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Varietal Improvement of the Oyster Mushroom, *Pleurotus ostreatus* for Commercial Cultivation in Northern Thailand

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Synopsis

Selected F_1 hybrids between a high-yielding stock of *Pleurotus ostreatus* and a stock tolerant of high temperature, both collected at the growers' farms in Northern Thailand, were crossed again with three such stocks selected from other hybrids between Thai and Japanese commercial stocks. From the hybrid population, four superior stocks were selected for commercial cultivation. Two were suitable for commercial cultivation at fluctuating temperatures 25°C or more in Thailand and the other two were suitable for commercial cultivation in air-conditioned rooms (lower than 20°C). This paper describes these results obtained by a cooperative project between Chiang Mai University (Thailand) and Kinki University (Japan) in 1995.

I Introduction

A cooperative project between Chiang Maj University (CMU) and Kinki University (KKU) has been in pregress for the past 6 years to advance agricultural technology in Northern Thailand. Some objectives of the project were to transfer breedjng technology for cultivated mushrooms from Japan to Thailand and to make available new varieties of mushrooms suitable for farmers' production in Northern Thailand. Workers at CMU had been making efforts to produce new varieties of oyster mushroom, *Pleurotus ostreatus*, before this project. However, results were not yet satisfactory. In the project, we have undertaken the introduction of Japanese mushroom germplasm into Thai varieties (Kinugawa *et al.*, 1996; Morimoto *et al.*, in preparation), and finally released new varieties promising for production in Thailand, because of their high yield and their tolerance of high temperatures. Here, the breeding procedure and results will be reported.

II Materials and Methods

Fungal stocks:

1. Varieties from CMU (CM1 and CM5).

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CM1 is a high-yielding variety that fruits at high temperature (25°C or more), producing whitish fruit-bodies. CM5 is a high-yielding variety at temperatures from 10°C to 18°C. Fruit-bodies are dark gray at the top and they form clusters. Pilei are broad (4.8-8.0 cm).

2. Varieties from KKU (KD1-KD3).

The KD varieties are high-yielding at wide range of temperatures lower than 20°C. They were selected at KKU from among F_1 hybrids between Japanese native MA91 and Thai NW89.

KD1 : mW26×mMA16
KD2 : mW30×mMA17
KD3 : mW43×mMA17

mW26, mW30, and mW43 are monokaryotic strains derived from NW89, and mMA16 and mMA17 are monokaryotic strains derived from MA91. NW89 is a high-yielding commercial variety adapted to the heat of tropical in Thailand (Kinugawa *et al.*, 1994, 1996). Fruit-bodies have whitish to pale brown tops and have a slightly tough texture. Most of the crop is harvested in several flushes. MA91 is a high-yielding commercial variety adapted to temperate climates. The largest part of the crop is harvested at the first flush. The fruit-bodies have a blackish top that turns dark gray with age, and the texture is slightly soft.

Culture media : Subculture and crossings of monokaryotic strains and dikaryotic stocks were done on PDA or MYP medium (Kinugawa *et al.*, 1994, 1996) in slants at 25°C.

Crossing : Parental monokaryotic strains were used to inoculate on opposite sides of a plate in a 9-cm petri dish. The margins of both colonies grew together, and a week or more later, we transplanted a tiny mycelial mass onto a slant and left it to grow.

Estimation of dikaryotization : Dikaryotization was taken to have occurred when clamp connections in mycelia were detected under a microscope.

Cultivation : Japanese and Thai parental stocks and their hybrids were cultivated on a sawdust substrate at CMU. The substrate (a mixture of 96 g of rice bran, 7.2 g of sugar, and 184 g of para rubber tree sawdust (with 64% water) was put into a 16.3×31.3 cm polypropylene bag with a mouth 3 cm across plugged with cotton. The bag was sterilized in hot steam at nearly 100°C in a petroleum dram for 4 to 6 hours. Spawning was done with wheat grain spawns in a clean-room. The stocks were cultivated in a well-aerated mushroom shack at temperatures 25°C or more or an air-conditioned cultivation room at lower than 20°C.

III Results

Cultivation of parental stocks.

1. Number of days needed for completion of spawn-run.

The mean number of days needed for the completion of spawn-run in bags was 36 days for KD1 and KD3, 40 days for KD2, and 32 days for CM1.

2. Mean number of days from spawning to harvest.

From spawning to harvest, KD1, KD3, and CM1 took 48, 62, and 38 days, respectively.

3. Pileus characteristics.

Size : KD1>KD3>CM1.
Color of top : KD1 and KD3 were darker than CM1.
Quality of texture : The texture of KD1 was of hjgh quality, that of KD3 was intermediate, and that of CM1 was low.

4. Yield during 30-day harvesting period.

The yields in cultivation are summarized in Table 1. The yield of KD1 per bag was nearly

Table 1. Yield of parental stocks

	KD1	KD3	CM1
No. of bags cultivated	50	60	60
No. of bags that fruited	11	26	47
% of bags fruited	22	43	78
Total yield (g)	851	1712	3458
Yield (g/bag fruited)	77	66	74

Table 2. Results of di-mon-crossing between Japanese and Thail stocks

Nuclear recipient		(mF_1d-1-n)						(mF_1d-3-n)			(mF_1d-5-n)	
		−1	−2	−7	−9	−11	−12	−1	−3	−4	−8	−9
Nuclear	KD1	+	+	+	−	+	+	+	+	−	+	+
donor	KD2	+	+	−	+	−	+	−	+	−	+	−
	KD3	+	+	−	+	+	+	−	+	+	+	+

+, with clamp connections; −, without clamp connections.

Table 3. Fruit-body yield of Thai-Japanese hybrid stocks in cool conditions and at room temperatures*.

hybrids	cool	room	hybrids	cool	room
mF_1d-1-n(xKD1)			mF_1d-1-n(xKD3)		
n= 7	+++	++	n= 1	+	+++
11	+++	+	2	+	+
12	++	+++	7	+	+
			9	+	+
mF_1d-3-n(xKD1)					
n= 1	++	++	mF_1d-3-n(xKD3)		
3	++	++	n=11	++	+
			12	++	+
mF_1d-5-9(xKD1)	+	+			
			mF_1d-5-9(xKD3)	+	+−
mF_1d-1-n(xKD2)					
n= 2	+	−			
2	+	−			

* Cool, at temperatures lower than 20°; room, at temperatures 25°C and more.
+++, high; ++, medium; +, low; −, no fruit-bodies.
Note: In $mF_1d-1-12$(xKD1); m means "monokaryotic", and F_1d means "mF_1 progeny after di-mon-crossing"; thus, $mF_1d-1-12$ is a nuclear recipient monokaryon, and KD1 is a dikaryotic nuclear donor in the di-mon-cross.

equal to that of CM1. However, the percentage of the total number of bags used that had fruit-bodies within 30 days was higher for CM1 than for KD1.

Di-mon-crossing between parental stocks.

1. CM1 was di-mon-crossed with the monokaryotic CM5.

Nineteen monokaryotic strains (mCM5s) were isolated through basidiospore dilution culture

of CM5 and di-mon-crossed with CM1. Among them, only 10 cross-combinations synthesized dikaryons, and five of these formed fruit-bodies. Three of the five, F_1d-1, -3, and -5, were selected for further studies. Monokaryotic strains were again obtained from the three F_1d strains: 12 mF_1d-1s, 8 mF_1d-3s, and 13 mF_1d-5s.

2. Di-mon-crossing between KD and mF_1d strains.

As monokaryotic parents, six mF_1d-1 strains (-1, -2, -7, -9, -11, and -12) and five mF_1d-3 strains (-1, -3, -4, -8, and -9) were selected. They were di-mon-crossed with KD1, KD2, and KD3. Among 33 cross-combinations, 24 combinations synthesized dikaryons (Table 2). Of the 24 synthesized stocks, 15 fruited at 15°C to 20°C in an air-conditioned room, and 11 fruited at fluctuating room temperatures 25°C or more. The results are shown in Table 3 with symbols based on the cross-combinations. mF_1d-1-12(×KD1) and mF_1d-1-1(×KD3) had high yield in fluctuating room temperatures higher than 25°C, and mF_1d-1-7(×KD1) and mF_1d-1-11(×KD1) were suitable for cultivation in an air-conditioned room lower than 20°C. The hybrids that fruited at lower temperatures tended to have darker tops than the hybrids that fruited at higher temperatures.

IV Discussions

As reported previously (Kinugawa *et al.* 1989, 1996), Thai commercial stocks of oyster mushrooms can fruit at temperatures fluctuating between 20°C and 35°C, and fruiting usually begins before the spawn-run ends. The texture of the fruit-bodies is rough and the top is whitish. Farmers using these stocks in Northern Thailand harvest the crops separately in several flushes. In contrast, Japanese commercial stocks currently in use fruit most plentifully at temperatures from 13°C to 15°C, and Japanese farmers usually harvest only in the first flush. The texture of the fruit-bodies is soft, and the pilei are circulary and uniform in shape, with smooth edges, and tops are blackish when young. Fruiting begins just after the spawn-run ends. The darker pileus color in Japanese stocks may be linked with the low-temperature fruiting and high-quality texture, and the whitish color in Thai stocks may be linked with tolerance of heat and an inferior texture. CM1, which fruits at a high temperature, has a rough texture and whitish pileus, and Japanese stocks that fruit at a lower temperature, have a high-quality texture and dark to blackish pilei. CM5 resembles the Japanese stocks. In other words, the optimum fruiting temperature, fruiting habit, texture, and pileus color seem to be linked. Morimoto *et al.* (in preparation) descrived such linkages as being genetically segregated in the F_2 hybrid population between Japanese and Thai stocks. In this study, the Japanese-Thai di-mon hybrids in most cases had combinations of characteristics that were consistent with these genetic linkages. The stocks selected here for Thai farmers were mF_1d-1-12(×KD1) and mF_1d-1-1(×KD3), which have the phenotypes of recombinants in their high-yield at temperatures 25° or more, in their regular-shaped fruit-bodies with high-quality texture and a whitish top color, and in their fruiting in several flushes with the first flush beginning before the spawn-run ends. These stocks could become leading commercial stocks in Thailand. (Note: In another article in this issue (Kinugawa *et al.* 1996), the cross-compatibility between Thai stock, (NW89), and a Japanese stock, PN87, is discussed, and the origin of Thai stocks is discussed from the viewpoint of phenotypic relationships with *Pleurotus* sp. Florida by Eger (1965, 1970)).

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北部タイでの商業的栽培に適したヒラタケ（*Pleurotus ostreatus*）菌株の品種改良

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要　　約

北部タイで生産者の栽培舎から栽培ヒラタケを採取し，その中から高温環境下でよく発生し多収性である菌株を選び交雑して優れたF1雑種菌株を得た。一方，タイ国産栽培用菌株と日本産栽培用菌株間で交雑し同様に高温環境下でよく発生し多収性のF_1雑種を得た。これら2種のF_1菌株間の交雑から商業栽培に適した4種の雑種菌株を得た。このうち，2種はタイ国の25°C以上の変温環境における商業栽培に適していた。他の2種は空調室（20°C以下）を使った商業栽培に適していた。以上は，近畿大学とチェンマイ大学間の協同研究における1995年度の成果である。