

MATING SYSTEM OF THE EDIBLE MUSHROOM, *Pleurotus cystidiosus*

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ABSTRACT

Mating system or sexual system determines sexual compatibility in fungi. The aim of this research was to determine the type of mating system of the edible commercial mushroom, *Pleurotus cystidiosus*. The experiments were carried out by crossing each pair of the 14 single spore isolates (SSIs, monokaryons) from one single fruiting body of *P. cystidiosus* in all combinations, on MEA plates, and incubated at 30°C for 3 weeks, followed by clamp connection examinations. The presence or absence of clamps indicates compatible or incompatible mating, respectively. The ratio number of compatible matings to the number of total matings of *P. cystidiosus* was determined to be 1:4. This ratio indicates that the sexuality of the species is an example of tetrapolar (bifactorial) heterothallism. The 14 SSIs were also separated into 4 groups according to the four mating types (A_1B_1 , A_1B_2 , A_2B_1 and A_2B_2) of the species.

KEYWORDS: mating system, mating type, incompatibility, tetrapolar, *Pleurotus* spp.

1. INTRODUCTION

Pleurotus cystidiosus together with many other species of the genus *Pleurotus* are the most widely cultivated mushrooms in Thailand and in many other countries in the world. The mating (breeding, sexual) systems of many species of this genus have been investigated by the authors. The present paper describes the mating system of *P. cystidiosus*.

Mating system in any species of mushroom has to be clearly understood in order to conduct rational genetic studies as well as breeding programmes. Most edible mushrooms are bifactorial or tetrapolar heterothallic species in which their mating system is controlled by two genes (A and B) and each gene contains (at least) two alleles (A_1, A_2 and B_1, B_2), enabling four mating types, or four types of basidiospores, (A_1B_1, A_1B_2, A_2B_1 and A_2B_2) to occur in one fruiting body [1-3]. Therefore, if a number (e.g. 10-14) of single spore isolates (monokaryons), from one single fruiting body, are crossed in pairs and in all combinations, on agar plates, the ratio of the number of compatible matings ($A \neq B$) to the number of total matings will be 1:4.

2. MATERIALS AND METHODS

Fruiting bodies of *P. cystidiosus*.

Spawn of *P. cystidiosus* on a PDA slant purchased from "Aranyik Mushroom Farm" in Thailand was inoculated into boiled soft sterilized sorghum grains in glass bottles at 30°C until well-grown mycelia were obtained. The infected grains (10-20 grains) were then

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transferred to autoclaved sawdust in plastic bags, especially prepared for mushroom growing, which were then incubated in a moist area until mycelia were well developed. The top of each bag was then cut open and the contents of the bag were sprayed with water regularly and kept moist until fruiting bodies (Fig. 1) were developed.

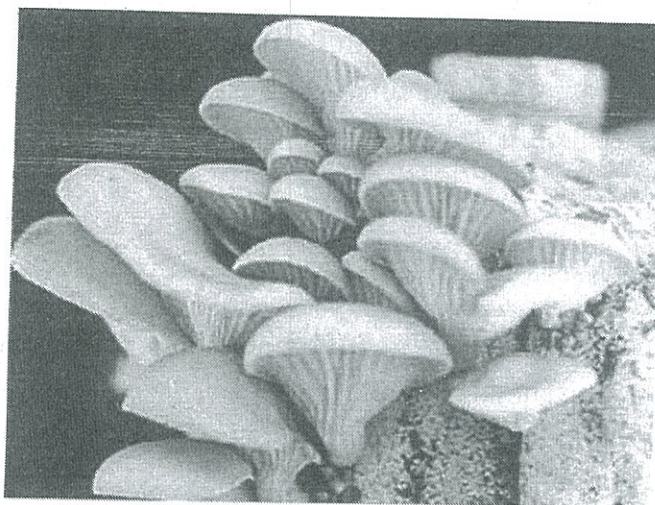


Figure 1 Fruiting bodies of *P. cystidiosus* growing on sawdust

Spore print and Single Spore Isolates

Spore prints from one single fruiting body were obtained by cutting a small piece (ca. 2.5×2.5 cm) of fresh mushroom cap and sticking it (with paraffin or sticky tape) gills-down on the sterilized lid of a Petri dish which was then put on top of a sterilized beaker covered with pieces (ca. 1×1 cm) of sterilized filter papers. The spores were allowed to be discharged onto the pieces of paper for 3-10 hours before the lid was replaced with a new sterilized one. The beaker with spore prints on pieces of paper was then kept in a refrigerator until single spore (basidiospore) isolation was performed. Single spore isolates (SSIs) on malt extract agar (MEA) slants were prepared as the method described in Peterson and Ridley [4].

Mating System Determination

Fourteen SSIs of *P. cystidiosus* were paired with each other in all combinations by placing the two inoculum discs (0.5 cm in diameter, using a cork borer) about 20 mm apart on a MEA plate at 30°C. Observations for clamp connections (Fig. 2) on mycelia within the contact zone were made under a light microscope at 400×. Presence or absence of clamps indicates compatible or incompatible matings, respectively. The ratio of number of compatible matings to the number of total matings of the 14 SSIs was determined. The morphology of each resulting "mated" colony described by Papazian [1] was also examined.

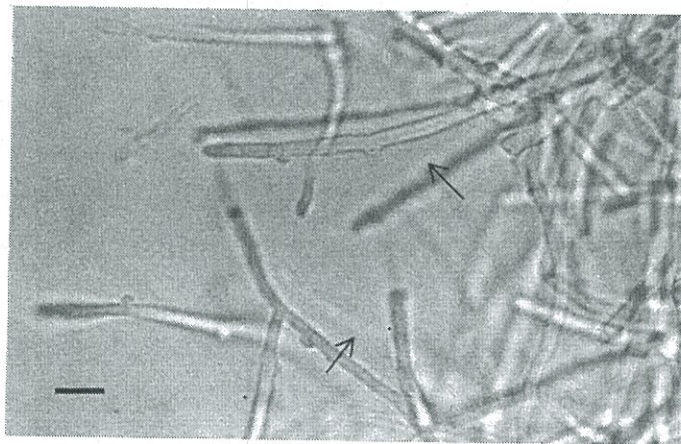


Figure 2 Clamp connections (arrows) of *P. cystidiosus* (400 $\times$), bar = 10 microns

3. RESULTS AND DISCUSSION

The results of the crosses of the 14 SSIs in all combinations are shown in Table 1. The ratio of the number of compatible matings to the number of total matings was 24:105 or 1:4.4 (ca. 1:4) which, therefore, indicates that the mushroom is a tetrapolar heterothallic species. In addition, from the mating results in Table 1, the 14 SSIs could be classified into 4 groups as follows: -

- Group 1: isolates 2, 3 and 14
- Group 2: isolates 4, 6, 8, 9, 10 and 12
- Group 3: isolates 5, 7 and 11
- Group 4: isolates 1 and 13

The morphology of the colony of each mating of the 14 SSIs (examples of some of the pairings were shown in Fig. 3) and the results in Table 1 were combined and rearranged into Table 2. The terms compatible, incompatible (overlap), hemicompatible-B [barrage with false clamps (Fig. 4) were found occasionally] and hemicompatible-A (flat) were designated and described by Papazian [1]. Each of the four groups of the 14 SSIs were then concluded to belong to each mating type as follows: -

- | | | |
|--|---|-------------------------------|
| Group 1 (isolates 2, 3 and 14) | = | A ₁ B ₁ |
| Group 2 (isolates 4, 6, 8, 9, 10 and 12) | = | A ₂ B ₂ |
| Group 3 (isolates 5, 7 and 11) | = | A ₂ B ₁ |
| Group 4 (isolates 1 and 13) | = | A ₁ B ₂ |

The mating system of *P. cystidiosus* determined in this study (tetrapolar heterothallism) is the same as those of the other species of *Pleurotus* (e.g. *P. ostreatus*, *P. sajor-caju* and *P. tuberrigium*) which we have been investigating in our laboratory (unpublished data). These results in mating type also agree with those reported by Moore [5], Petersen and Krisai-greihuber [6], Roxon and Jong [7] and Isikhuemhen *et al.* [8], respectively. The results, therefore, to some extent indicate that species belonging to the same genus are likely to have the same mating system. The 14 SSIs possessing the four mating types (A₁B₁, A₁B₂, A₂B₁ and A₂B₂) will be used for further hybridization studies either by conventional method or protoplast fusion.

Table 1 Compatibility results of crossings between the 14 SSIs of *P. cystidiosus* on MEA at 30°C

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	-	-	-	-	+	-	+	-	-	-	+	-	-	-
2		-	-	+	-	+	-	+	+	+	-	+	-	-
3			-	+	-	+	-	+	+	+	-	+	-	-
4				-	-	-	-	-	-	-	-	-	-	+
5					-	-	-	-	-	-	-	-	+	-
6						-	-	-	-	-	-	-	-	+
7							-	-	-	-	-	-	+	-
8								-	-	-	-	-	-	+
9									-	-	-	-	-	+
10										-	-	-	-	+
11											-	-	+	-
12												-	-	+
13													-	-
14														-

+ = compatible mating [(true) clamp connections were detected]

- = incompatible mating [no (true) clamp connections were detected]

Table 2 Details of incompatibility of matings of the 14 SSIs of *P. cystidiosus* derived from Table 1

		A ₁ B ₂		A ₁ B ₁			A ₂ B ₁			A ₂ B ₂					
		1	13	2	3	14	5	7	11	4	6	9	8	10	12
A ₁ B ₂	1	*	*	***	***	***	+	+	+	**	**	**	**	**	**
	13		*	***	***	***	+	+	+	**	**	**	**	**	**
A ₁ B ₁	2			*	*	*	**	**	**	+	+	+	+	+	+
	3				*	*	**	**	**	+	+	+	+	+	+
	14					*	**	**	**	+	+	+	+	+	+
A ₂ B ₁	5						*	*	*	***	***	***	***	***	***
	7							*	*	***	***	***	***	***	***
	11								*	***	***	***	***	***	***
A ₂ B ₂	4									*	*	*	*	*	*
	6										*	*	*	*	*
	9											*	*	*	*
	8												*	*	*
	10													*	*
	12														*

+ = compatible mating (true clamps were detected)

* = incompatible mating (overlap)

** = hemicompatible-B (barrage)

*** = hemicompatible-A (flat)

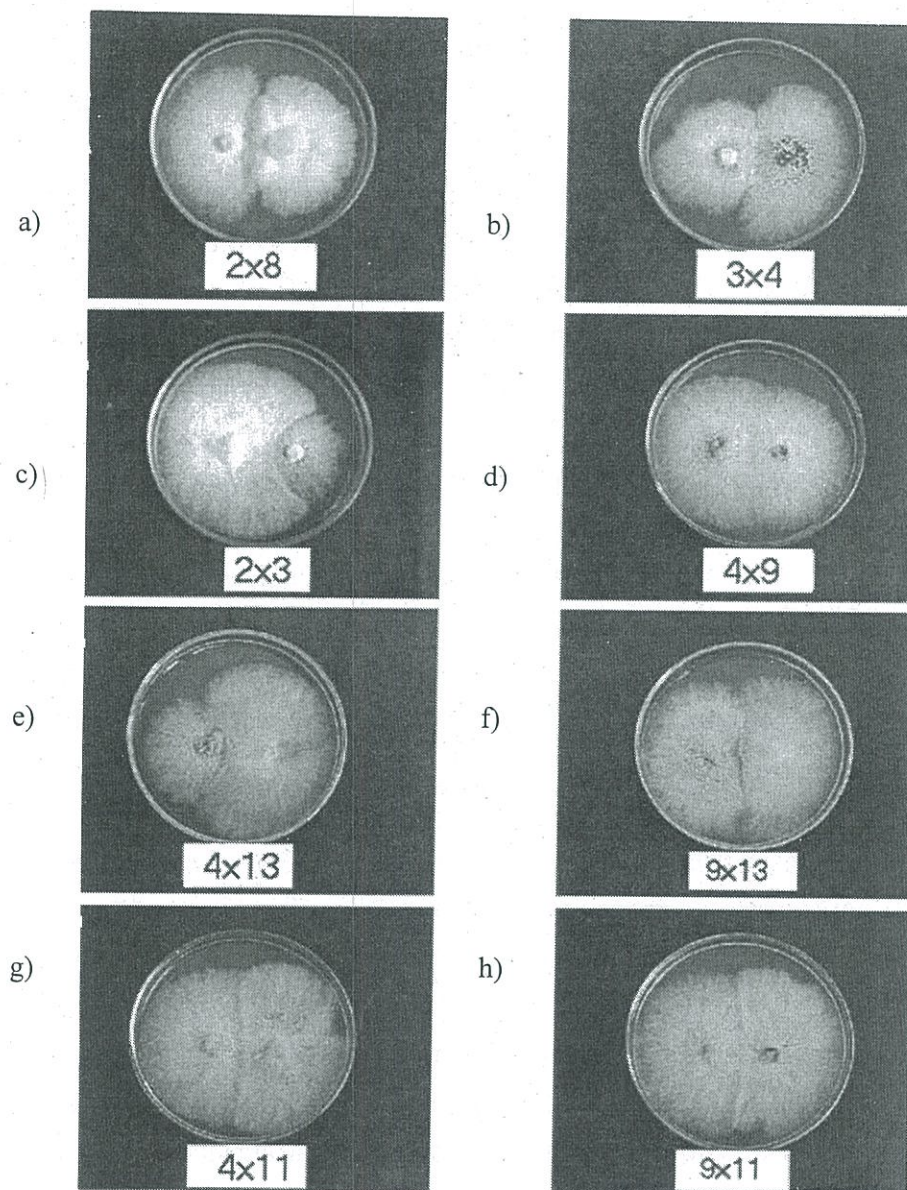


Figure 3 Colony characteristics of some pairs of the matings of the 14 SSIs of *P. cystidiosus* on MEA at 30°C.

- a) (crossing between isolates 2 and 8) and b) (crossing between isolates 3 and 4) represent compatible matings ($A \neq B \neq$)
- c) (crossing between isolates 2 and 3) and d) (crossing between isolates 4 and 9) represent incompatible matings ($A = B =$, overlap)
- e) (crossing between isolates 4 and 13) and f) (crossing between isolates 9 and 13) represent hemicompatible-B matings ($A \neq B =$, common B, barrage)
- g) (crossing between isolates 4 and 11) and h) (crossing between isolates 9 and 11) represent hemicompatible-A matings ($A = B \neq$, common A, flat)

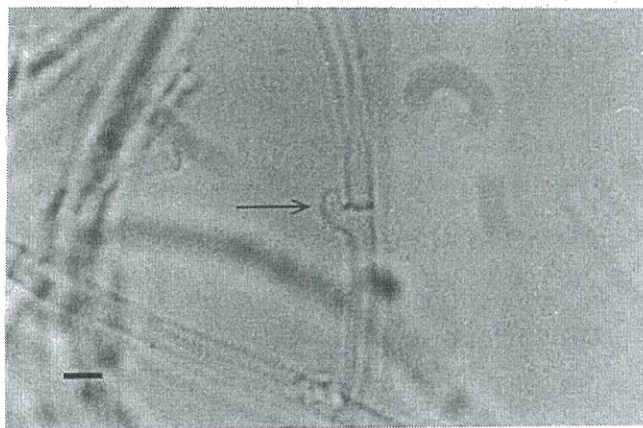


Figure 4 False clamps (arrow) from a $A \neq B$ crossing of *P. cystidiosus* (1000 $\times$), bar = 5 microns

4. ACKNOWLEDGEMENTS

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