Elementary Research Report on the Cross Breeding of *Pholiota nameko*

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Abstract: We have studied on cross breeding of *Pholiota nameko* since 2000. Ninety-five crossed strains were identified by collecting spores from four parents, germinating the spores, cross breeding, antagonistic testing, and fruit body production *in vitro*. Strains No. 115 and No.64 were selected for small-scale testing and several basic points of repeat small scale test. Mono lump yield of No.115 is 1.61kg and 44.6% higher than the compare strain. Mono lump yield of No.64 is 1.54kg and 37.5% higher than the compare strain. Both of them possess good quality fruit bodies and stable heredity.

Key words: Antagonistic test, cross strain, dikaryotic mycelia, monokaryotic mycelia, *Pholiota nameko*, parental strain selection

1 Introduction

Pholiota nameko has been cultivated in China for more than 20 years and the country is now the world's largest producer with a total output exceeding that of Japan. However, our strains of *P. nameko* have been used for a long time and evidence various degrees of degeneration phenomena have appeared, leading to reduced output and poor mushroom quality. Since 2000, we have undertaken explorative studies on cross breeding in *P. nameko.* Two new strains, No.115 and No.64 were bred in 2003 and 120,000 bag cultures of strains 115 and 64 were cultured in 2004. The average yield per bag was more than 1.8 kg and 1.6 kg for strains 115 and 64, respectively. The average yield per bag was higher than the repetition of the small-scale test in the several basic points.

2 Materials and Methods

2.1 Selection of parent strains

Seven strains were selected as parents for the crossbreeding test. They produced good fruiting bodies in large-scale cultivation and showed clear differences with respect to properties. One wild *P. nameko* strain was also selected as a parent strain. Spores of only four of these parent strains germinated to produce a fungal colony. Essential features of these four parents are shown in Table 1.

Table 1. Comparison of properties of parent strains of *P. nameko*

Strain	Fruiting type	Growth type	Av. yield (g/bag)	Av. Wt. of fruit body (g)	Pileus color
Ph01	early ripeness	Grows thickly	1130	3.2	orange-tangerine
Ph13	early ripeness	Grows thickly	1250	2.1	tangerine
Ph11	very early ripeness	Grows singly	1310	1.9	orange
Ph08	early ripeness	Grows thickly	1330	2.6	orange-tangerine

Healthy and strong fruit bodies were harvested from the first flush of a culture bag when the pileus was about 80% opened. The fruit body was placed on a filter paper and spores collected and transferred to a test tube.

2.2 Spore germination

Spores suspensions were diluted to different spore densities, an aliquot of each dilution spread on to a dish containing potato dextrose agar (PDA), and the cultures incubated until the spores germinated and produced a fungal colony. The mono-colonies were collected and transferred to PDA test tube slants. Once the surface of the agar slant was completely covered, hyphae were taken and examined microscopically to identify monokaryotic mycelium.

2.3 Crossing of monokaryotic mycelium

Paired crossing was undertaken between monokaryotic mycelium according to a cross plan. Fifty-nine crossed pairs were observed microscopically. When two monokaryic mycelia came into contact with each other, hyphae from both parts of the junction line were observed microscopically. A piece of mycelium taken from areas of hyphal union was sub-cultured on to PDA slants. One hundred and eighteen cross tests pieces were examined altogether.

2.4 Antagonistic test

Dikaryotic mycelia taken from the area of hyphal union were antagonistic to the parent strains. These were determined as cross strains.

2.5 Observation of fruiting in the test tube

Strains exhibiting antagonistic reactions were sub-cultured on to PDA slants and, after the mycelium had covered the entire slant surface, the tubes were incubated at 15-20°C in diffuse light to induce fruiting. The fruiting ability of each strain was recorded.

2.6 Small-scale test

The small-scale test was divided into two steps. In the first step, we simulated fruiting test in logs of sawdust medium 55 × 35 × 5cm.The second step: Strains giving yields above 1000 gms were cultured at three locations in 2002. Each strain was cultured using ten bags.

2.7 Repetition of small-scale test at several locations

We increased the number of cultures for the strains selected in the small-scale test, observing and studying adaptability and stability. The test was carried out during 2003 in five villages located within the producing area. Cultured strains were: 115 (1640 bags), 64 (910 bags), 120 (1310 bags), 111 (1410 bags) 134 (960 bags) and CK (910 bags); a total of 7140 bags.

2.8 Quality appraisal

We sampled ten samples from each of the above strains during the second fruiting flush. Parameters measured were: pileus diameter, stipe length, stipe diameter, average fruit body weight, pileus colour and rate of expansion (quick or slow).

3 Result and Analysis

3.1 Identification of dikaryon mycelia

We examined 118 positions of 59 cross pairs and determined 116 positions with lock union.

3.2 Assessment of antagonistic phenomenon

The antagonistic test was carried out on 116 cross-culture mycelia with lock union. Ninety-five cross culture mycelia showed an antagonistic reaction to two parents, five cross culture mycelia were antagonistic to one parent but not to the other, and 14 cross culture mycelia exhibited obvious antagonism to one parent but less obvious antagonism to the other. Two cross culture mycelia showed no antagonism to either parent. Therefore, producing the antagonism rate was 81.9%. We regarded 95 cross culture mycelia showing the antagonistic phenomenon to parents as cross strains.

3.3 Fruiting in the test tube

Ninety-five strains fruited under one or more of the temperature and light regimes applied in this experiment. However, there was considerable variability with respect to the number of fruiting bodies, size and colour that reflected the diversification of the different parental crosses.

3.4 Results of small-scale test

Among the 95 strains tested, many grew slowly and were discarded because of contamination with other fungi. Fifty strains fruited normally in the culture lumps. Ten strains produced yields in excess of 1000 gm. These included: No.133, 1200g; No.96, 1200g; No.92, 1300g; No.105, 1850g; No.64, 1300g; No.101, 1050g; No. 131, 1150g; No.120, 1050g; and No.115, 1080g. Small scale testing of ten strains was initiated 13th March, 2000. Each strain grew normally after sowing the mycelia but strains Nos.133, 96, 92, 105, 112, 131 and No. 101 produced fruiting bodies in the summer. Tests on these strains were terminated due to sundry fungal infections. Only strains 115, 64 and 120 were conserved from spring to autumn. Their average yields were 970g, 869g and 707g, respectively. Three strains produced two flushes of fruit bodies because of cold current. Therefore, the yield of each strain was lower in the small-scale test. Moreover, Strains 111 and 134 fruited early in the institute fruiting test and their average yield per lump was 1303g and 1035g, respectively. The small-scale test for these two strains together with strains 115, 64 and 120 was repeated at several locations.

3.5 Repeat of small-scale test at different locations

3.5.1 Mycelia growth and culture bag conservation rate

Mycelium inoculation was carried out between 3-12 March, 2003 at each location. Mycelia growth was assessed during the first ten days of May, and the bag conservation rate (i.e. number of original bags that have developed normally and have not become contaminated) was investigated during the last ten days of August (Table 2). The results show that strains 115 and 64 grew faster than CK and other strains, and the bag conservation rate was also higher in the case of these strains.

Table 2. Mycelia growth and lump conservation rate

Strain	Bag inoculation date	Full colonisation date	Growing period (days)	No. of bags inoculated	No. of bags conserved	Bag conservation rate (%)
115	10/03/03	04/05/03	56	1640	1624	99.0
64	10/03/03	05/05/03	57	1080	1072	99.3
120	11/03/03	05/05/03	56	1310	1292	98.6
111	12/03/03	09/05/03	59	1410	1354	96.0
134	12/03/03	09/05/03	59	960	920	95.8
CK	11/03/03	07/05/03	58	910	874	96.0

3.5.2 Yields derived from three repetitions (I-III) of the small-scale test
The data are shown in Table 3.

Table 3. Yields derived from three repetitions of the small-scale test

Strain	No.115	No.64	No.134	No.111	No.120	CK
I	1.62	1.46	1.19	1.09	0.87	0.99
II	1.67	1.78	1.15	1.12	1.05	1.17
III	1.53	1.38	1.08	0.93	1.10	1.21
Average value	1.61	1.54	1.14	1.05	1.01	1.12

Values are expressed as kg.

An analysis of variance carried out on data shown in Table 3 is presented in Table 4. The results reveal a marked difference between the strains. The rate of growth of strains 115 and 64 is 44.6% and 37.5%, respectively.

Table 4. Analysis of variance on the average yield of five strains of *P. nameko*

Source of dispersion	Degrees of Freedom	Sum of Squares	Mean square	F value	F 0.01
Treatments	t-1=5	1.0193	0.2039	13.32**	5.06
Error	t(r-1)=12	0.1836	0.0153		
Total sum	rt-1=17				

3.6 Appraisal of fruit body quality

Table 5 shows the results of the appraisal of fruit body quality for five strains and strain CK in 2003. The data show that the stipe diameter, average pileus diameter and average weight of fruit body for strain 115 was generally higher than for other strains. However, the stipe length was shorter than in the case of CK and other strains.

Table 5. Survey and appraisal of fruit body quality from cross-breeding strains

Strain	Average pileus diameter (mm)	Stipe length (mm)	Stipe diameter (mm)	Average wt. per fruit body (gm)	Pileus color	Easy or non expansion
115	19.8	17.7	8.6	2.8	orange-tangerine	Non
64	17.8	24.0	7.8	2.7	orange	Non
120	17.6	19.1	8.6	2.5	tangerine	Easy after the Second flush
134	16.0	25.0	6.0	2.2	orange	Easy
111	18.6	32.8	6.8	2.1	tangerine	Easy
CK	18.1	23.9	9.1	2.6	orange-tangerine	Non

3.7 Analysis of affects of cross breeding in *P. nameko*

In this test, the affinity rate between the different parents was 81.9%. The characteristics of each strain were variable. Five strains were diverse with respect to yield and shape. Strains 115 and 64 possess bigger pilei, thicker stipes, and the average fruiting body weight was higher. Strain 115 was bred through crossing wild strain, ph01, and the earliest strain, ph11. Strain 115 retained strain ph11's characteristic of precocity, and also showed the thick growth and less easy expansion characteristics of the wild strain. Strain 64 was bred by crossing strains Dan hua 8 (Strain CK) and ph13. Strain 64 produced the same healthy and strong mycelia, hemispheric fruit body and high yield that are characteristic of Dan hua 8, but also the thick mycelial growth, fruit body density and less easy expansion of strain ph13. The shortcoming of strain 115 is the short stipe of fruit bodies in the second flush. The pileus of strain 64 is also slightly small.

4 Discussion

The results of this study revealed an affinity rate of 81.9% among the different parents. Monokaryotic mycelium from different parents possessed a higher affinity. Crossed strains exhibited bigger differences in fruiting times, fruit body shape, growing state, yields, and resistance to disease. Cross generations showed diversity, and the selective range was enlarged. Although the life cycle of *P.nameko* is complex, the selected strains expressed more stable heredity. Therefore, cultivating fine strains *P .nameko* is feasible by means of cross breeding.

Normally, crossed mycelia are obtained from the contact position of the two parents. We obtained crossed mycelia from both place of the contact position, thereby enlarging the selective range and increasing the purity of the strains, and decreasing the probability of variation during preservation and reproduction.

By studying cross breeding for four years, we have bred two strains, Nos.115 and 64. Their average one bag yield is 1.61 kg and 1.54 kg, respectively when grown at several different locations. Based on results from repeat small-scale tests, yields of strains 115 and 64 were 44.6% and 37.5% higher, respectively than the standard strain (Dan hua 8). Both 115 and 64 possess good qualities and stable heredity.