CHAPTER 7

GENETICS AND BREEDING OF SPORE-DEFICIENT STRAINS IN *AGROCYBE CYLINDRACEA* AND *LENTINUS EDODES*

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1. INTRODUCTION

Spores discharged from cultivated mushrooms may give rise to several problems: (a) respiratory allergies among mushroom growers which are known to be caused by antigens present on the spore surface, (b) reduced commercial value because of spores deposited on mushrooms, (c) increased populations of mites and other microorganisms feeding on fungal spores in the cultivation rooms, (d) erosion of genic variation in natural populations of the mushroom species. To solve these problems, spore-deficient strains may be useful.

In this paper, the cytology and genetics of a sporeless mutant of *Lentinus edodes* are presented (Hasebe *et al.*, 1991). In relation to this mutant, problem (d) is discussed in relation to the large-scale outdoor cultivation of shiitake. Secondly, induction of spore-deficient mutants of *Agrocybe cylindracea* by UV-irradiation and their use in breeding is described. In addition, spore deficiency resulting from matings between geographically separated strains of *A. cylindracea* (Japanese and European) is introduced.

2. SPORELESS MUTANT OF *LENTINUS EDODES*

During genetical analysis of a *Lentinus edodes* mutant bearing aberrant clamp connections (Murakami *et al.*, 1987), a spontaneously occurring sporeless mutant (TMI-1597) was found. Its sporeless fruitbodies were of normal morphology but a spore print could not be collected. When examined under scanning electron microscope , basidia on a hymenium of a wild type fruitbody matured asynchronously (Fig. 1). The basidia usually formed four sterigmata whose apices subsequently swelled and developed into basidiospores. In the sporeless mutant, basidia at different developmental stages were also present, but only up to the stages of sterigma formation; sterigmata developed but their distal ends rarely swelled (Fig. 2). The majority of autolyzed basidia bore

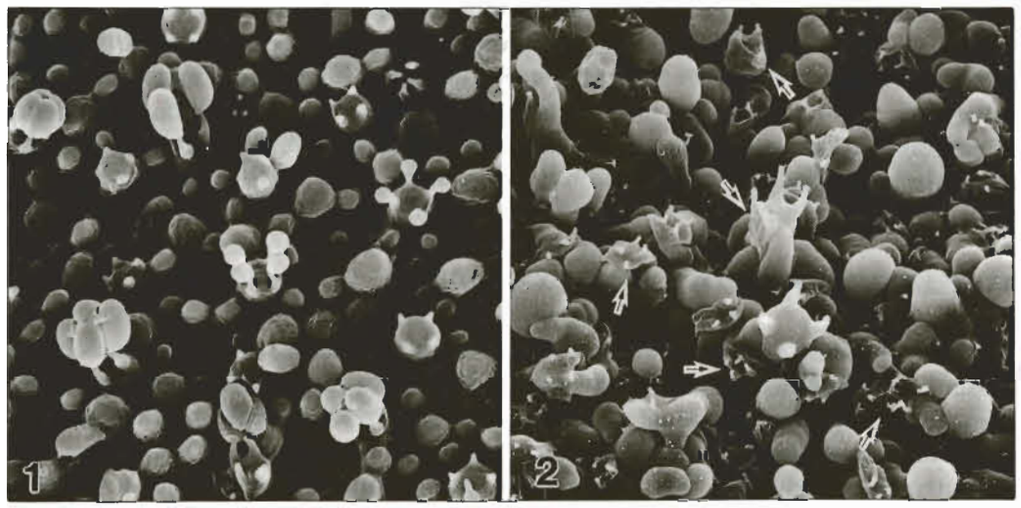
remnants of sterigmata only (Fig. 2).

Nuclear behaviour during basidiospore formation in the sporeless mutant was studied under the light microscope, using the HCl-Giemsa staining protocol. Nuclear fusion and meiosis in the mutant were completed normally ending in a stage with tetrads of nuclei. After meiosis, sterigmata developed on the basidium but swelling of the distal ends was not usually evident. Murakami & Takemaru (1985) reported that a post-meiotic mitosis took place in *L. edodes* corresponding to the pattern C defined by Duncan and Galbraith (1972): a single post-meiotic mitotic division occurs in each basidiospore while the spore is still attached to the sterigma, and then one of the daughter nuclei will move back to the basidium.

We also examined the post-meiotic nuclear behaviour in abortive basidia of normal fruitbodies and found that the post-meiotic mitosis took place within basidia which thus became eight-nucleate. However, we could not find any eight-nucleate basidia in the sporeless mutant. It is apparent that during the period between meiosis and post-meiotic mitosis, the cytoplasm of the basidium degenerated to such an extent that the tetrads of nuclei were unable to undergo further division.

When the component homokaryons (Sp1 and Sp2) of the sporeless mutant were each mated to the wild-type homokaryon #9, both the resulting dikaryons formed normal basidiocarps bearing numerous basidiospores. From a basidiocarp of Sp1 x #9 dikaryon, 193 single spore isolates were taken, and all of these homokaryons were crossed with Sp2 to make 193 dikaryons. One hundred of these dikaryons produced sporeless basidiocarps and 93 produced normal ones (Table 1). These results indicated that deficiency in sporulation is caused by a single recessive gene, *spo*. Linkage analysis showed that the *spo* gene was not linked to either A or B incompatibility factor.

Respiratory allergies caused by the inhalation of shiitake spores have been reported (Kondo, 1969; Sastre *et al.*, 1990), and this problem appears to have escalated with the increase in indoor cultivation of shiitake. On the other hand, the large-scale outdoor cultivation of shiitake by the bedlog method may reduce the extent of genetic variation in natural populations. Circumstantial evidence



FIGURES 1 & 2. Scanning electron micrographs of gill surfaces of a wild strain and the sporeless mutant of *Lentinus edodes*. 1. Wild type. Various stages of sporulation are shown. 2. Sporeless mutant. Arrows point to autolyzed basidia bearing remnants of sterigmata.

for this problem is provided by the observation that the number of alleles at both A and B incompatibility loci is significantly lower when calculated using data from western Japan (A = 40-65, B = 63-100) (Tokimoto *et al.*, 1973) compared to values based on data collected in Hokkaido (A = 130-152, B = 192-205). Traditionally, there have been many large-scale shiitake farms in western Japan, but only a few in Hokkaido. We speculate that genic variation of *L. edodes* has been significantly reduced by the spores discharged from major cultivars. Use of sporeless cultivars would, therefore, be effective in reducing these problems.

3. BREEDING SPORE-DEFICIENT STRAINS OF *AGROCYBE CYLINDRACEA*

This breeding programme is aimed primarily at reducing problems (a) and (b) referred to in the Introduction, because this mushroom produces numerous brown-pigmented basidiospores.

3.1. Induction of Spore-deficient Mutants

Dikaryotic hyphal fragments from a strain (2493) were irradiated with a Toshiba 10-watt

TABLE 1. Genetic analysis of the basidiospore progeny derived from the cross between Sp1 (genotype: *spo A1 B1*), one of the component homokaryons of the sporeless mutant, and the wild-type homokaryon #9 (genotype: + *A2 B2*) of *Lentinus edodes*.

Mating type	Sporeless	Wild type	Total
A1B1	20	19	39
A2B2	36	26	62
A1B2	19	19	38
A2B1	17	17	34
A1Brec	6	4	10
A2Brec	2	8	10
Total	100	93	193

Analysis:

Segregation of

					X^2 (1:1)	P
Sporeless	*spo*	100	+	93	0.25	0.750 - 0.500
A-factor	*A1*	87	*A2*	106	1.87	0.250 - 0.100
B-factor	*B1*	73	*B2*	100	4.21	0.050 - 0.025

Recombination between

	Parental	Recomb.		
spo and *A*	96	97	0.01	- 0.900
spo and *B*	82	91	0.47	0.500 - 0.250
A and *B*	101	72	4.86	0.050 - 0.025
B-factor*	173	20	121.29	0.005 -

* Recombination value = 10.4%.

germicidal lamp for 30-40 sec at a distance of 10cm. The irradiated fragments were then plated on CY-2 medium containing 20g glucose, 0.5g $MgSO_4 \cdot 7H_2O$, 0.46g KH_2PO_4, 1g K_2HPO_4, 2g polypeptone, 2g yeast extract and 20g agar per litre of water, and incubated at 25°C. The UV treatment and the incubation were performed in darkness. Lethal rate was more than 95%. Viable colonies were then isolated and transferred on to fresh CY-2 medium. A few days later, the recovered colonies were examined for clamp connections. The isolates obtained were inoculated on slants of CY-2 medium and placed under conditions suitable for fruiting. The fruitbodies produced were examined for production of basidiospores. This fungus produced fruitbodies on CY-2 medium; the fruitbodies were very small but otherwise normal in their morphology and spore productivity. However, since germination of basidiospores did not occur on CY-2 medium, PSA medium (extract of 200gm fresh pototoes, 20 gm sucrose and 20gm agar per liter of water) was used for germination tests. Final assessment of the morphology of fruitbodies, cultural characteristics and deficiency of spore formation were done using plastic bottles filled with sawdust medium (400g of air-dried sawdust wetted evenly with a liter of tap water, containing 80gm of rice bran). From 11,500 colonies treated with UV irradiation, 24 spore-deficient mutants and 233 strains with reduced sporulation were detected. Imbernon & Labarere (1989) reported that UV treated strains of *Pleurotus ostreatus* and *P. pulmonarius* exhibited some abnormality in morphology and yields of fruitbodies. In our experiment, pilei of the spore-deficient mutants were either very thin at the margin or small in size, or their stipes were slender and became bent.

3.2. Strain Improvement

In several fungi, di-mon mating is useful for introducing one nucleus of a dikaryon into another homokaryon. However, in *A. cylindracea*, this method is not useful because of lack or delay of the nuclear migration through the homokaryotic mycelia. The sporeless mutants obtained produced very few basidiospores, which did not germinate well (< 2%). About a thousand basidiospore progenies gathered from all these mutants were mated with four wild tester homokaryons having different mating types derived from the strain 2943. Resultant dikaryons were then grown on sawdust medium and examined for spore deficiency. It was found that 153 progenies carried the spore deficient characters, indicating that these spore deficiencies were dominant characters. From the result obtained, several spore-deficient homokaryons which had the ability to produce normal fruitbodies were selected.

3.3. Hybrid Construction

To change the colour of fruitbodies according to the consumer's preference is one of the important aims for breeders. When the basidiospore progeny derived from a strain 2944 was crossed with the homokaryon from strain 2943, the fruitbodies produced from the resultant dikaryons were divided into two types: yellowish or brownish, at the ratio of 1:1 (unpublished data). The selected homokaryons from strain 2944 were then mated with previously selected spore-deficient homokaryons. Fruitbodies of resultant dikaryons were examined for their yield and morphology. Figure 3 shows the results obtained with selected strains (#43 and #44) and the control strain 2943. There were no significant differences between the three strains in their fruitbody yields.

Spore-deficient strains are now available for *A. cylindracea* and matings between spore-deficient progeny and homokaryons derived from a wild-type strain have improved cultural characteristics.

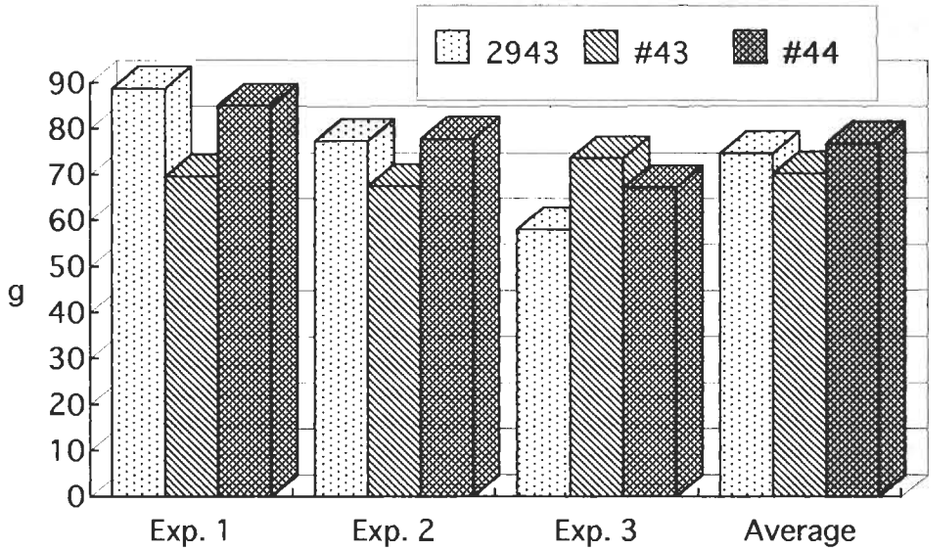
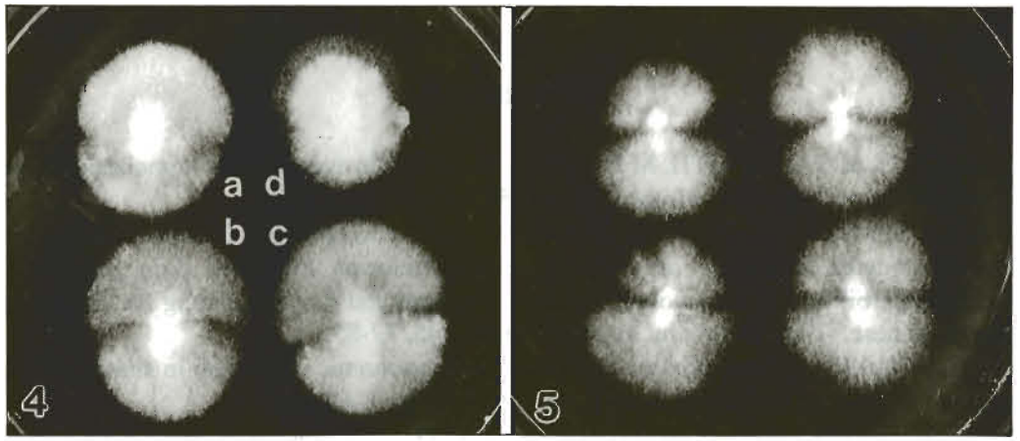


FIGURE 3. Fruitbody yield of selected strains (#43, #44) and control strain (2943) in *Agrocybe cylindracea*. The Y axis indicates mean fresh weight per 800ml bottle. Eight duplicates for respective strain in each yield experiment.

3.4. Spore Deficiency Resulting from Matings between Geographically Separated Strains

Marmisse (1989) examined the genetic variation in basidiocarp production within several strains of *A. aegerita* (= *A. cylindraea*). We also carried out similar experiments using several European and Japanese strains. During this study, we found curious and interesting phenomena. We confirmed the bifactorial incompatibility system in this mushroom (Esser *et al.*, 1974). As shown in Fig. 4, fully compatible matings were visibly distinguishable from other non-compatible matings by the denser and white, but relatively smaller, dikaryotic colony formed in the contact zone.



FIGURES 4 & 5. Mating reactions in *Agrocybe cylindracea*. 4. Intrastrain crosses. a: common-AB, b: common-A, c: common-B, d: compatible. 5. Compatible mating reactions in the interstrain crosses between European and Japanese strains.

FIGURE 6. Fruitbodies of a dikaryon derived from the intercross between a European strain and a Japanese strain in *Agrocybe cylindracea*. Gills are fairly white because of significantly reduced number of basidiospores.

Compatible matings of intracrosses between European strains and between Japanese strains all showed this response. However, in all intercrosses between European and Japanese strains, the compatible mating reaction was very different. In such crosses, barrage like reaction, characterized by a contact zone of scanty mycelium bordered by somewhat more congested hyphae, was always observed (Fig. 5). Clamped dikaryotic hyphae were not observed in the margin of these colonies. When the central regions of the contact zone were cut and reinoculated on to fresh medium, most of the inocula produced dikaryotic hyphae.

Most of the resultant dikaryons readily produced fruitbodies of normal morphology but with significantly reduced numbers of basidiospores. The degree of spore production of fruitbodies varied with the combination of homokaryons. As shown in Fig. 6, gills of some dikaryotic strains were white because of much reduced spore production. The germination frequency of the basidiospores derived from all of these basidiocarps was relatively high, but most of the germ tubes soon ceased to grow. Thus less than 4% of the germinated spores formed colonies. The reduction in compatibility, spore production and viability in the basidiospore progeny stated above, may have an important implications for microevolution in fungi.

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