 ML 12, Japan) 14 days after removal bag.

16 Weeks vegetative growth; production at 16 ^{0}C.

Strain 16 (sh 03/07 ML 12, Japan) 10 days after removal bag.

12 Weeks vegetative growth; production at 16 ^{0}C.

Strain 17 (sh 03/08 A567, Japan) 10 days after removal bag.

16 Weeks vegetative growth; production at 16 ^{0}C.

Strain 17 (sh 03/08 A567, Japan) 7 days after removal bag.

Strain 17 (sh 03/08 A567, Japan) 10 days after removal bag.

16 Weeks vegetative growth; production at 16 ^{0}C.

Strain 17 (sh 03/08 A567, Japan) 9 days after removal bag.

12 Weeks vegetative growth; production at 16 ^{0}C.

Strain 18 (sh 03/09, MM 1, Japan) 14 days after removal bag.

16 Weeks vegetative growth; production at 16 ^{0}C.

Strain 18 (sh 03/09, MM 1, Japan) 18 days after removal bag.

12 Weeks vegetative growth; production at 16 ^{0}C.

Strain 19, S600, commercial) 10 days after removal bag.

Strain 19, S600, commercial) 14 days after removal bag.

16 Weeks vegetative growth; production at 16 ^{0}C.

Strain 19, S600, commercial) 7 days after removal bag.

12 Weeks vegetative growth; production at 16 ^{0}C.

Strain 20 (sh 03/13 Somycel 4087, commercial) 10 days after Removal bag.

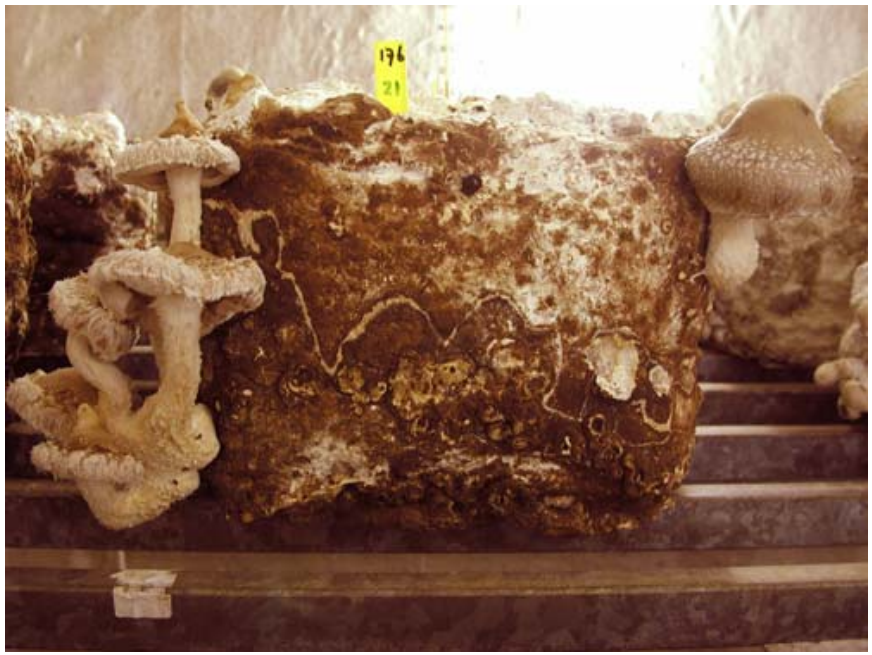
Strain 20 (sh 03/13 Somycel 4087, commercial) 10 days after Removal bag.

16 Weeks vegetative growth; production at 16 ^{0}C.

Strain 20 (sh 03/13 Somycel 4087, commercial) 7 days after Removal bag.

Strain 20 (sh 03/13 Somycel 4087, commercial) 9 days after Removal bag.

7* weeks vegetative growth; production at 16 ^{0}C.



Strain 21 (Mycelia 3782, commercial). 5 Weeks after spawning the first mushrooms were produced. Plastic was removed. Bags transferred to production room 7 weeks after vegetative growth and picture is taken 12 days after transfer to production room (12 weeks after spawning).

*: The logs were transferred after 7 weeks to the production room. The strain started to produce 5 weeks after spawning.

7* weeks vegetative growth; production at 16 ^{0}C.

Strain 21 (Mycelia 3782, commercial). 5 Weeks after spawning the first mushrooms were produced. Plastic was removed. Bags transferred to production room 7 weeks after vegetative growth and picture is taken 19 days after transfer to production room (12 weeks after spawning).

*: The logs were transferred after 7 weeks to the production room. The strain started to produce 5 weeks after spawning.

12 Weeks vegetative growth; production at 16 ^{0}C.

Strain 22 (Mycelia 3715, commercial) 10 days after removal bag.

16 Weeks vegetative growth; production at 16 ^{0}C.

Strain 22 (Mycelia 3715, commercial)
7 days after removal bag.

Strain 22 (Mycelia 3715, commercial)
9 days after removal bag.

12 Weeks vegetative growth; production at 24 ^{0}C.

Strain 1 (4B Su Xiang, China) 6 days after removal bag.

Close up.

16 Weeks vegetative growth; production at 24 ^{0}C.

Strain 1 (4B Su Xiang, China) 4 days after removal bag.

12 Weeks vegetative growth; production at 24 ^{0}C.

Strain 3 (sh 02/02, 2477, China) 10 days after removal bag.

Close-up.

16 Weeks vegetative growth; production at 24 ^{0}C.

Strain 3 (sh 02/02, 2477, China) 7 days after removal bag.

Strain 3 (sh 02/02, 2477, China) 9 days after removal bag.

16 Weeks vegetative growth; production at 24 ^{0}C.

Strain 8 (sh 02/07, China) 7 days after removal bag.

Close-up

12 Weeks vegetative growth; production at 24 ^{0}C.

Strain 20 (Somycel 4087) 6 days after removal bag.

Close-up

16 Weeks vegetative growth; production at 24 ^{0}C.

Strain 20 (Somycel 4087) 7 days after removal bag.

Close-up

2^{e}-flush, production at 16 ^{0}C.

Strain 1 (4B Su Xiang, China). Green molds.

2^{e}-flush, production at 16 ^{0}C.

Strain 3 (sh 02/02, 2477, China) green molds.