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Cross-compatibility between Thai and Japanese Oyster Mushrooms and the Inheritance of Fruiting Habits

Kenjiro KINUGAWA*, Eiji TANESAKA*, Akira NAGATA* and Kazuo WATANABE**

Synopsis

Full cross-compatibility was confirmed in di-mon mating between cultivated stocks of Japanese *Pleurotus ostreatus* and a Thailand *Pleurotus* sp. still taxonomically unidentified. The characteristics of the Thai stock used are discussed, and the conclusion reached that it is probably identical to *Pleurotus* sp. Florida. Hybrid stocks are equal to or superior to their parents in fruit-body yield in cultivation. The Japanese parent used may be useful to increase the yield of the hybrids through heterosis. Fruiting habits including fruiting ability at a high temperature (25 C) and some fruit-body characteristics evidently were passed to the hybrids from the Thai parent, and the dark top color of the pileus originated from the Japanese parent. These findings suggest that Japanese commercial stocks of *Pleurotus ostreatus* have breeding characteristics suitable for use in improvement of the Thai stock.

I Introduction

Oyster mushrooms (*Pleurotus ostreatus*, *P. pulmonarius*, *P. abalonus*, and *P. sajor-caju*) are widely cultivated in Thailand. From 1989 to 1995, joint expeditions were made in Northern Thailand by a mission sent by Kinki University (KKU), Japan, and Chiang Mai University (CMU), Thailand. Many commercial stocks of oyster mushrooms were collected, and the collections were studied for physiological traits, with special attention to the fruiting response to temperature, in the laboratories of both universities. Some of the results of the expeditions have already been published (Kinugawa *et al*. 1989, 1991, and 1994). *P. ostreatus* also is widely cultivated in Japan. However, the fruiting response of Thai commercial stocks to temperature is different from that of Japanese stocks: the Thai stocks are adapted to a tropical or subtropical climate, with few being wild species, and the Japanese stocks are adapted to the temperate zone, in which wild species usually grow in the cool autumnal season.

Here, the cross-compatibility between a Thai commercial stock of *Pleurotus* sp. and a Japanese commercial stock of *P. ostreatus* is studied, and their fruiting habits are compared mainly from the viewpoint of breeding.

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* Lab. of Genetics and Plant Breeding, Faculty of Agriculture, Kinki University, Nakamachi, Nara 631, Japan (衣川堅二郎, 種坂英次, 長田 彰, 農学科遺伝育種学研究室)
** Forest Experiment Station of Nara Prefecture (渡辺和夫, 奈良県林業試験場)

II Materials and Methods

Materials used. PN87 is a commercial stock widely cultivated in Japan. In commercial cultivation, the spawn usually run at 20°C to 23°C. In a week after the end of the spawn-run, exhausted spawn are removed (Kinkaki), the cultures are transferred to a fruiting room at 15°C, and fruiting occurs in clusters. The top of the fruit-body is blackish at a young stage. Most of the crop is obtained at the first flush. The optimum temperature for mycelial growth is 24-25°C. This stock was obtained from the Naganoken Nōson-Kōgyō Kenkyūsho (Institute for Research into Rural Industries in Nagano Prefecture).

NW89, a commercial stock of Thailand, was purchased in Bangkok, Thailand. The cultures, in polypropylene bags, are usually placed in mushroom shacks under alternating temperatures (10-28°C in winter, 23-30°C in summer, with a maximum of 34°C, in Chiang Mai ; Kinugawa *et al.*, 1989). Fruiting begins with the spawn-run and several flushes follow. The fruit-bodies are usually pale brown to whitish when young. The optimum temperature for mycelial growth is about 25°C. This kind of oyster mushroom is cultivated widely in Thailand.

Culture medium. MYP medium (Bandoni and Johri, 1972), used for subculture and monospore isolation, consisted of 7 g of malt extract (Difco), 0.5 g of Soytone (Difco), 0.1 g of yeast extract (Difco), and agar (15 g for slants and 10 g for plates) in 1,000 ml of distilled water. The medium was sterilized at 120°C for 15 min. All stocks and strains were subcultured on slants at 25°C.

Di-mon mating. mPNn (n=1 to 18) were monokaryotic isolates isolated by the dilution plating method of basidiospores taken from a spore print of a fruit-body of PN87. Eight isolates were selected at random and used for di-mon mating with NW89. Mycelia of an mPNn were planted on one side of a plate and several days later mycelia of NW89 were plated on the other side. The margins of both colonies grew together, and a week or more later, a tiny mycelial mass was taken from the monokaryotic colony, transplanted onto a slant, and allowed to grow afterwards. The mycelia on which clamp connections were microscopically detected were assumed to be dikaryotized (F_1d's).

Cultivation and cultivation substrate. For comparison of fruiting abilities, F_1d's and their parental stocks were cultivated at 25°C and 80-90% RH in an air-conditioned cultivation room of the Forest Experiment Station of Nara Prefecture. The process of cultivation was the same as that for PN87 (see Materials and Methods). A mixture of sawdust and rice bran in a ratio of 4 : 1 (v/v) with 65% water content was prepared in 800 ml polypropylene bottles, sterilized for 60 minutes at 120°C, and used as the cultivation substrate.

III Results

Di-mon mating. NW89 could dikaryotize all of the monokaryotic mPN's used, suggesting a close relationship between NW89 and PN87 (Table 1).

Fruiting abilities of F_1d progenies. In preliminary experiments, at a constant temperature (14-15°C), both PN87 and NW89 were capable of normal fruiting, but at a constant temperature of 25°C or more, the fruiting occurred only for NW89. NW89 fruited under a wider range of temperatures than PN87. Accordingly, for comparison of the fruiting abilities of PN87, NW89, and their hybrids (F_1d's) large-scale cultivation was done at a constant temperature of 25°C in this experiment. NW89 and six of the 10 F_1d's examined produced normal fruit-bodies ; PN87 and the four other F_1d's did not. Table 2 shows the results for the first two harvests in cultivation. Fertile stocks were characterized by even fruiting in several flushes, and with moderate fruiting even at high temperatures. Fruit-body yields of F_1d hybrids were approximately equal to or higher than

Table 1. Success of di-mon matings

dikaryon	monokaryon	results
	PN87- 1	+
	- 2	+
	- 3	+
	- 5	+
NW89	- 8	+
	- 9	+
	-10	+
	-11	+
	-12	+
	-14	+

+, dikaryotization

Table 2. Results of cultivation

Hydrid and parent	First flush			Second flush			Total yield (g/bottle)	Color of top
	Yields (g/bottle)	SE	Days to harvest	Yields (g/bottle)	SE	Days to harvest		
F_1d-1	64.0	5.8	25.5	52.5	7.4	29.7	116.5	*c*
-2	-			-			-	
-3	71.6	4.2	22.2	48.6	4.7	30.5	120.2	*cc*
-5	58.1	5.7	23.9	56.3	9.4	29.9	114.4	*cc*
-8	43.8	5.4	24.6	68.8	6.4	31.9	112.6	*ccc*
-9	73.9	5.3	23.0	31.6	3.4	30.7	105.5	*cc*
-10	-			-			-	
-11	-			-			-	
-12	28.2	3.3	25.9	68.7	9.6	34.1	96.9	*cc*
-14	-			-			-	
PN87	-			-			-	
NW89	57.4	5.2	24.1	43.1	4.1	29.9	100.5	*c*

Mean of 10 replicates, fresh weight. -, no fruiting.
SE, standard error of the mean.
Color of the top: *c*, almost white; *cc*, whitish-gray; *ccc*, pallid gray.

those of their Thai parent, NW89. The four sterile hibrids and PN87 fruited in another experiment at lower temqeratures. The fruit-bodies had some variations in pileus color in different hybrids, ranging from white to pallid gray. Any large fruit-bodies that developed were fully fertile, and dispersed many basidiospores.

IV Discussion

European and Japanese stocks of *Pleurotus ostreatus* are adapted to temperate climatic zones, and the optimum temperature for fruiting is 15°C or less. The stocks cultivated in Thailand are

adapted to the subtropical and tropical zones, and fruiting takes place easily at 25°C or more, at which temperatures fruiting of the Japanese stocks is completely suppressed. Jacquat and Bertossa (1990) reported that Thai *Pleurotus* sp. was introduced from Bhutan for commercial production, but it is difficult to believe that a stock adapted to a hot climate was native to the cool hilly highlands of Bhutan. Physiological traits of *Pleurotus* sp. from Florida (*Pleurotus* sp. Florida) have been reported by Eger (1965, 1970, 1976, and 1978) and by Zadražil (1978). It can fruit at high temperatures, up to 26-27°C, and its fruit-bodies have a somewhat trumpet-like shape (cf. the photograph in Eger, 1976) and have a pileus of pallid yellow or white (Zadražil, 1978). According to Eger (1976), *Pleurotus* sp. Florida has spread among mushroom growers throughout the world. The physiological and morphological traits of Thai stocks appear to resemble those of *Pleurotus* sp. Florida.

Eger *et al.* (1976) proved that the European *P. ostreatus* is cross-compatible with *Pleurotus* sp. Florida, although crossability is not great. Bresinsky *et al.* (1987) reported full cross-compatibility (interbreedability) between stocks of *P. ostreatus* from Germany and Japan. In our study, a stock of Japanese *P. ostreatus*, PN87, proved to be highly cross-compatible in di-mon mating with the Thai NW89. All of these stocks should be included in the species *Pleurotus ostreatus*. NW89 may be a stock of *Pleurotus* sp. Florida which was originally introduced to Thailand through Bhutan or Taiwan.

The F_1d hybrids gave higher fruit-body yields than their parents, indicating a good breeding potential for hybridization. The Japanese stock may help increase the yields of the hybrids through heterosis. Fruiting pattern (separate harvesting in several flushes), fruit-body shape (slightly trumpet-shaped), texture (a little hard and rough), and the whitish top of fruit-bodies evidently were passed to the hybrids from NW89 rather than from PN87. The darker top color was probably from PN87. The breeding potential of Japanese stocks will be useful in the improvement of Thai stocks, because in the current cultivation process, Japanese growers prefer to harvest only in the first flush for economic reasons, and most Japanese prefer fruit-bodies with a blackish top and soft feeling to the tongue.

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タイ国産ヒラタケと日本国産ヒラタケの間の交雑和合性ときのこ形成特性の遺伝

衣川堅二郎，種坂英次，長田　彰，渡辺和夫

要　約

日本産ヒラタケ（*Pleurotus ostreatus*）商業菌株と従来未同定であったタイ国産ヒラタケ商業菌株の間には，ダイーモン交雑をしたとき十分な交雑和合性のあることが確かめられた。そして，タイ国産ヒラタケ菌株の特長について，それがアメリカ合衆国フロリダ州を原産地とし世界へ広まっているヒラタケ（*Pleurotus* sp. Florida）であろうとの見地から考察した。両者間の雑種菌株は，栽培したとき，きのこ収量において両親と同等かより優れていた。ここで使用した日本産菌株は雑種強勢を通じて雑種の多収性に寄与しているもののようであった。また，雑種の，高温下で発茸する能力やいくつかの子実体形質などの特長は明かにタイ国産菌株に，菌傘表面が暗色になる形質は日本産菌株に起源するものであった。以上の結果は，使用した日本産ヒラタケ商業菌株がタイ国産商業ヒラタケ菌株の特性の改良に寄与することを示している。