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Progress Report (1991-1993) of the Joint Research Program of Kinki and Chiang Mai Universities on the Promotion of Mushroom Research

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Inhabitants of minor tribes (hill people) in hilly areas along the Myanmar border of northern Thailand in particular often cultivated poppy plants, and earn money by the illegal production and trading of large amounts of opium. The opium is then illegally exported to all parts of the world, giving rise to immeasurable social difficulty (Walker, 1986). Thai people face another serious problem in agriculture: Impoverishment of rice farmers that has resulted from a recent drastic decrease in the price of rice.

A Joint Research Program by Kinki University (Japan) and Chiang Mai University (Thailand) was started with the belief that promotion of other profitable agricultural technolgies or new varieties of crops, if adopted by small farmers, will contribute to efforts in Thailand to resolve the current problems. The final goal of our efforts are 1) the eradication of poppy cultivation by replacement of poppy with other crops, and 2) the rescue of impoverished rice farmers by the introduction of profitable legal crops to increase their net income. Any success with our second goal should facilitate the achievement of our first goal.

The staff listed below has undertaken research on the improvement of mushroom cultivation jointly and separately in our two universities.

Kinki University:	Dr. Kenjiro Kinugawa (Professor, Laboratory of Genetics and Plant Breeding, Faculty of Agriculture)
	Mr. Shuzo Fukada (Research Associate, Laboratory of Genetics and Plant Breeding, Faculty of Agriculature)
	Mr. Eiji Tanesaka (Research Assistant, Laboratory of Genetics and Plant Breeding, Faculty of Agriculture)
	Miss. Mieko Okada (Research Assistant, Laboratory of Genetics and Plant Breeding, Faculty of Agriculture)
Chiang Mai University:	Dr. Wichian Phusawang (Associate Professor, Faculty of Horticulture)
	Dr. Nuchnart Jonglaekha (Associate Professor, Faculty of Plant Pathology)
	Mr. Smarn Chinbenjaphol (Lecturer, Faculty of Horticulture)

Mushrooms, including shiitake (*Lentinus edodes*), oyster mushrooms (*Pleurotus* spp.), and the common mushroom (*Agaricus brunnescens*), are now cultivated in Chiang Mai and Chiang Rai Provinces, and sold at retail shops in Bang Kok, Chiang Mai, Chiang Rai, and in other cities as well as in country districts. Leading growers are growing mushrooms on a large scale in several or sometimes more than ten mushroom houses, but there are still many small-scale growers in these provinves. Prices of mushrooms are as a rule (Table 3), higher than those of other

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vegetables, but undeveloped technology and unadapted spawn have prevented much further increases in cultivation. Our main objective, at present, is to introduce more efficient mushroom cultivation to small-scale farmers in villages and the hills.

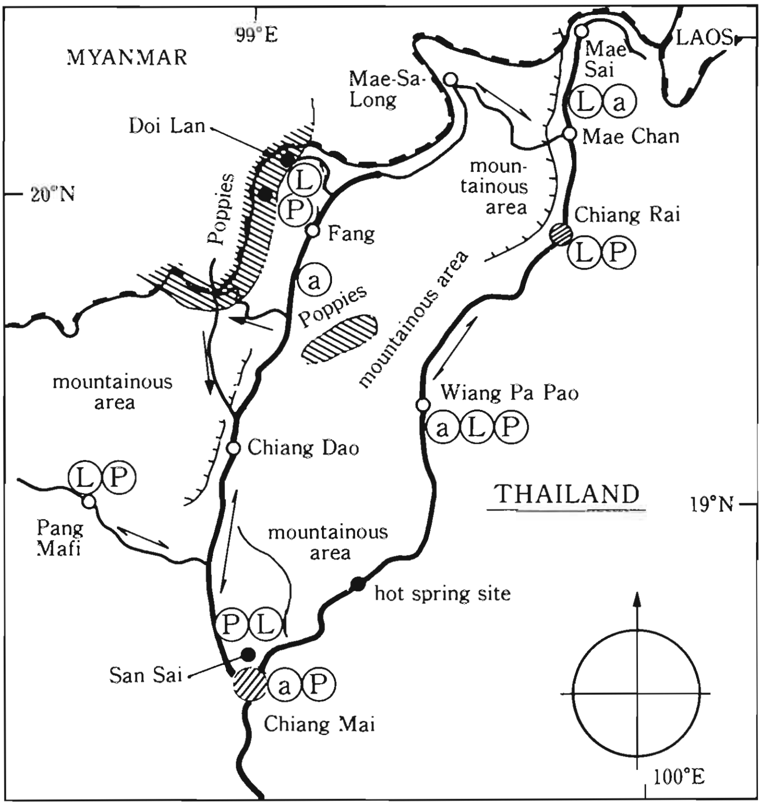
Our principal strategies are first to develop efficient technology and high-yield spawns while training experts as advisers for the growers, second to increase growers' incomes through improvements in their technology of growing, and third to increase the number of growers among small-scale farmers, including those inhabiting poppy-growing areas.

After the Scientific Mission sent by Kinki University to Thailand in January 1991 (SMKT91) following SMKT88, SMKT89 and SMKT90, Kinki University sent three other missions to Chiang Mai University as experts in mushroom studies:

SMKT92-1, Jan. 6-12, 1992
SMKT92-2, Aug. 5-11, 1992
SMKT93, Jan. 6-12, 1993

The participants discussed the results obtained up to that time and plans for the future with staff members of Ciang Mai University.

The missions have made joint expeditions during each visit with their counterparts in Thailand. The groups visited mushroom growers along a route stretching from Chiang Mai through Wiang



Cultivation sites :
Ⓛ; *Lentinus edodes*,
Ⓟ; *Pleurotus* spp.,
ⓐ; *Agaricus brunnescens*.

Fig. 1. Itinerary of the missions.

Pa Pao, Chiang Rai, Mae Sa Long (in SMKT92-1), Doi Lan, and Fang, back to Chiang Mai. The map of the itinerary is shown in Fig. 1. During the trips, the present technical and economic conditions faced by growers (in particular, growers of *L. edodes* and *Pleurotus* spp.) and yearly changes in their cultivation procedures and patterns have been observed and recorded.

The project work achieved by the end of January 1993 is outlined below.

By Kinki University

1) Financial (grant) support was donated to Chiang Mai University

Recipient	Year	Barts donated	Cumulative total
Dr. Jonglaekha	1989	100,000	100,000
and	1990	100,000	200,000
Dr. Phusawang	1991	100,000	300,000
	1992	100,000	400,000
	1993	80,000	480,000

2) Spawns of *L. edodes* and *Pleurotus* spp. gathered in northern Thailand were analyzed genetically.
3) F_1 progenies between Thai and Japanese stocks were used for breeding of new varieties.
4) Current features of mushroom cultivation done in northern Thailand were investigated with the cooperation of Dr. Phusawang and Mr. Chinbenjaphol (also refer to Progress Report 3).
5) Technical training was done for researchers of Chiang Mai University in the Laboratory of Genetics and Plant Breeding, Kinki University.
   Dr. Nuchnart Jonglaekha, from Oct. 7 to Oct. 31, 1991.
   Mr. Smarn Chinbenjaphol, from Nov. 11 to Dec. 10, 1992.

By Chiang Mai University

1) Experimental facilities for use in the project, including mushroom houses, sterilizers, laboratory equipment, and glassware, were installed with payment from the grants described above.
2) Physiological studies were done for improvement of cultivation procedures (refer to Progress Report 4).
3) Workshops on mushroom cultivation technology for farmers' groups were done in collaboration with the Royal Project, Thailand. Technical extension services for the growers were continued.
4) Other studies useful for the project were done.

Progress reports about some of the results obtained in this period follow.

Progress Report 3

Section 1. Outline of Mushroom Cultivation in Northern Thailand

by

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The climate in northern Thailand is better for growing mushrooms than that of southern Thailand, except for the straw mushroom (*Volvariella volvacea*), which is adapted to the hotter climate of the south. Chiang Mai is a major trading center for vegetables, including mushrooms. Farmers of Chiang Mai and Chiang Rai Provinces bring their crops, including mushrooms, to Chiang Mai markets, wholesalers send vegetables to Bang Kok and elsewhere, including Malaysia. Retail dealers of Tak and Sukhothai, in the south, usually come to Chiang Mai and stock up on vegetables, including mushrooms. The farmers have not organized into cooperatives, and they can ship their crops directly to Bang Kok only with great difficulty. Typical features of mushroom cultivation observed in northern Thailand are described below.

1. *Agaricus brunnescens* Pk. (= *A. bisporus* Sing.)

A. brunnescens is cultivated during the cool season from September or October to February. It is grown on compost mainly composed of rice straw with some minerals and organic materials as supplements. Composting and the subsequent process of cultivation in most mushroom houses are similar to those followed generally in other countries. Reddish tropical soil obtained from the surrounding area is used as casing soil after it is brought to neutral pH. A typical example of cultivation used by one grower near Wiang Pa Pao is shown in Table 1. Most mushroom houses are hand-made huts, with roofs and walls thatched with bundles of graminaceous straw supported by bamboo poles and beams (Fig. 2).

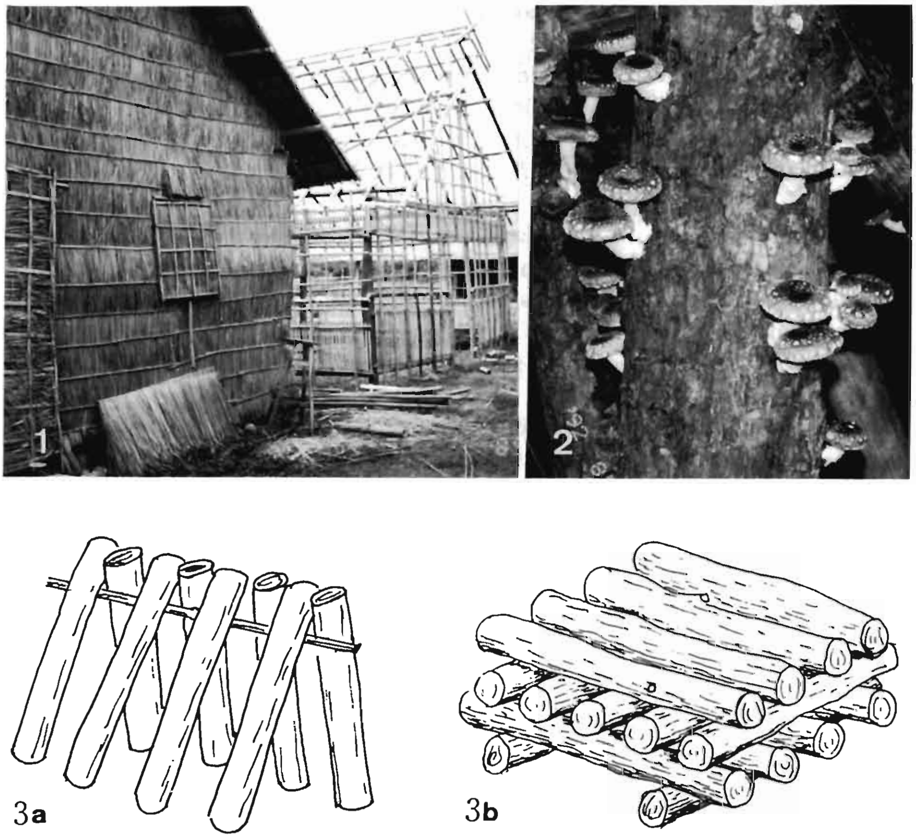

Fig. 2. 1. Mushroom house of *A. brunnescens* cultivation (Chiang Rai Pref.). 2. Fruit-bodies of *L. edodes* which develop on the logs (Doi Lan). 3. Stacks of logs for *L. edodes* cultivation : a. lean-to stacks ; b. crib-stacks.

Table 1. Flow chart for cultivation of *Agaricus brunnescens*.

Cutting of paddy-straw
Wetting
Stacking + lime
↓ 2 days
Turning + urea (1%),
↓ 4 days
Turning + $(NH_4)_2SO_4$ (2%),
↓ 4 days
Turning + superphosphate (2%),
↓ 4 days
Turning + $CaCO_3$ (2%), turning
↓ 4 days
Loading, top heat for 8 h
Spawning
↓ Spawn-run
Casing
↓ + watering
Flushing (9-10 flushes)
(Four flushes for 60% harvest)

2. *Pleurotus* spp.

Oyster mushrooms, including *Pleurotus ostreatus*, *P. pulmonarius*, *P. sajor-caju*, and a botanically unidentified species (? *P.* sp. *abalone* by Zadražil, 1978; cv. Black Beauty) are cultivated in Chiang Mai and Chiang Rai Provinces. According to local farmers growing *Pleurotus* spp. including *P. sajor-caju*, most of the spawns cultivated originally came from Bhutan through Yun-Nan, China. Fungal stocks were isolated from spawns and fruit-bodies which were collected in mushroom houses in Chiang Mai, San Sai, Wiang Pa Pao, Chiang Rai, and Mae Sa Long, and purchased on road near Fang (Table 4). These stocks were crossed with each other and with taxonomically identified stocks in Japan, making possible identification of the species. However, *P. pulmonarius* and *P. sajor-caju* were easily crossed with each other, suggesting that the taxonomic relationship between the two should be reexamined (see Section 2).

We could not find any wild stocks of *Pleurotus* spp. or *L. edodes* in forests in Chiang Mai and Chiang Rai Provinces, although Dr. Sanchai Tontyaporn (a mycologist in the Department of

Table 2. Composting of 'Black Beauty'.

Cutting of paddy straw
Wetting and stacking
↓ + urea (1%)
Covering with sheet
↓ 4 days
Uncovering
Turning + $Mg(SO_4)_2$ (0.02%)
↓ 2 days
Turning + lime (0.5%)
↓ 2 days
Composting finished

Agriculture, Bang Kok) found one wild stock of *L. edodes*, and a mission sent to northern Thailand by the Farmers' Cooperative Association of Nagano Prefecture, Japan, to collect mushrooms in 1992 found wild stocks of *P. pulmonarius* and *P. ostreatus*. All the commercial stocks of *L. edodes* and *Pleurotus* spp. in Thailand seemed to be of foreign origin.

Compost made of rice-straw is used as the medium for "Black Beauty" and sawdust is used in medium for other species, with some exceptions. Both kinds of media are prepared in polypropylene bags, inoculated, and arranged on shelves in huts similar to those used in the cultivation of *A. brunnescens*. The best sawdust for cultivation is from the para rubber tree (*Hevea brasiliensis*), and the next best is from the rain tree (*Samanea saman*). For a recipe for sawdust medium and a cultivation scheme for *P. ostreatus* and *P. pulmonarius*, refer to Kinugawa et al. (1989). A recipe of rice straw compost for "Black Beauty" is in Table 2.

3. *L. edodes* (Berk.) Sing.

Since 1989 we studied several growers of *L. edodes* along the route from hot spring site (on Rt. 1019) to Mae Sai (along Myanmer border), Mae Sa Long, and Doi Lan (very close to the Myanmer border). Most of these farmers grew fungi on hardwood logs in the past, and the trees used were not identified taxonomically. Some growers said that all wood used was a species of maple imported from Myanmer across Mae Nam Kok (the Kok River), and farmers in Doi Lan reported using a species of oak growing in the area. Because of a Prohibition by Thai law against civilians cutting state-owned trees down without special permission, the legal gathering of domestic logs for cultivation is virtually impossible in Thailand; almost all forests are on state-owned land in that country.

Under shelters with thick grass-thatched roofs that decrease direct sunlight and heat, farmers arranged wood logs inoculated in crib stacks or lean-to stacks (Fig. 2; refer also to Przybylowicz and Donoghue, 1988) to achieve good spawn-runs and fruiting. The shelters also protected against rain, so logs were watered every day. In spite of such shelters, in lowlands, the heat was great, resulting in poor yields and small fruit-bodies with long stalks and thin caps. The logs dcayed rapidly. and were heavily contaminated with undesirable fungi and bacteria. By 1993 (SMKT93), most of the growers in lowland had abandoned the used of logs for cultivation and were using sawdust medium in bags. In 1991 and 1992, many stacks of logs almost neglected were observed in shelters near Chiang Rai and in Mae Sa Long; the logs abandoned before the visit of SMKT93 in 1993.

In August 1992 (SMKT92-2) in Mae Sa Long, a stack of a number of cultures on sawdust medium in bags was observed under a shelter. Most of the medium was contaminated. Half an year later (SMKT93), cultivation had greatly improved, with little contamination apparent.

In August 1992 (SMKT92-2), we visited a farmer's cooperative in Doi Lan, in a high mountainous area northwest of Fang. They cultivated *L. edodes* on logs and on sawdust medium. We saw many logs stacking in cross stripes (crib-stacks) and rows of many logs leaning in A-shapes formed by alternate logs (lean-to stacks), on which many small fruit-bodies with long legs and thin caps had developed (Fig. 2). The farmers said that they had inoculated about 10,000 *Quercus* logs (they are not yet checked taxonomically). For each fruiting cycle, 500 logs were steeped in water overnight at the end of eight months of incubation. The logs were then taken into a shelter, wrapped with wet sheets for primordial development, and left on the ground for four days. After the sheets were removed, the logs were arranged in A-shapes with support by bars (lean-to stacks) under the shelter. After harvest, which lasted three to four days, the logs were taken out of the shelter and stacked in cross stripes for two months' rest outside. Sawdust spawns were supplied by the Royal Project in Chiang Mai (25 barts/bottle), and the usual harvest was 20 kg/day (50-60 kg/day in the rainy season) per 500 logs in a single fruiting cycle. The logs were ordinarily exhausted after being used 4 times, but large logs could support fruiting for four to five years. For the cultivation of *L. edodes*, the climate of Doi Lan seemed to be suitable (it is cool,

Table 3. Retail prices of vegetables and fruits in a market near Chiang Mai.

Vegetable or fruit	Price in Barts	Vegetable or fruit	Price in Barts
Oyster mush.[1]	20-40/kg	Shiitake[1]	60-120/kg
Common mush.[1]	30-35/kg	Ear mush.[1]	30/kg
Straw mush.[1]	60/kg	Yam beans	5/kg
(*V. volvacea*)		Spinach	20/kg
Chili pepper	1/3 parts	*Chryptotaenia japonica*	20/kg
Eggplant	2/5 parts	Cabbage	6-8/kg
Ipomoea aquatica	8/kg	*Brassica chinensis*	8/kg
Cucumber	40/kg	Tomato	8/kg
Sesame seed	2/2 bags	Potato	18/kg
Egg	14/10 parts		
Cauliflower	10/kg		
Mean daily wage	60/day (man)		

[1] fresh

Table 4. List of the fungal stocks of *Pleurotus* collected in northern Thailand.

Species	Strain	Origin	Year of accession	Identification[1]
Standard stocks				
Pleurotus ostreatus	PN87	Nagano Pref.	1987	
P. pulmonarius	PLPU	IFO 31345	1988	
P. sajor-caju	PSCK	Kagoshima Pref.	1990	
P. sajor-caju	PSCN	Nagano Pref.	1989	
Unidentified species				
Pleurotus sp.	PT88	San Sae	1988	*P. pulmonarius*
P. sp.	PT89	〃	1989	〃
P. sp.	OG89	purchased in BK	1989	〃
P. sp.	OB89	〃	1989	〃
P. sp.	OW89	〃	1989	*P. ostreatus*
P. sp. (from Bhtan)	AB89	〃	1989	?
P. sp. (from Taiwan)	BB89	〃	1989	?
P. sp. (*P. sajor-caju*)	PuB91	San Sae	1991	*P. pulmonarius*[1]
P. sp. (*P. sajor-caju*)	TPL93-3	Wiang Pa Pao	1993	〃
P. sp. (*P. sajor-caju*)	TPL93-3t	〃	1993	*P. ostreatus*
P. sp. (*P. sajor-caju*)	TPL93-4	〃	1993	*P. pulmonarius*[1]
P. sp. (*P. sajor-caju*)	TPL93-5	Chiang Rai	1993	〃

(*P. sajor-caju*) ; tentatively identified when collected. [1] tentative identification estimated by crossing with standerd stocks. BK, Bang Kok.

especially at night) for fruiting at least in the month that we visited.

In other method, the farmers arranged bags on the ground under a separate shelter. The bags were always purchased from another grower after a spawn-run ended. The bags were opened and watered every day. Members of the cooperative were waiting for fruiting at the time of our visit. In January 1993 (SMKT93), we visited them again and ascertained their success.

It was unusually hot in April and May of 1992 in northern Thailand. On Aug. 8, 1992, SMKT92-2 visited a grower near Mae Chan, north of Chiang Rai, who had many cultures on sawdust mdium. He had inoculated the cultures on April 4 of the same year, and arranged the cultures under shelters, but there were no signs of fruiting at the time of our visit. Even in northern Thailand, the hottest season (April to May) will inevitably strike *L. edodes* cultivation in the low lands. It is necessary for growers in the lowlands to have summer shelters in the cooler highlands to avoid problems caused by hot weather.

4. Prices of mushrooms in the market

Table 3 is a price list of vegetables and fruits surveyed in a market in Chiang Mai. The prices of mushrooms were higher than those of other vegetables and fruits.

Section 2: Identification by Genetic Analysis of *Pleurotus* Mushrooms in Northern Thailand

by

Kenjiro KINUGAWA, Eiji TANESAKA and Mieko OKADA
(Kinki University)

SMKT missions obtained many isolates from oyster mushrooms under cultivation during each survey carried out northern Thailand in 1988 and 1989 (Table 4). Not all of mushrooms have been identified taxonomically yet. Taxonomic identification on the basis of fruit-body color and morphological characteristics including spore size was not possible, because all of the materials to be identified were grown artificially and fruit-body color is variable in every known *Pleurotus* species, and according to Imazeki and Hongo (1989), spore sizes overlap in different species: 6-9×3.0-3.5 μm in *P. cornucopiae*, 6-9×3.0-4.5 μm in *P. salmoneostramineus*, 6-10×3-4 μm in *P. pulmonarius*, and 7.5-11.8×3-4 μm in *P. ostreatus*.

In this experiment, the possibility of crossing various combinations of two stocks was studied. If two or more organisms can be crossed, it means that they have a gene pool in common, so the organisms should be placed in the same species if species is defined based on genetics.

Results

1. In 1989, monokaryotic strains were produced by monobasidiospore culture of the stocks PN87 (standard stock of *P. ostreatus* in Kinki University) and PT88 (Thailand), and crossings between the stocks were attempted with 167 cross-combinations of mon-mon mating. However, crossing failed in all of the combinations. PT88 was not *P. ostreatus* (Fig. 3).

2. Because of the close resemblance of fruit-bodies of PT88 and some other stocks to those of *P. pulmonarius*, the standard stock of this species was used in crossing experiments. Results due to di-mon mating (Buller, 1931) are shown in Fig. 3 and Table 5. OW89, TPL93-3, and TPL93-3t were *P. ostreatus*, and all other stocks were *P. pulmonarius*. We thank Mr. K. Yokoyama of Shiga University (Japan) for his advice on the fruit-body characteristics of *P. pulmonarius*. The

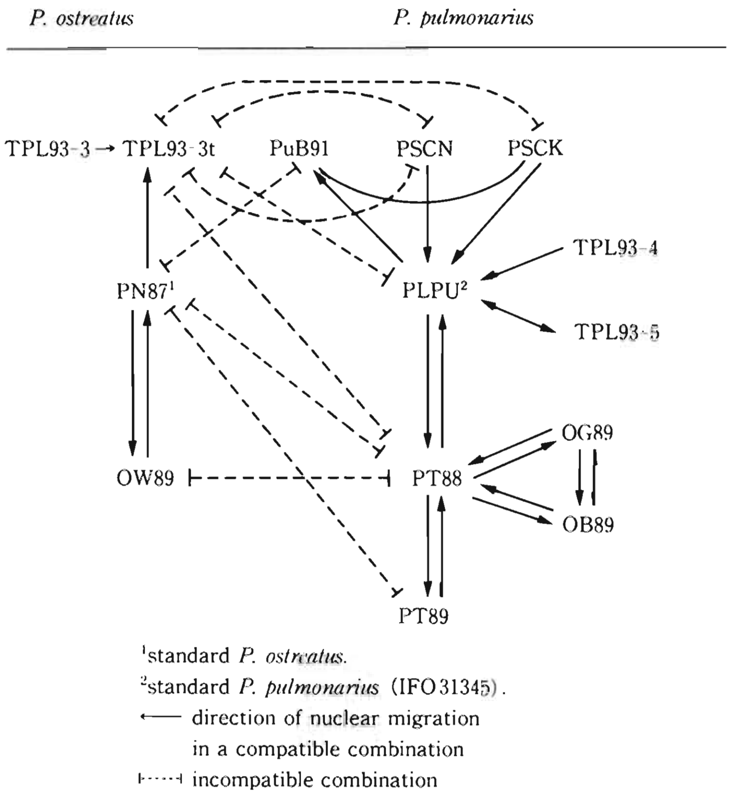

Fig. 3. Relationships between stocks of *Pleurotus* in terms of possibility of cross.

Table 5. Results of di-mon crossing between *Pleurotus* stocks.

1. mPuB91 (monokaryons of PuB91) and various dikaryotic stocks

mPuB91	Dikaryon				
	PN87	PLPU	PSCK	AB89	BB89
1	−	+	+	−	−
2	−	+	+	−	−
3	−	+	+	−	−
4	−	+	+	−	−
5	−	+	+	−	−
6	−	+	+	−	−
7	−	+	+	−	−
8	−	+	+	−	−
9	−	+	+	−	−
10	−	+	+	−	−
11	−	+	+	−	−

+ dikaryotized.

2. mTPL93-3t (monokaryons of TPL93-3t) and various dikaryotic stocks

mTPL93-3t	Dikaryon								
	PSCK	PN87	PSCN	PLPU	PT88	TPL93-3	TPL93-4	TPL93-5	TPL93-3t
1	−	+	−	−	−	−	−	−	−
3	−	+	−	−	−	+	−	−	+
5	−	+	−	−	−	+	−	−	+
6	−	+	−	−	−	+	−	−	−
7	−	+	−	−	−	−	−	−	+
9	−	+	−	−	−	−	−	−	+

+ dikaryotized.

3. mPLPU (monokaryons of PLPU) and various dikaryotic stocks

mPLPU	Dikaryon					
	PSCN	PSCK	TPL93-4	TPL93-5	PLPU	PT88
1	+	+	+	+	+	+
2	+	+	+	+	+	+
3	+	+	+	+	+	−
4	+	+	+	+	−	−
5	+	+	+	+	+	+
6	+	+	+	−	−	−
7	+	−	−	+	+	−
8	+	+	+	+	−	+
9	−	−	−	−	−	−
10	+	+	+	+	+	−

+ dikaryotized.

relationship between PuB91 and *P. pulmonarius* will be discussed in the next section.
3. Electrophoretic banding patterns of proteins extracted from mycelia and fruit-bodies of PT88, PT89, and PN87 were compared. the former two are *P. pulmonarius* and the latter is *P. ostreatus*. The patterns for PT88 and PT89 were similar, but different from the latters for PN87 (Fig. 4).

Section 3. Crossing of fungal stocks of *P. sajor-caju* in Japan and Thailand with *P. pulmonarius*

by

Kenjiro KINUGAWA, Eiji TANESAKA and Mieko OKADA
(Kinki University)

Stocks of *P. sajor-caju* were isolated from fruit-bodies cultivated in Nagano Prefecture (PSCN) and Kagoshima Prefecture (PSCK), Japan, and also from those in northern Thailand (listed in Table 4) . A taxonomic check has not been done with these cultivated samples, because wild samples have not been available. Corner (1981) did not accept the combination *P. sajor-caju*

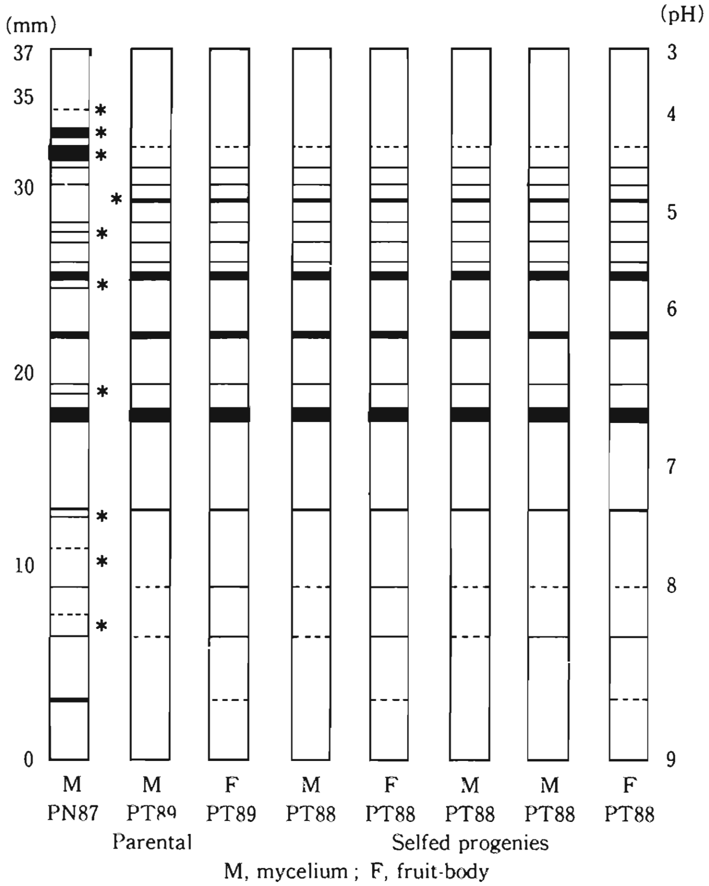

Fig. 4. Electrophoretic patterns of proteins extracted from fruit-bodies of *P. ostreatus* and *P. pulmonarius*. Asterisks show bands in PN87 not found in PT88 or PT89.

(Fr.) Sing.

These stocks could be crossed with each other, and could not be crossed with PN87 (*P. ostreatus*) ; the stocks crossed easily with *P. pulmonarius* (stock PLPU ; Table 5). The stocks that were suggested to be *P. sajor-caju* in Japan and Thailand had a gene pool in common with *P. pulmonarius*. In this report, all stocks that could be crossed with PLPU are tentatively put in the category of *P. pulmonarius* complex.

Section 4. Physiological Traits of PN87 (*P. ostreatus*) and PT88 (*P. pulmonarius*) in Culture

by

Kenjiro KINUGAWA, Eiji TANESAKA and Mieko OKADA
(Kinki University)

Some physiological traits in culture of a stock of *P. pulmonarius* (PT88) cultivated in Thailand were studied, and compared with those of a stock of *P. ostreatus* (PN87) cultivated in Japan. *P. pulmonarius* is not been cultivated commercially in Japan.

Results

1. Extention growth of mycelia at various temperatures on MYP medium (pH 6.5), composed of 7 g of malt extract (Difco), 1 g of soytone (Difco), 0.5 g of yeast extract (Difco), and 10 g of agar in 1,000 ml of distilled water was measured. Growth of PN87 and PT88 was maximum at about 25°C, and faster than the PN87 at 20°C and 25°C (Fig. 5).
2. When culture was in liquid MYP (malt, yeast, and soytone) medium (agar omitted) at 25°C, the total amount of organic carbon and nitrogen in the medium decreased earlier in PT88 than in PN87. At this temperature, fruiting occurred in PT88, but not in PN87.
3. During the spawn-run on a 4 : 1 mixture of sawdust and rice bran medium at 25°C, the amount of CO_2 released per hour by PT88 increased more rapidly than the amount released by PN87. The amount released by PT88 and PN87 peaked in 14 days and reached a minimum in 42 days after spawning. PT88 probably decays wood more rapidly than PN87 (refer to Tanesaka et al., 1993).
4. PT88 had earlier fruiting and fruit-bodies of better shape at 20°C or lower temperature than at 21°C.

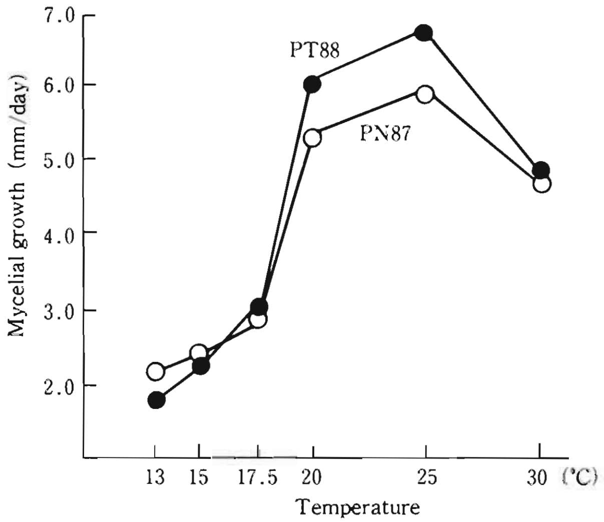

Fig. 5. Mycelial growth of *P. ostreatus* (PN87) and *P. pulmonarius* (PT88) at various temperatures.

Section 5. Characterization of Progenies of PT88 Self-Crossed and of PT88 and PT89 Di-Mon-Crossed

by

Kenjiro KINUGAWA, Eiji TANESAKA and Mieko OKADA
(Kinki University)

Both PT88 and PT89 are cultivated stocks of *P. pulmonarius*. They were isolated from San Sae near Chiang Mai (Fig. 1 and Table 4). The stocks were cultivated again in Japan, and spore prints were obtained.

A mass of basidiospores was taken with the tip of a needle from a spore print of a PT88 fruit-body, suspended in sterilized water, and plated on MYP plates. After several days of incubation, many colonies had grown. Tiny pieces of mycelia from the colonies were then transplanted into MYP slants. Some were found to be dikaryotic by the detection of clamp connections of septa of their mycelia, and others were found to be monokaryotic. Dikaryotic mycelia also arise from auto-mating between spores in proximity at plating, when they are compatible with each other. Monokaryons of PT88 are designated as mT8- and dikaryons are designated as PT88s-, or s- for short. Di-mon mating were brought about between mT8- and PT89, resulting in dikaryotic mycelia with cytoplasm from PT88 and conjugate nuclei stemming from a nucleus of mT8- and a nucleus of PT89. Dikaryons thus formed are designated as mT8 -(×PT89) d1, or T8T9d1, or d for short. In this report, the term "strain" is used for monokaryotic mycelia and the term "stock" is used for dikaryotic mycelia, as suggested by Ainsworth et al. (1971).

Results

1. Stocks of PT88s- and T8T9d- were cultivated at the same time at 20°C on a 4 : 1 mixture of sawdust and rice bran as the medium in 800 ml culture bottles, and their spawn-run time (days), days until fruiting, fruit-body yield per bottle (grams fresh weight), and characteristics of the fruit-bodies were studied. In a separate experiment, the rate of mycelial growth on the MYP plate medium at 25°C was measured. The results were analyzed by principal component analysis for comparison of characteristics of the stocks. The days until the start of fruiting and the rate of mycelial growth were main contributers to the first principal component, Z1, and the yield and the

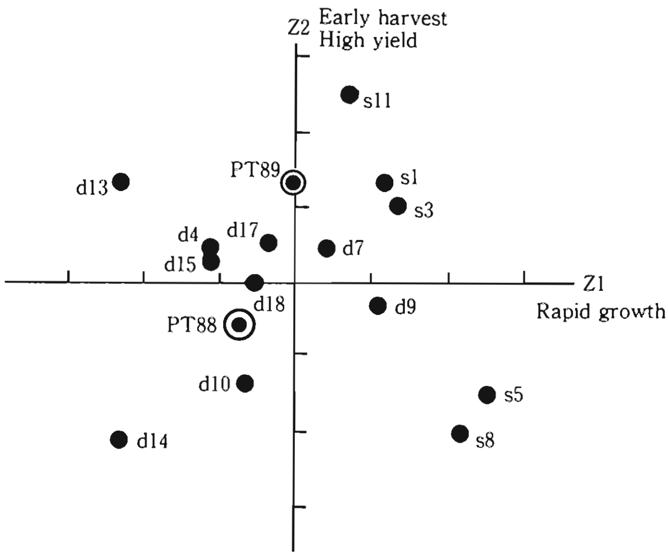

Fig. 6. Scatter diagram of various stocks of *P. pulmonarius* plotted by their first and second principal component scores. Stocks: PT88, PT89, progenies by selfing of PT88 (s1, etc.), and progenies by di-mon crossing of mPT88- (monokaryons from PT88) and PT89 (d1, etc.).

time until harvest the main contributers to the second principal component, Z2. Characteristics of stocks are summarized as their plotted locations in Fig. 6.
2. All of the PT88s- stocks fruited earlier and had more rapid mycelial growth than the parental PT88, and many gave higher yields than the parent. The best stock, overall, was PT88s-11.
3. Most of the T8T9d- stocks are scatter between their parental stocks, PT88 and PT89, in the principal-component diagram.
4. Monokaryons were obtained from the single basidiospore cultures of PT88-11, the most promising stock. By crossing of them with each other, dikaryotic selfed progenies PT88s2- occurred. Two or three of the total of 13 progenies surpassed their parental PT88s-11 in having thicker fruit-bodies, darker caps, earlier fruiting, higher yield, and faster mycelial growth.

Progress Report 4

Pysiology of *Lentinus edodes* and *Pleurotus* sp. on Medium of Sawdust, Rice Bran, and Sucrose

by

Wichian PHUSAWANG and Smarn CHINBENJAPHOL
(Chiang Mai University)

Experiment 1. Effects of rice bran and sugar on the mycelial growth of shiitake

Shiitake (*Lentinus edodes*) can be grown in bags filled with a medium made of a mixture of sawdust and nutrients. The most common materials added to sawdust by farmers are rice bran and raw sucrose, and these materials always stimulate mycelial growth. In this experiment, the basic physiological responses of the mycelia to these nutrients were investigated.

Materials and Methods

Dried sawdust from para-rubber trees was mixed rice bran and sucrose. The proportions of the additives were 4%, 8%, and 12% rice bran and 0.3%, 0.6%, and 0.9% sucrose. The medium was used to fill test tubes (15×240 mm) 10 cm deep. Inocula were placed on the top of the media, and the length to which mycelia grew in the first two weeks after inoculation was measured in centimeters, with three replicates.

Results and Discussion

The highest levels of sucrose without rice bran inhibited mycelial growth (Fig. 7). The growth at 0.9% sucrose was significantly lower than at the other two levels (least significant difference (LSD) 5%, 0.30).

Increased levels of rice bran without sucrose increased mycelial growth (Fig. 8). Growth on 12% rice bran was significantly greater than that at 4% (LSD 5%, 0.30).

Growth by mycelia varied with different proportions of rice bran to sucrose (Fig. 9). In the medium containing 12% rice bran, increased sucrose decreased mycelial growth linearly. The

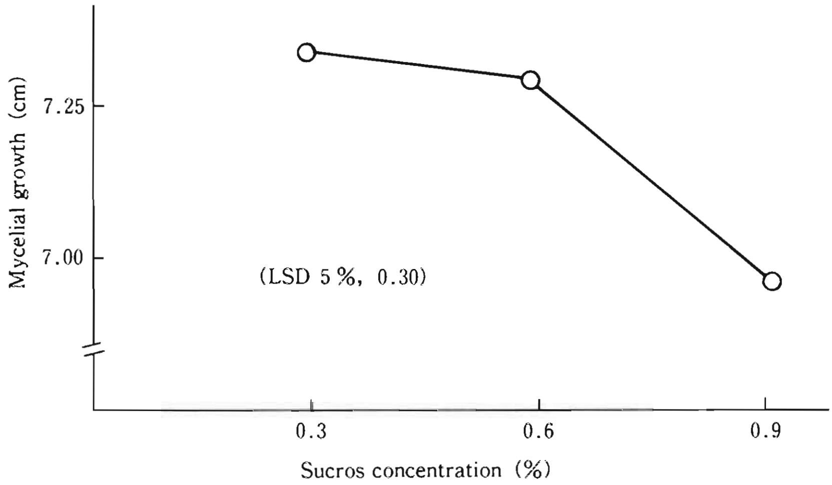

Fig. 7. Mycelial growth of *L. edodes* with various proportions of sucrose in a medium of sawdust from para-rubber trees.

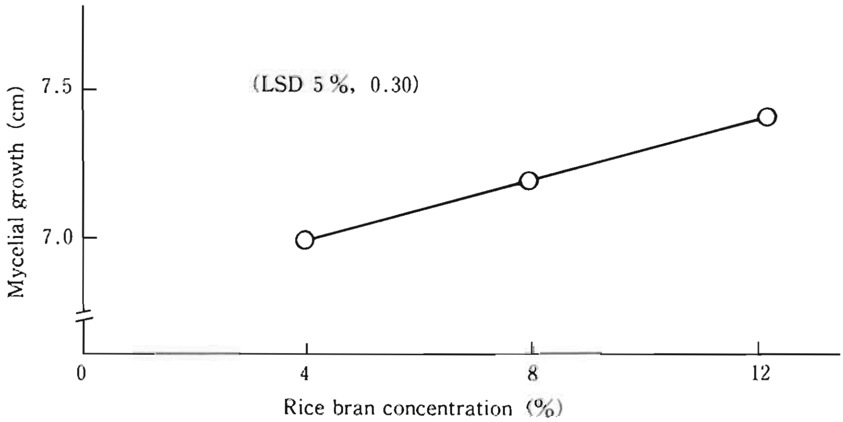

Fig. 8. Mycelial growth of *L. edodes* with various proportions of rice bran in a medium of sawdust from para-rubber trees.

growth of mycelia with 4% and 8% rice bran and different amounts of sucrose changed in a more complicated pattern. Growth on 4% rice bran was greatest with 0.6% sucrose of the sucrose concentrations tested.

Experiment 2. pH changes in medium in bags during cultivation of *L. edodes*

pH changes in media used for shiitake cultivation outside of Thailand have been reported. Therefore, a study was done of pH changes in a medium often used for commercial cultivation in Thailand.

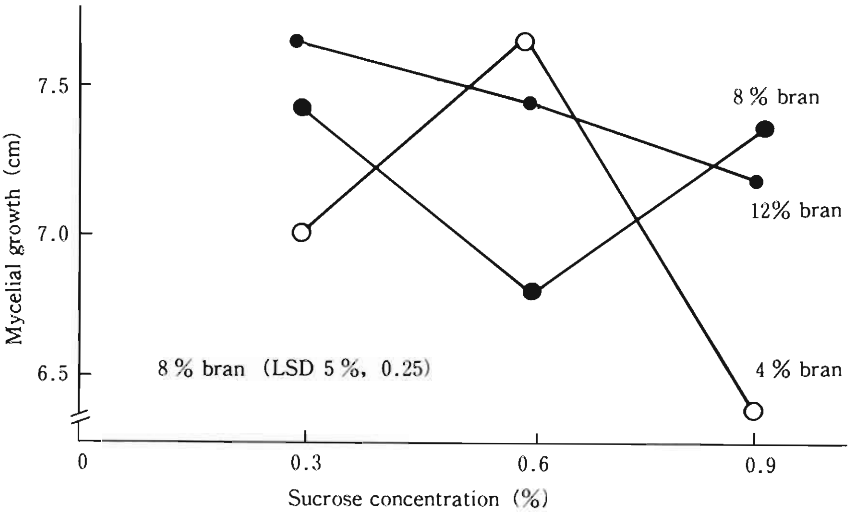

Fig. 9. Mycelial growth of *L. edodes* in a medium of sawdust from para-rubber trees with different proportions of rice bran and sucrose.

Materials and Methods

Three bags of a medium were selected randomly from the production lots of C.P. Co. near Pang Mafin, northwest of Chiang Mai. The pH was measured at the stages of cultivation listed below.

stage	
1	Just before sterilization
2	Just after sterilization
3	One month after inoculation, when growing margin of mycelia has reached halfway through bag
4	Two months after inoculation, when mycelia have grown to fill the bag
5	Four months after inoculation, at the first harvest
6	Seven months after inoculation, at the final harvest

Results and Discussion

Mean pH values of medium were 5.90 and 5.83 just before and after sterilization, respectively. At 1, 2, 4 and 7 months after inoculation, the pH was 5.73, 5.13, 6.03 and 6.57, respectively (LSD 10%, 0.69). It seemed that active vegetative growth of the mycelia produced an acidic substance(s). The media pH dropped significantly up to 2 months after inoculation then rose during the following 5 months. Active mycelial growth continued for the first two months, and harvest was possible during the next 5 months. The rise in pH during the final peiod of cultivation, probably due to breakdown of the middle lamella between cells of the wood, releasing calcium ions into the medium.

Experiment 3. Effects of different proportions of two kinds of sawdust from para rubber trees and rain trees in the medium on the yield of *Pleurotus* mushrooms

In northern Thailand, sawdust from para-rubber trees has been used in the medium for *Pleurotus* mushroom cultivation. The sawdust must be transported about 1500 km from the southern part of Thailand, increasing the cost, which was already high. Sawdust from rain trees is cheap and widely available in northern Thailand. Therefore, the effect of the proportions of para-rubber sawdust and rain tree sawdust on the yield of these mushrooms was examined.

Materials and Methods

The ratios of sawdust from para-rubber trees to sawdust from rain trees examined were 5 : 1, 4 : 2, 3 : 3, 2 : 4, and 1 : 5. The experiment was done four times with six bags in each experimental group. The mean yield was calculated on the basis of a 15-day harvest.

Results and Discussion

At the proportions listed above, the yield was 109, 100, 93, 84 and 81 g/bag, respectively (LSD 5%, 17.16) The yield decreased as the proportion of rain tree sawdust increased. The spawn used prepared by the C.P. Co. with a medium composed mainly of para-rubber tree sawdust. It is possible that lack of adaptation by the mycelia to rain tree sawdust accounted for the decreased yield when the proportion of rain tree sawdust was increased.

Experiment 4. Effects of time of sawdust fermentation on the yield of *Pleurotus* mushrooms

Sawdust is usually been stacked in an open yard for from one to three months before being used for cultivation. During time, fermentation is unavoidable. This experiment was done to find the effects of the length of sawdust fermentation on the yield of *Pleurotus* mushrooms.

Materials and Methods

After 1% organic nitrogen was added to sawdust of para-rubber trees, the sawdust was stacked in an open yard for 1.5, 2.0 and 3.0 months, before being prepared for use as a medium. The experiment was done three times with 12 bags in each experimental group. The yield was based on the total amount of mushroom obtained in a 45-day harvest period.

Results and Discussion

The longer the fermentation period, the faster was the mycelial growth (Fig. 10). This result suggested that the sawdust contained a substance(s) harmful to mycelial growth or fruiting of the fungus, and that this substance(s) degraded during fermentation. Another possible explanation is that some substance, probably ammonia gas, is produced by mycelial metabolism, but the

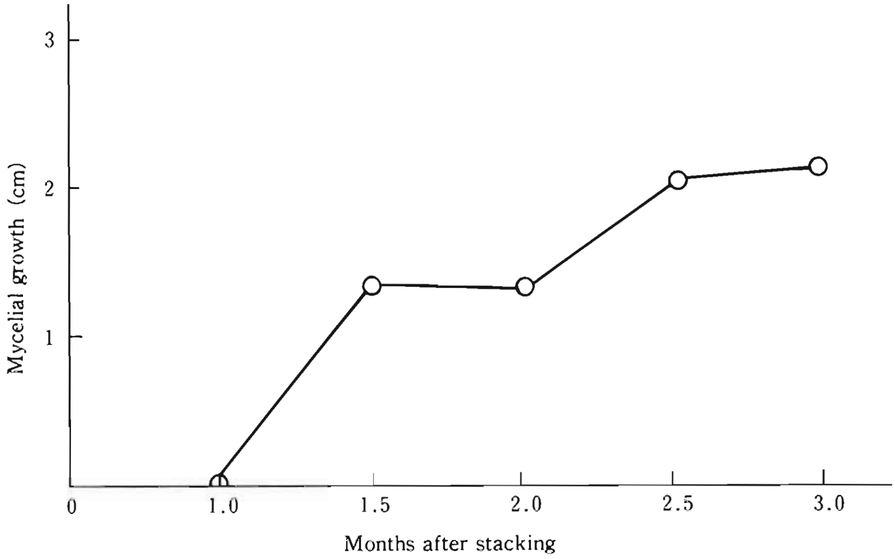

Fig. 10. Effect of the length of sawdust fermentation before preparation of a sawdust medium on mycelial growth of *Pleurotus* sp.

concentration of the substance is decreased when fermented sawdust is used.

Experiment 5. Comparison of the growth of *Pleurotus* mycelia on media containing sawdust from para-rubber trees or rain trees

Para-rubber tree sawdust is expensive in northern Thailand, as explained above. This study was done to find if this expensive sawdust can be replaced by the less expensive sawdust from rain trees.

Materials and Methods

Bags were filled with 1 kg of a medium based on sawdust of para-rubber trees or rain trees ("para-rubber medium" and "rain tree medium" hereafter). The pH of the medium was measured before and after sterilization, on day 11 after inoculation, and weekly thereafter. Inoculation was done on the day after sterilisation. The experiment was done four times with three bags in each experimental group. Means were based on these measurements.

Results and Discussion

The pH of the medium and the mycelial growth (The depth into which mycelia grew) are shown in Table 6. The pH was higher in para-rubber medium than in rain tree medium at all times. For both media, sterilization raised the pH, probably because of breakdown of the middle lamella of cells of the wood, releasing calcium ions into the medium. Mycelial growth was better in rain tree medium than in para-rubber medium, and the difference was significant on day 11. As

Table 6. Differences in pH of the medium and mycelial growth on sawdust from para-rubber trees or rain trees.

Days after sterilization	pH in medium with sawdust from			Mycelial growth[1] in medium with sawdust from		
	para-rubber	rain tree	P	para-rubber	rain tree	P
Before	7.69	7.12	<0.01	–	–	
Immediately after	7.90	7.88	ns	–	–	
11	6.64	6.38	<0.05	4.68	6.33	<0.05
18	6.64	6.38	ns	11.45	12.35	ns
25	5.88	5.77	<0.05	+	+	

[1] from the top of the medium to the margin of the colony.
+, margin of the colony reached the bottom of medium.
P, probability in unpaired t-test. ns, not significant.

far as mycelial growth concerned rain tree sawdust can replace para-rubber medium in the cultivation of *Pleurotus*.

Experiment 6. Effects of washing of rain tree sawdust with boiling water on growth of *Pleurotus* mycelia

This experiment was done to find if mycelial growth can be accelerated by the use of medium made with sawdust washed with boiling water. Washing might made fermentation unnecessary.

Materials and methods

Rain tree sawdust was washed with boiling water for 15 min and dried. Nonwashed sawdust was used as the control. After the necessary additions to the medium, test tubes in 24×150 mm were filled 10 cm deep with the medium. Means were calculated from the results of three test

Table 7. Effects of washing of sawdust in boiling water on mycelial growth and pH of the medium.

Days after sterilization	Length (cm) of mycelia in sawdust that is			pH of medium that is		
	unwashed	washed	P	unwashed	washed	P
Immediately after	–	–	–	6.50	6.48	ns
7	3.4	4.0	<0.01	5.55	5.36	<0.01
14	+	+	–	5.00	4.85	<0.01

+, colony reached the bottom of medium.
P, probability in unpaired t-test. ns, not significant.

tubes. The experiment was repeated four times.

Results and Discussions

Results are shown in Table 7. Washing of the sawdust increased mycelial growth on the medium seven days after sterilization, and decreased the pH of the medium. A harmful substance(s) in the sawdust seemed to be removed by the washing. After washing for removal of a harmful substnce(s) in rain tree sawdust, this sawdust can replace sawdust of para-rubber trees as the main component of a medium for *Pleurotus* cultivation.

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きのこ研究促進に関する近畿大学およびチェンマイ大学合同研究事業の進捗報告（1991-1993）

衣川堅二郎[1]・ビチアン　プーサワン[2]・サマン　チンベンジャフォル[2]
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摘　　要

近畿大学およびチェンマイ大学は，タイ王国のきのこ栽培に関する研究事業を共同して進めてきた。本報は1991年から1993年１月までの研究の進捗状況報告である。

タイ王国には，１）山岳小数部族の一部のケシ栽培，２）米価の低落を一原因とする稲作農民の疲弊，などの困難がある。一方，都市市場では，きのこが他の野菜類にくらべ高価で販売されている。そこで，現地のきのこ生産法を改良しその地に適した品種の開発に成功して，これを普及できれば低地の小規模農民の中に収入を増加する家族のでる可能性があり，山岳民族の中にもこれにならう家族がでればケシからきのこ栽培への転換がいささかでも促進されるものと考察した。ここでは，事業の期間内の経過，北部タイ地方のきのこ生産現況の調査結果の一部，および上記考察にそう基礎研究の結果の一部を以下のとうり報告する。

期間内に近畿大学担当教員は３回チェンマイを訪れ（SMKT92-1，SMKT92-2，SMKT93），チェンマイ大学担当者との打ち合わせ，共同調査，および資料収集を行なった。チェンマイ大学からは，２名の担当者（Dr. Nuchnart　Jonglaekha および Mr. Smarn Chinbenjaphol）が近畿大学農学部に研修のため各１箇月間滞在した。

進捗状況報告はつぎの各項について行なった。

近畿大学：　北部タイにおけるきのこ生産の概要。北部タイで現在栽培されているヒラタケ属の主な種の同定（*P. ostreatus*, *P. pulmonarius*, *P. sajor-caju* ?）。所謂 *P. sajor-caju* と *P. pulmonarius* との関係。日本産 *P. ostreatus* の一菌株（PN87）とタイ国産 *P. pulmonarius* の一菌株（PT88）の培養的性質の比較，および菌糸体ときのこの蛋白質の等電点電気泳動型比較。タイ国産 *P. pulmonarius* の菌株 PT88の自家交配による子孫および PT88と PT89の交配による子孫の特性分析。

チェンマイ大学：　シイタケ菌の菌糸体成長に対するコメヌカと蔗糖の影響。シイタケ袋培養中におこる培養基の pH 変化。培養基中の para-rubber tree と rain tree おがくずの比が培養する *Pleurotus* 属きのこの収量に及ぼす影響。おがくずの発酵期間の長さがそれをつかって培養した *Pleurotus* 属きのこの収量に及ぼす影響。*Pleurotus* 属きのこの菌糸体成長に及ぼす para-rubber tree と rain tree おがくずの特性の比較。沸騰水によるおがくずの洗浄が *Pleurotus* 属きのこの菌糸体成長に及ぼす影響。