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SHORT COMMUNICATION

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Morphological mutation of *Lentinula edodes* mycelium, particularly detectable in the dikaryotic state

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Abstract A morphological mutation particularly detectable in the dikaryotic state was found in *Lentinula edodes*. The mutant dikaryon was readily distinguishable from the normal dikaryon by the irregularly branched short hyphae, very slow hyphal growth, and sparse aerial hyphae. Genetic analysis revealed that expression of this mutation was controlled by a single recessive gene, *mor-13*. Linkage analysis showed that the *mor-13* was not linked to either the incompatibility factors (*A* and *B*) or the five kinds of *mor* genes that were segregated independently of each other in a previous study.

Key words Aberrant mycelial growth · *Lentinula edodes* · Morphological mutation

Shiitake, *Lentinula edodes* (Berk.) Pegler, is the major edible mushroom in Asia, particularly in China and Japan. Through breeding, many excellent strains have been developed and widely used. One of the serious problems in breeding and maintenance of Shiitake strains is mutation of important genes, and thus genetic studies of this fungus have practical importance. Several different mutations have been reported in *L. edodes*, e.g., those inducing nutrient deficiency (Murakami and Tsuneda 1982; Hasebe 1991), aberrant clamp connections (Murakami et al. 1987), sporeless hymenium (Hasebe et al. 1991), haplophasic lethal factors (Hasebe et al. 1992), and abnormal colony or basidiocarp morphology (Komatsu and Kimura 1964a,b, 1968; Murakami and Takemaru 1975; Hasebe et al. 1982, 1987). Sixteen homokaryotic, morphological mutants were classified into four groups based on their hypal growth habits and colony morphology. Of these, 15 mutants were caused by a single recessive gene, and 1 carried 2 independent recessive genes. Nonallelic relations were recognized in 12 genes, and these were designated as *mor* (*mor-1* to *mor-12*) (Hasebe et al. 1987; Hasebe 1991). We recently found a new morphological mutation, particularly detectable in the dikaryotic state. In this article, we report the morphological characteristics and the genetics of this mutation.

This mutation was found in the crosses between basidiospore progeny of a commercial strain and was deposited at the Tottori Mycological Institute Culture Collection (TMIC-1822). Colony morphology and mycelial growth of the wild-type and the mutant dikaryons on PDA medium at 25°C are shown in Fig. 1. The mutant dikaryon was readily distinguishable from the wild-type dikaryon (W-15 × W-22) by short and abnormally branched hyphae, very slow hyphal growth, and sparse aerial hyphae of the colony. Aerial hyphae of the component homokaryon (1822a) of the mutant dikaryon were somewhat denser than those of the wild-type (W-15). However, no significant difference was recognized in the hyphal growth habits between the homokaryons of the two (Fig. 2). Therefore, it was difficult to accurately distinguish the mutant-type from the wild-type by their hyphal growth and colony morphology in the homokaryotic state. This mutation did not belong to any of the four groups, into which Hasebe (1991) classified 16 homokaryotic morphological mutants based on their hyphal growth habits and colony morphology.

When the component homokaryons (1822a, mating type A^xB^x; 1822b, mating type A^mB^m) of the mutant were mated to the wild-type homokaryons (W-15, A^yB^y; M620, A^nB^n), the resulting dikaryons (1822a × W-15 and 1822b × M620) showed wild-type morphology. Single basidiospore isolates were taken from the basidiocarps of these two dikaryons, and all these homokaryons were crossed with a compatible homokaryon of the mutant (#83, A^lB^l), which has been selected from basidiospore isolates from the cross between mutant (1822a) and wild-type (M4, A^lB^l) homokaryons. As shown in Table 1, approximately half the dikaryons in each

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cross exhibited mutant morphology. These results indicate that the expression of this mutation is controlled by a single recessive gene; we hereby call this *mor-13*. Linkage analysis of the basidiospore progeny of the 1822b × M620 dikaryon showed that the *mor-13* gene was not linked to either the *A* or the *B* incompatibility factor (Table 2). Five linkage groups have been established by the analyses of 12 kinds of recessive genes for homokaryotic morphological mutations (*mor-1* to *mor-12*) and 11 kinds of nutrient-requiring genes (Hasebe 1991). Therefore, a crossing experiment was carried out between the *mor-13* gene and five kinds of *mor* genes that were segregated independently of each other in a previous study (Hasebe 1991). When the mutant homokaryon 1822a (genotype, *mor-13*) was mated to the homokaryon (#78) carrying *mor-3, mor-4, mor-9, mor-10*, and *mor-12* genes, the resulting dikaryon showed normal colony morphology, indicating nonallelic relation. Linkage analysis for the cross between 1822a and #78 showed that the *mor-13* gene was not linked to *mor-3, mor-4, mor-9, mor-10*, or *mor-12* (Table 3). On the other hand, a recent study demonstrated 11 linkage groups based on the results of amplified fragment length polymorphism (AFLP) analysis (Terashima et al. 2002). A total of 203 AFLP markers are

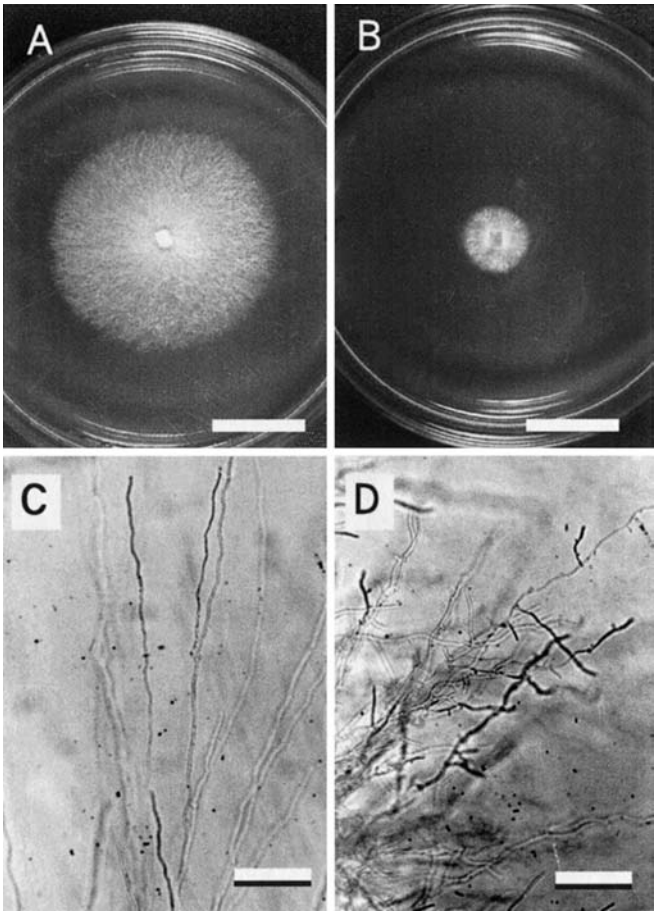

Fig. 1. Mycelial (**A**, **B**) and hyphal (**C**, **D**) morphologies of wild-type and mutant dikaryon of *Lentinula edodes*. **A,C** Wild-type dikaryon (W-15 × W-22); **B,D** mutant-type dikaryon (TMIC 1822). *Bars* **A,B** 2 cm; **C,D** 100 μm

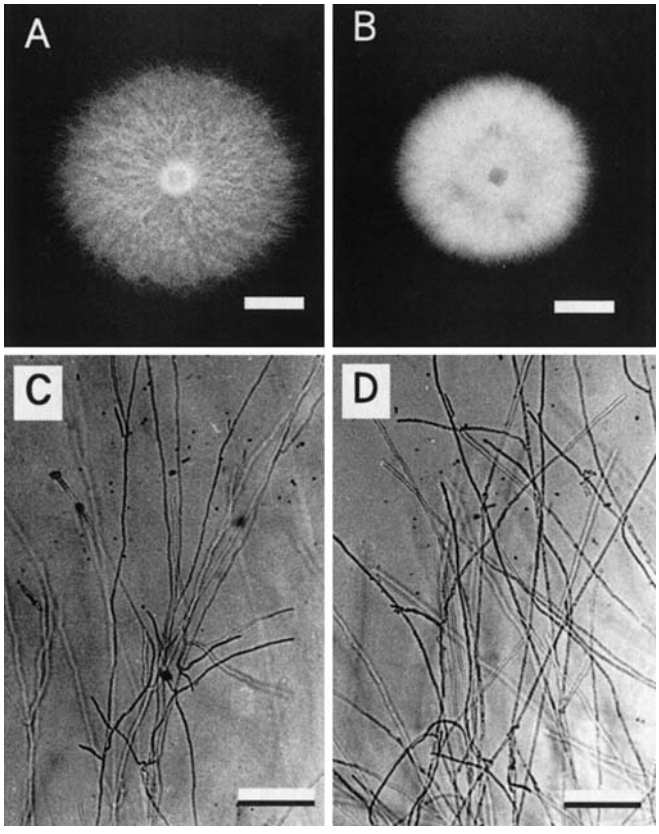

Fig. 2. Mycelial (**A**, **B**) and hyphal (**C**, **D**) morphologies of wild-type and mutant homokaryon of *Lentinula edodes*. **A,C** Wild-type homokaryon (W-15); **B,D** mutant-type homokaryon (1822a). *Bars* **A,B** 1 cm; **C,D** 100 μm

Table 1. Segregation in the basidiospore progeny from the crosses between mutant (1822a and 1822b) and wild-type (W-15 and M620) homokaryons of *Lentinula edodes*

Cross	Mutant[a]	Wild-type[a]	χ^2 (1:1)	*P*
1822a × W-15	95	112	1.40	0.25–0.10
1822b × M620	125	117	0.26	0.75–0.50
Total	220	229	0.18	0.75–0.50
Homogeneity[b]			1.48	0.25–0.10

[a] Genotype was identified by colony morphology of the dikaryon resulting from the cross between a basidiospore isolate and a compatible mutant homokaryon (#83)

[b] χ^2 value of homogeneity ($\chi^2 = 1.48$, *d.f.* = 1, $0.25 > P > 0.10$) was calculated by 1.40 + 0.26 – 0.18 = 1.48; this indicates that basidiospore progenies of the two crosses tested belong to the same population in which the mutant and wild-type are expected to segregate in the ratio of 1:1

Table 2. Genetic analysis of the basidiospore progeny derived from the cross between mutant 1822b (genotype, *mor-13* A^mB^m) and wild-type homokaryon M620 (genotype, + A^nB^n) of *Lentinula edodes*

Mating type	Mutant[a]	Wild-type[a]	Total
A^mB^m	27	25	52
A^nB^n	28	29	57
A^mB^n	34	21	55
A^nB^m	27	30	57
A^mB^{rec}	4	7	11
A^nB^{rec}	5	5	10
Total	125	117	242

Analysis						
Gene		Segregation			χ^2 (1:1)	*P*
Mutant	*mor-13*	125	+	117	0.26	0.75–0.50
A-factor	A^m	118	A^n	124	0.15	0.75–0.50
B-factor	B^m	109	B^n	112	0.04	0.90–0.75
Gene pair	Parental		Recombinant			
mor-13 and *A*	129		113		1.06	0.50–0.25
mor-13 and *B*	104		117		0.76	0.50–0.25
A and *B*	109		112		0.04	0.90–0.75
B-factors[b]	221		21		165.29	<0.005

[a] Genotype was identified by colony morphology of dikaryon resulting from the cross between a basidiospore isolate and a compatible mutant homokaryon #83 (genotype, *mor-13* A^lB^l)
[b] Recombination value = 8.7%

Table 3. Linkage analysis between *mor-13* gene and five kinds of *mor* genes in the basidiospore progeny derived from the cross between 1822a (genotype, *mor-13*) and #78 (genotype, *mor-3, mor-4, mor-9, mor-10*, and *mor-12*) of *Lentinula edodes*

Gene pair	Segregation		χ^2 (1:1)	*P*
	Parentals	Recombinants		
mor-13–mor-3	100	83	1.58	0.30–0.20
mor-13–mor-4	89	94	0.14	0.80–0.70
mor-13–mor-9	95	88	0.24	0.70–0.50
mor-13–mor-10	95	89	0.27	0.70–0.50
mor-13–mor-12	94	89	0.14	0.80–0.70

distributed in the AFLP map. Further studies are needed to determine the location of the marker genes including *mor* genes and nutrient-requiring genes on the AFLP linkage map.

To examine whether commonly used commercial strains carry the *mor-13* gene, 10–20 basidiospore isolates were taken from each of eight cultivars developed by four different spawn companies and were mated to the tester homokaryons carrying the *mor-13* gene to make eight dikaryotic populations corresponding to the progenies of the cultivars. The mutant and wild-type dikaryons appeared approximately in the ratio of 1:1 in each of the seven populations, and only wild-type dikaryons appeared in one population. These results indicated that, of eight cultivars tested, seven strains are heterozygous for the *mor-13* gene. It seems that this mutation did not occur independently in each of the seven cultivars during maintenance of strains, but occurred in one cultivar, possibly in one of the earliest cultivars, and was widely distributed among many cultivars through cross-breeding. There is a possibility that the *mor-13* gene or closely linked gene(s) may participate in the expression of some superior agronomic traits, such as colonization in the substrates, fruiting quality, and mushroom productivity.

References

Hasebe K (1991) Genetic studies on mutants and agronomic characters in Shiitake, *Lentinus edodes* (in Japanese with English summary). Rep Tottori Mycol Inst 29:1–69

Hasebe K, Tokimoto K, Komatsu M (1982) "Dwarf" mutant of *Lentinus edodes* (Berk.) Sing (in Japanese with English summary). Rep Tottori Mycol Inst 20:113–116

Hasebe K, Murakami S, Komatsu M (1987) Genetic analysis of some morphological mutations in homokaryotic mycelia of *Lentinula edodes* (in Japanese with English summary). Rep Tottori Mycol Inst 25:56–61

Hasebe K, Murakami S, Tsuneda A (1991) Cytology and genetics of a sporeless mutant of *Lentinus edodes*. Mycologia 83:354–359

Hasebe K, Murakami S, Tsuneda A (1992) Cytology and genetics of a dikaryotic strain of *Lentinus edodes* carrying two haplolethal factors. Can J Bot 70:601–606

Komatsu M, Kimura K (1964a) Studies on abnormal fruit-bodies of the hymenomycetous fungi. III. Fruit-bodies with brownish gills of *Lentinus edodes* (Berk.) Sing (in Japanese with English summary). Rep Tottori Mycol Inst 4:21–28

Komatsu M, Kimura K (1964b) Studies on abnormal fruit-bodies of the hymenomycetous fungi. IV. Sterile fruit-bodies of *Lentinus edodes* (Berk.) Sing. (in Japanese with English summary). Rep Tottori Mycol Inst 4:29–36

Komatsu M, Kimura K (1968) Studies on abnormal fruit-bodies of hymenomycetous fungi. V. Fruit-bodies with white pilei of *Lentinus edodes* (Berk.) Sing. (in Japanese with English summary). Rep Tottori Mycol Inst 6:9–17

Murakami S, Takemaru T (1975) "Puff" mutation induced by UV irradiation in *Lentinus edodes* (Berk.) Sing. Rep Tottori Mycol Inst 12:47–51

Murakami S, Tsuneda A (1982) Adenine-requiring mutant of *Lentinus edodes*, extremely susceptible to attack by *Trichoderma* species. Rep Tottori Mycol Inst 20:54–62

Murakami S, Hasebe K, Tsuneda A (1987) A mutant of *Lentinus edodes* forming aberrant clamp connections. Rep Tottori Mycol Inst 25:49–55

Terashima K, Matsumoto T, Hayashi E, Fukumasa-Nakai Y (2002) A genetic linkage map of *Lentinula edodes* (shiitake) based on AFLP markers. Mycol Res 106:911–917