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NOTE

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Biological and inherited characteristics of a newly identified *Lentinula edodes* strain capable of forming a fruiting body without a shift to low temperature

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Abstract A strain of the basidiomycete *Lentinula edodes* (Shiitake) was newly identified from the mushroom library of Mori Sangyo Co., Ltd., Japan. This strain, named MIL-LEW-M13-1, is capable of forming the fruiting body on sawdust-based medium without a reduction in temperature. Mating experiments with a monokaryotic mycelium of *L. edodes* strain that does require low temperature for fruiting–body formation suggest that the unique property of the MIL-LEW-M13-1 strain is a dominant trait that can be inherited by its progeny in a nucleus-dependent manner.

Key words Basidiomycete · Fruiting body formation · *Lentinula edodes* · Temperature dependent

Fruiting–body formation is the most conspicuous and complex process in cell differentiation of the basidiomycetes. The popular edible mushroom *Lentinula edodes* (Shiitake) (Shishido 1992) forms its fruiting body on the logs of broad-leaved trees. Thus, for commercial production of *L. edodes* mushrooms, logs and sawdust-based media are frequently used. Fruiting–body formation in this fungus is induced by several environmental factors, including light, moisture, and a decrease in temperature from 25°C to 17°–18°C (Ishikawa 1967; Matsumoto and Kitamoto 1987). Fruiting body production of a typical strain of *L. edodes* on sawdust-based medium occurs as follows: the dikaryotic mycelium grows vegetatively in the sawdust-based medium at 25°C for about 40 days under light exposure and high moisture. The cultivated mycelium is flooded with water at 17°–18°C for 24 h and then incubated at 17°–18°C (Matsumoto and Kitamoto 1987). Many primordia are formed on the surface of the sawdust-based medium after about 10 days, several of which grow into mature fruiting bodies. This entire process of forming a mature fruiting body of *L. edodes* usually takes at least 2 months.

In this study, we tried to produce fruiting bodies of many strains of *L. edodes* (Mori Sangyo Co., Ltd., Japan) in sawdust-based medium at 25°C for about 40 days (data not shown). One of them, the MIL-LEW-M13–1 strain, exhibited primordia formation on the 40th day, in contrast to FMC2 (mating type, *A1B1* + *A2B2*), a more typical *L. edodes* strain (Katayose et al., 1986) that only initiated the fruiting process upon transfer to 18°C. To investigate further fruiting body formation in MIL-LEW-M13-1, the dikaryotic mycelium was inoculated into 150 g sawdust-based medium [28% *Fagus crenata* sawdust (w/w), 7% corn bran (w/w), 65% water (w/w)] in a 300-ml culture flask and cultivated at 25° ± 1°C under 65% moisture and 1000–1300 lux of light for 12-h periods. The average time (in 13 independent experiments) elapsed before primordia formation (more than one per flask) was 39.2 ± 2.8 days. After about 50 days, mature fruiting bodies appeared on the medium. When the MIL-LEW-M13-1 strain was cultured under these conditions but without any light exposure, no fruiting bodies were formed for more than 80 days in all the samples. These results suggest that white light is necessary for the formation of fruiting bodies in the MIL-LEW-M13-1 strain, cultivation at 18°C is not.

Next, we investigated whether the progeny of the MIL-LEW-M13-1 strain dominantly inherit the ability to form fruiting bodies without low-temperature cultivation. Eighteen basidiospores were isolated from a MIL-LEW-M13-1 fruiting body, and the mating type of each spore was determined by crossing with each other and with FMC2-1.1 (*A1B1*) and FMC2–1.2 (*A2B2*), monokaryons derived from the FMC2 strain (Yasuda and Shishido 1999). Mating ex-

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Table 1. Genetic and biological analyses of MIL-LEW-M13-1 strain of *Lentinula edodes*

Spores isolated			Mating strain	Numbers of dikayotic mycelia forming primordium[a]	Days required for primordium formation
Mating types	Numbers				
A3B3	6			4	32.8 ± 4.9
A3B4	4		FMC2-1.1	4	36.0 ± 9.4
A4B3	3	×	*A1B1*	2	33.0
A4B4	5			5	35.4 ± 3.8
Total	18			15 (83.3%)	34.5 ± 5.5

[a] The 18 dikaryotic mycelia obtained by mating with FMC2-1.1 (*A1B1*) were cultivated in sawdust-based medium at 25°C under 65% moisture and light exposure

Table 2. Genetic and biological analyses of CSM2 strain of *L. edodes*

Spores isolated			Mating strain	Numbers of dikayotic mycelia forming primordium[a]	Days required for primordium formation
Mating types	Numbers				
A1B1	5			3	57.0 ± 7.9
A1B3	5		FMC2-1.2	2	56.5
A3B1	5	×	*A2B2*	2	49.0
A3B3	6			3	54.0 ± 10.0
Total	21			10 (47.6%)	54.4 ± 7.5

[a] The 21 dikaryotic mycelia obtained by mating with FMC2-1.2 (*A2B2*) were cultivated in sawdust-based medium at 25°C under 65% moisture and light exposure

periments were done on potato dextrose agar (PDA) plates by incubation at 25°C for about 10 days. Clamp connections, which occur in the dikaryon ($A\pi\ B\pi$), were examined by microscopy, and barrages, which occur in the common-B heterokaryon ($A\pi\ B=$), were observed visually according to the method reported by Raper (1966). We found that the 18 basidiospores were grouped into four mating types: *A3B3*, *A3B4*, *A4B3*, and *A4B4* (Table 1). The 18 dikaryotic mycelia (F_1 progeny) produced by mating with FMC2-1.1 strain, termed CSM1–CSM18, were cultivated on sawdust-based medium at 25°C under 65% moisture and light exposure at 1000–1300 lux. Fifteen of the 18 dikaryons were found to be capable of forming a fruiting body, with an average time required for primoridum formation of 34.5 ± 5.5 days (see Table 1). These results indicate that most of the F_1 progeny dominantly inherited the genotype of the MIL-LEW-M13-1 strain.

The 21 basidiospores isolated from one of the F_1 progeny, the CSM2 strain (*A1B1* + *A3B3*), were able to form fruiting bodies without cultivation at 18°C. From the mating experiments, they were found to be grouped into four mating types: *A1B1*, *A1B3*, *A3B1*, and *A3B3*. The 21 derivatives were mated with monokaryotic mycelia of the FMC2-1.2 strain (*A2B2*). Each the obtained dikaryons (F_2 progeny) were cultivated in sawdust-based medium at 25°C. Ten of these 21 dikaryons formed fruiting bodies without cultivation at 18°C, with an average period required for primordium formation (54.4 ± 7.5 days) that was longer than that of the MIL-LEW-M13-1 strain as well as that of the F_1 progeny (Table 2). Furthermore, these 10 strains were found to include all four mating types (Table 2). These results suggest that the locus of the genotype derived from MIL-LEW-M13-1 strain is not linked with the mating locus, and that the property derived from the MIL-LEW-M13-1 strain is dependent on the nucleus, not the cytoplasm, for inheritance. To confirm this hypothesis, the mycelia of the CSM2 strain (*A1B1* + *A3B3*) were mated with FMC2-1.1 (*A1B1*) according to the di-mon mating protocol reported by Buller (1931). The four dikaryotic mycelia (*A1B1* + *A3B3*) isolated by this method were cultivated in sawdust-based medium at 25°C. All these dikaryons of *L. edodes* were found to form fruiting bodies, with an average period of 30.4 ± 1.2 days. These results suggest that the ability to form fruiting bodies without low-temperature cultivation exhibits nuclear inheritance. Further genetic and molecular biological research on *L. edodes* is required to clarify the gene locus that encodes this unique property of the MIL-LEW-M13-1 strain.

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