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Research Article

# Isolation and Characterization of Monokaryotic Strains of *Lentinula edodes* Showing Higher Fruiting Rate and Better Fruiting Body Production

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**Abstract** The effects of monokaryotic strains on fruiting body formation of *Lentinula edodes* were examined through mating and cultivation of the mated dikaryotic mycelia in sawdust medium. To accomplish this, monokaryotic strains of *L. edodes* were isolated from basidiospores of the commercial dikaryotic strains, Chamaram (Cham) and Sanjo701 (SJ701). A total of 703 matings (538 self-matings and 165 outcrosses) were performed, which generated 133 self-mates and 84 outcross mates. The mating rate was 25% and 50% for self-mating and outcross, respectively. The bipolarity of the outcross indicated the multi-allelic nature of the mating type genes. The mating was only dependent on the A mating type locus, while the B locus showed no effect, implying that the B locus is multi-allelic. Next, 145 selected dikaryotic mates were cultivated in sawdust medium. The self-mated dikaryotic progenies showed 51.3% and 69.5% fruiting rates for Cham and SJ701, respectively, while the fruiting rate of the outcross mates was 63.2%. The dikaryotic mates generated by mating with one of the monokaryotic strains, including A20, B2, E1, and E3, showed good fruiting performance and tended to yield high fruiting body production, while many of the monokaryotic strains failed to form fruiting bodies. Overall, these findings suggest that certain monokaryotic strains have traits enabling better mating and fruiting.

**Keywords** Fruiting rate, *Lentinula edodes*, Mating, Monokaryotic strains

New mushroom strains have been generated by common breeding methods, including mating [1-3], protoplast fusion [4], and molecular genetic transformation [5, 6]. Mating of different mycelia, which is the most widely applied technique, involves random fusion of hyphae from two monokaryotic mycelia with different mating types. This method eventually generates dikaryotic mycelial cells, but only if both mycelia contain compatible mating type genes [2]. In the tetrapolar mating system, mushrooms form four different haploid basidiospores and their mating is regulated by mating type genes in two independent genomic loci, A and B [7-9]. Mating type locus A encodes a transcription factor containing homeodomains, while the B locus expresses pheromones and pheromone receptors [10, 11]. Successful mating requires compatible pairing of the two loci, therefore, the rate of the actual mating is only 25% [2]. When the newly formed dikaryotic mycelial strains are ready, they are cultivated to produce fruiting bodies, which are used to screen for strains with good commercial and cultivation traits. However, dikaryotic strains do not always produce fruiting bodies [12, 13]. The fruiting rate of the newly formed strain, which is the ratio of fruiting body-forming strains per total dikaryotic strains, is highly dependent on environmental conditions and mushroom species. *Lentinula edodes* in this study shows that the fruiting rate is somewhere between 50% and 70%. When combined with the mating rate, the actual fruiting rate from the total mates is at most 18%; accordingly, it is necessary to make long-term efforts for the development of new varieties of mushroom.

Diversification of genetic pools is one strategy to overcome the 25% mating rate in the tetrapolar mating system. Many studies have shown that mushroom mating type genes can be present as multiple alleles, even within the same species [10, 11, 14-16]. Sixteen and 15 alleles at loci A and B, respectively, were reported from 12 strains of *Pleurotus eryngii*, suggesting that *P. eryngii* can have at least 240 (15 × 16) different compatible mating pairs when different pairs

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of monokaryotic strains are used [3]. Random mutagenesis by chemical or physical means does not change the mating rate, but can be applied to enhance the diversity of the genetic pool [13, 17, 18]. A temperature-adapted strain of *P. ostreatus* and a high β-glucan producing *Hypsizygus marmoreus* were recently developed using a combination of mycelial mating and chemical mutagenesis [17, 19]. Therefore, diversification of the genetic pool by either vast collection from the wild or physicochemical mutagenesis is necessary for generation of new strains and varieties of mushrooms.

*L. edodes* is one of the most cultivated mushrooms in East Asia. This mushroom was traditionally cultivated on the logs of oak trees; however, the trend is now moving toward sawdust medium cultivation owing to its advantages for mass production, including low cost, ease of environmental control, and relatively short cultivation period. Even with the sawdust cultivation method, at least 3 months of mycelial development period are required for induction of fruiting body formation. Moreover, the harvesting period can last for several months with multiple rounds of physical stimulation. Therefore, development of new cultivars of *L. edodes* normally takes more than a year just for the first round in the developmental procedure and is thus extremely laborious and time-consuming.

In this study, we investigated the dikaryotic mycelia of *L. edodes* generated by random mating of basidiospore-derived monokaryotic mycelia. The influence of the monokaryotic mycelia was then examined in terms of the mating and fruiting characteristics, and certain monokaryotic strains were found to have a tendency to generate dikaryotic strains with better fruiting rate and fruiting body production yield.

## MATERIALS AND METHODS

**Strains and isolation of monokaryotic mycelia.** The *L. edodes* strains, Chamaram (Cham) and Sanjo 701 (SJ701), were obtained from the Forest Mushroom Research Center, Korea. Basidiospores were collected from the fruiting bodies of the Cham and SJ701 strains and were suspended in 1-mL potato dextrose broth (PDB; Vent Tech Bio Co., Eumseong, Korea), then spread on potato dextrose agar (PDA; Oxoid Ltd., Hampshire, England). The agar plates were subsequently incubated at 25°C for 3 days, after which the monokaryotic mycelial strains were isolated from the independent mycelial colonies. The monokaryotic strains from the Cham strain were designated as A strains, while those from the SJ701 strain were designated D strains.

To generate more genetic diversity, the basidiospores were treated with 0.01 vol% methymethane sulfonate (MMS; Sigma-Aldrich Co., St. Louis, MO, USA), an alkylating agent, using a previously described method [13]. The survival rate of the germinated spores was 93% at 0.01 vol% MMS. Rapid growing monokaryotic mycelia were isolated from PDA plates and the monokaryotic strains from the Cham strain were designated as B strains, while those from the SJ701 strain were E strains.

**Mating for the generation of dikaryotic strains.** Monokaryotic mycelia were screened based on the growth rate, which was determined by the radial diameter (cm) of mycelial grown on PDA for 7 days at 25°C. The selected monokaryotic mycelia were subjected to a mating experiment, in which mating was conducted by placing mycelial blocks (3 × 3 mm) of two opposite strains 1 cm apart on the same PDA plate. Success of mating was confirmed by the formation of clamp connections under the light microscope after incubation for 7 days at 25°C.

**Mycelial propagation and ripening in sawdust substrate.** The mated dikaryotic mycelia were grown in PDB for 14 days at 25°C, after which 10 mL of each culture broth was inoculated onto a sterile sawdust substrate consisting of oak tree sawdust (380 g), rice bran (20 g), and water (600 mL) in a polyethylene bag. The inoculated substrate bags were capped with cotton plugs and then placed in the culture room. The substrates were incubated for 30 days at 25°C in the dark to promote vegetative growth. When the mycelia were fully propagated through the substrate, the incubation temperature was shifted to 20°C with white light irradiation and the incubation was prolonged for an additional 70 days to induce ripening and browning of the mycelia.

**Induction of fruiting body production.** At the end of the ripening period, production of the fruiting body was induced by soaking treatment in which the substrate was immersed in water for 12 hr at 19°C. The treated substrate was then transferred to a production room with a temperature maintained at 23°C during the day and 15°C at night and a relative humidity of 80%. The first round harvest occurred 10 to 20 days after induction. The harvest was performed three times which took an additional 30~50 days, depending on the mushroom strains. All the fruiting bodies were collected and their fruiting characteristics were examined.

## RESULTS AND DISCUSSION

**Mating and fruiting characteristics of the mated dikaryotic mycelia.** Fast growing monokaryotic mycelial strains were selected from the germinating basidiospores of the cultivated strains. Sixteen A and 14 D monokaryotic strains for Cham and SJ701, respectively, were initially isolated. Additionally, 17 B and 19 E of Cham and SJ701 monokaryotic strains, respectively, were isolated after treatment with low level MMS. Self-mating (selfing) was performed by 272 (16 A strains × 17 B strains) and 266 (14 D strains × 19 E strains) crosses for the Cham and SJ701 strains, respectively. Outcrosses were also performed using 11 Cham-derived and 15 SJ701-derived monokaryotic strains. The mating rates of selfings for Cham and SJ701 were 24.3% and 25.2%, respectively (Table 1). Outcrosses between

**Table 1.** Overall fruiting and cultivation characteristics of the mated dikaryotic strains

| | | Cham × Cham (A × B) | SJ701 × SJ701 (D × E) | Cham × SJ701 (A or B) × (D or E) |
|---|---|---|---|---|
| Mating | No. of total matings | 272 | 266 | 165 |
| | No. of the mated strains with clamp connections | 66 | 67 | 84 |
| | Mating rate (%) | 24.3 | 25.2 | 50.9 |
| Fruiting | No. of the selected dikaryotic strains from the mating | 39 | 59 | 57 |
| | No. of the fruiting body-forming strains | 20 | 41 | 36 |
| | Fruiting rate (%) | 51.3 | 69.5 | 63.2 |
| Substrate utilization | Average initial substrate weight (g) | 1,151.8 ± 96.6 | 1,052.6 ± 74.5 | 1,050.7 ± 37.9 |
| | Average final substrate weight (g) | 513.4 ± 95.8 | 288.2 ± 62.0 | 412.3 ± 82.8 |
| | Average fruiting body weight (g) | 102.1 ± 58.7 | 115.9 ± 51.8 | 118.1 ± 50.6 |
| | Fruiting body yield (%) | 8.9 | 11.0 | 11.2 |
| | Unused substrate (%) | 44.6 | 27.4 | 39.2 |
| | Substrate used for maintenance (%)[a] | 46.5 | 61.6 | 49.5 |
| | Total (%) | 100.0 | 100.0 | 100.0 |

[a]The rate of the substrate for maintenance was estimated by the subtraction of the fruiting body weight plus the weight of the unused substrate from total substrate weight.

Cham and SJ701 showed a mating rate of 50.9%, indicating that the bipolar mating system functions in the outcross, while the tetrapolar mating system controls the self-mating. The mated dikaryotic mycelia were cultivated to produce fruiting bodies, and the rates of fruiting body-forming strains among the mated strains (the fruiting rate) were highly dependent on the mushroom strains. Only half of the Cham strains could produce fruiting bodies with the fruiting rate of 51.3%, whereas the SJ701-derived strains showed a fruiting rate of 69.5% (Table 1). The strain-dependent difference in fruiting rates may reflect the differential effect of environmental conditions such as temperature and humidity on the fruiting process. The fruiting of the parental Cham strain has been known to be highly sensitive to light and thus should be incubated in dark condition during the mycelia propagation stage. The fruiting rate for outcrosses between these two strains was 63.2%, indicating that the fruiting rate can be enhanced through mating with better fruiting strains.

**Substrate utilization and fruiting body yield.** Cultivation characteristics of the fruited dikaryons were examined in terms of fruiting body yield and substrate utilization, and the results are summarized in the 'Substrate utilization'

**Table 2.** Selected self-mating results for the Cham strains

| | | A3[a] | A5 | A18 | A20 | A15 | A2 | A7 | A8 | A9 | A14 | A1 | A6 | A13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A1B1[b] | | | | A1B2 | | | A2B1 | | | A2B2 | | |
| B7 | A1B1 | × | × | × | × | × | × | × | × | × | × | NF | 5[c] | NF |
| B13 | | × | × | × | × | × | × | × | × | × | × | NF | 54 | 200 |
| B19 | | × | × | × | × | × | × | × | × | × | × | NF | NF | NF |
| B4 | A1B2 | × | × | × | × | × | 84 | 131 | NF | 38 | 8 | × | × | × |
| B6 | | × | × | × | × | × | NF | NF | NF | NF | NF | × | × | × |
| B16 | | × | × | × | × | × | 16 | 124 | 86 | 40 | NF | × | × | × |
| B1 | A2B1 | × | × | × | × | 121 | × | × | × | × | × | × | × | × |
| B5 | | × | × | × | × | 71 | × | × | × | × | × | × | × | × |
| B10 | | × | × | × | × | NF | × | × | × | × | × | × | × | × |
| B18 | | × | × | × | × | NF | × | × | × | × | × | × | × | × |
| B20 | | × | × | × | × | NF | × | × | × | × | × | × | × | × |
| B2 | A2B2 | 132 | 123 | 176 | 208 | × | × | × | × | × | × | × | × | × |
| B8 | | 105 | NF | 88 | 134 | × | × | × | × | × | × | × | × | × |
| B9 | | NF | NF | 55 | 100 | × | × | × | × | × | × | × | × | × |
| B12 | | 155 | NF | 48 | 121 | × | × | × | × | × | × | × | × | × |
| B15 | | 50 | NF | NF | 176 | × | × | × | × | × | × | × | × | × |
| B17 | | NF | NF | 109 | 150 | × | × | × | × | × | × | × | × | × |

Cham, Chamaram; ×, not mated; NF, mated but no fruiting.

[a]Monokaryotic strain number.

[b]Mating type of the monokaryotic strain.

[c]Weight of fruiting bodies (g).

section in Table 1. The progenies of Cham strains were highly inefficient at utilization of the substrate. Indeed, only 8.9% of the substrate was used for fruiting body production, while 44.6% remained in the spent media. The remaining 46.5% appeared to be utilized for the cellular maintenance. Interestingly, the self-mates of the SJ701 strain efficiently utilized the substrate with only 27.4% of the initial substrate remaining at the end of the cultivation, and 11% being used for the fruiting body production. The SJ701 strains appeared to have high metabolic activity, with 61.6% of the substrate mass being utilized for maintenance. The cultivation characteristics of the outcross strains were between those of two parental strains.

**Mating and fruiting behaviors.** *L. edodes* has a tetrapolar mating system regulated by mating genes contained in the

**Table 3.** Selected self-mating results for the SJ701 strains

| | | D13 | D15 | D16 | D9 | D10 | D17 | D2 | D4 | D5 | D12 | D14 | D6 | D11 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A1B1 | | | A1B2 | | | A2B1 | | | | | A2B2 | |
| E6 | A1B1 | × | × | × | × | × | × | × | × | × | × | × | 71 | 14 |
| E12 | | × | × | × | × | × | × | × | × | × | × | × | NF | 23 |
| E15 | | × | × | × | × | × | × | × | × | × | × | × | 38 | NF |
| E17 | | × | × | × | × | × | × | × | × | × | × | × | 39 | 19 |
| E20 | | × | × | × | × | × | × | × | × | × | × | × | NF | 110 |
| E3 | A1B2 | × | × | × | × | × | × | 110 | 54 | 140 | 154 | 96 | × | × |
| E4 | | × | × | × | × | × | × | NF | 14 | NF | 151 | NF | × | × |
| E8 | | × | × | × | × | × | × | NF | 72 | 153 | NF | 109 | × | × |
| E10 | | × | × | × | × | × | × | NF | NF | NF | NF | NF | × | × |
| E13 | | × | × | × | × | × | × | 136 | 154 | 87 | 71 | 203 | × | × |
| E2 | A2B1 | × | × | × | 88 | 100 | NF | × | × | × | × | × | × | × |
| E7 | | × | × | × | NF | NF | NF | × | × | × | × | × | × | × |
| E14 | | × | × | × | NF | 78 | NF | × | × | × | × | × | × | × |
| E16 | | × | × | × | 103 | 183 | NF | × | × | × | × | × | × | × |
| E1 | A2B2 | 105 | 121 | 135 | × | × | × | × | × | × | × | × | × | × |
| E5 | | 120 | 119 | 41 | × | × | × | × | × | × | × | × | × | × |
| E9 | | 59 | 189 | 139 | × | × | × | × | × | × | × | × | × | × |
| E18 | | 87 | 69 | NF | × | × | × | × | × | × | × | × | × | × |
| E19 | | 134 | 120 | 27 | × | × | × | × | × | × | × | × | × | × |

SJ701, Sanjo701; ×, not mated; NF, mated but no fruiting.

**Table 4.** Outcrossing monokaryotic strains from the Cham and SJ701 dikaryotic strains

| | | | Monokaryotic strains from Cham | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | B13[a] | B19 | B6 | B16 | B1 | B5 | B8 | B15 | B17 |
| | | | A1B1[b] | | A1B2 | | A2B1 | | A2B2 | | |
| Monokaryotic strains from SJ701 | E6 | A1B1 | × | × | × | × | 150[c] | 177 | ○ | ○ | ○ |
| | E12 | | × | × | × | × | 183 | 187 | ○ | ○ | ○ |
| | E15 | | × | × | × | × | 160 | 124 | ○ | ○ | ○ |
| | E20 | | × | × | × | × | 132 | 125 | ○ | ○ | ○ |
| | E3 | A1B2 | × | × | × | × | ○ | ○ | 149 | 142 | 229 |
| | E4 | | × | × | × | × | ○ | ○ | 82 | NF | 96 |
| | E8 | | × | × | × | × | ○ | ○ | 167 | 134 | 179 |
| | E10 | | × | × | × | × | ○ | ○ | NF | NF | 114 |
| | E7 | A2B1 | NF | NF | ○ | ○ | × | × | × | × | × |
| | E14 | | 30 | NF | ○ | ○ | × | × | × | × | × |
| | E16 | | 8 | NF | ○ | ○ | × | × | × | × | × |
| | E9 | A2B2 | ○ | ○ | NF | 47 | × | × | × | × | × |
| | E5 | | ○ | ○ | 15 | 56 | × | × | × | × | × |
| | E19 | | ○ | ○ | 40 | 120 | × | × | × | × | × |

Cham, Chamaram; SJ701, Sanjo701; ○, mated but not cultivated; ×, not mated; NF, mated but no fruiting.

[a]Monokaryotic strain number.

[b]Mating type of the monokaryotic strain.

[c]Weight of fruiting bodies (g) produced from the dikaryotic mycelium by mating.

two independent multiallelic mating loci, A and B [20, 21]. Both the Cham and SJ701 strains showed typical tetrapolar mating behavior. Only a monokaryotic strain with a compatible mating type could mate with a partner monokaryotic strain during self-mating (Tables 2 and 3). The successful mates were cultivated in sawdust medium and their fruiting characteristics were examined. As described above, many of the dikaryotic mates failed to produce fruiting bodies (NF) (Tables 2 and 3), with the dikaryotic mates generated by mating with one of the monokaryotic strains, including A1, B6, B19, D17, E7, and E10, producing no fruiting bodies. However, the monokaryotic strains, including A20, B2, D13, D15, E1, E3, E5, E9, E13, and E19, always underwent successful mating with any partner monokaryotic strains. The A20, B2, E1, and E3 strains not only showed good fruiting performance, but also tended to yield high fruiting body production. These results suggest that certain monokaryotic strains have traits enabling better mating and fruiting characteristics. Moreover, these findings indicate that screening of monokaryotic strains with better traits is important and prerequisite to increase the efficiency of development of new mushroom strains.

Outcrosses between the monokaryotic strains from Cham and SJ701 showed intriguing bipolar mating behavior. Specifically, mating was only dependent on the A mating type locus, while the B locus did not show any influence (Table 4). Both mating type loci A and B of *L. edodes* have been reported to be multi-allelic [10, 11]; therefore, these results imply that the B locus contains allelic variations in the mating type genes. Similar to selfing, cultivation of some of the dikaryotic mates showed that the monokaryotic strains B1 and B5 of Cham and E3, E8, E6, E12, E15, and E20 of SJ701 always had superior fruiting and production yield. Interestingly, the mating between monokaryotic strains with the A2B1 mating type from Cham and those with the A1B1 from SJ701 resulted in perfect fruiting and good production yield. It is not clear whether mating between monokaryotic strains with certain mating types always results in better fruiting characteristics in the dikaryotic strain; accordingly, additional studies for further verification are needed.

**Analysis of fruiting bodies.** Phenotypical characteristics of the fruiting bodies of all mates were examined and the morphology of some of the fruiting bodies is shown in Fig. 1. The cross mates, including B2 × E13, B2 × A20, B8 × A20, and B8 × E8, produced fruiting bodies with better characteristics (bigger pileus diameter and higher production yield), whereas the mates in the second and the third columns in Fig. 1 produced fruiting bodies with no commercial merits, small, under developed, or deformed pileus. The characteristics of fruiting bodies produced from mating of selected monokaryotic strains are summarized in Table 5. Mates with the monokaryotic strains A20 and B5 produced fruiting bodies with commercial merits (fruiting bodies with high production yield, big pileus, short stipe length,


**Fig. 1.** Morphology of fruiting bodies produced from the dikaryotic strains generated by mating of selected monokaryotic strains.

etc.) only through selfing, whereas those with B1 and E8 produced the fruiting bodies only through outcross. The B2 and E3 strains consistently generated the dikaryotic mates producing better fruiting bodies either through selfing or outcross.

There were no correlation between the monokaryotic strain and the cultivation period, except for the B1 mates which required relatively constant 82~85 days of cultivation period. The fastest mate to the completion harvest was E8 × B15 with 48 days followed by B5 × E12 and E3 × D12 with 50 days and 53 days, respectively. Considering the production yield, however, the B5 × E12 mate would be the better choice for further exploration. Individual weight of fruiting body is an important factor deciding commercial value. Generally, the individual weight of commercial fruiting body is around 30 g for which many of our mates suffice. The dikaryotic mates with some of the monokaryotic strains, including A20, B1, E3, and E8, produced fruiting bodies with relatively constant dimension (pileus diameter × stipe length), suggesting that the genetic traits can be inherited from the monokaryotic strain.

Overall, investigation of the effects of monokaryotic strains on fruiting body formation of *L. edodes* in this study demonstrates that certain monokaryotic strains have traits enabling better mating and fruiting and thus indicates that screening of monokaryotic strains with better traits is

**Table 5.** Characteristics of fruiting bodies produced from mating of selected monokaryotic strains

| Host mono | Partner mono | Cultivation period (day)[a] | Total harvest (g) | Individual weight (g)[b] | Pileus (cm) × Stipe length (cm) | No. of FB[c] | Mating method |
|---|---|---|---|---|---|---|---|
| A20 | B8 | 83 | 134 | 26.8 ± 5.6 | (5.4 ± 0.5) × (2.8 ± 0.3) | 5 | Selfing |
| | B15 | 61 | 176 | 25.1 ± 3.6 | (5.2 ± 0.4) × (2.4 ± 0.2) | 7 | |
| B1 | E6 | 84 | 150 | 16.7 ± 2.6 | (4.3 ± 0.3) × (3.3 ± 0.3) | 9 | Outcross |
| | E12 | 82 | 183 | 26.1 ± 5.1 | (5.1 ± 0.4) × (3.1 ± 0.2) | 7 | |
| | E15 | 85 | 160 | 22.9 ± 4.2 | (5.1 ± 0.4) × (3.0 ± 0.2) | 7 | |
| | E20 | 82 | 132 | 33.0 ± 8.9 | (5.4 ± 0.5) × (4.0 ± 0.5) | 4 | |
| B2 | A5 | 63 | 123 | 61.5 ± 1.5 | (8.3 ± 0.2) × (6.3 ± 0.3) | 2 | Selfing |
| | A18 | 85 | 176 | 35.2 ± 5.1 | (6.3 ± 0.5) × (3.7 ± 0.1) | 5 | |
| | A20 | 85 | 208 | 23.1 ± 3.7 | (5.0 ± 0.4) × (2.7 ± 0.2) | 9 | |
| | E13 | 64 | 196 | 32.7 ± 4.9 | (6.0 ± 0.5) × (3.5 ± 0.2) | 6 | Outcross |
| | D9 | 58 | 147 | 22.4 ± 3.9 | (4.8 ± 0.5) × (2.9 ± 0.3) | 7 | |
| B5 | E6 | 64 | 177 | 25.3 ± 6.6 | (5.4 ± 0.6) × (4.2 ± 0.6) | 7 | Selfing |
| | E12 | 50 | 187 | 31.2 ± 4.3 | (6.3 ± 0.4) × (4.2 ± 0.2) | 6 | |
| | E15 | 89 | 124 | 31.0 ± 8.4 | (5.5 ± 0.8) × (4.8 ± 1.1) | 4 | |
| | E20 | 80 | 125 | 31.3 ± 6.7 | (5.6 ± 0.5) × (4.1 ± 0.5) | 4 | |
| E3 | D2 | 46 | 110 | 27.5 ± 6.8 | (5.2 ± 0.5) × (2.4 ± 0.4) | 4 | Selfing |
| | D5 | 87 | 140 | 35.0 ± 20.2 | (5.6 ± 1.4) × (3.6 ± 0.9) | 4 | |
| | D12 | 53 | 154 | 22.0 ± 6.0 | (4.9 ± 0.7) × (3.2 ± 0.4) | 7 | |
| | B8 | 50 | 149 | 29.8 ± 5.2 | (5.5 ± 0.3) × (3.7 ± 0.4) | 5 | Outcross |
| | B15 | 79 | 142 | 28.4 ± 10.0 | (5.2 ± 0.7) × (3.9 ± 0.8) | 5 | |
| | B17 | 66 | 229 | 28.6 ± 3.7 | (5.6 ± 0.5) × (3.7 ± 0.4) | 8 | |
| E8 | B8 | 84 | 167 | 27.8 ± 4.4 | (5.5 ± 0.3) × (3.3 ± 0.4) | 6 | Outcross |
| | B15 | 48 | 134 | 33.5 ± 2.7 | (5.8 ± 0.3) × (3.6 ± 0.4) | 4 | |
| | B17 | 75 | 179 | 29.8 ± 3.8 | (5.5 ± 0.4) × (3.3 ± 0.4) | 6 | |
| E13 | D2 | 51 | 136 | 43.5 ± 4.3 | (6.8 ± 0.3) × (3.7 ± 0.6) | 3 | Selfing |
| | D4 | 63 | 154 | 25.7 ± 4.8 | (5.3 ± 0.3) × (3.6 ± 0.4) | 6 | |
| | D14 | 64 | 203 | 50.8 ± 18.1 | (7.2 ± 1.3) × (4.9 ± 0.5) | 4 | |
| Underdeveloped strains | | | | | | | |
| E5 | B6 | 87 | 15 | 5.0 ± 1.5 | (1.8 ± 0.4) × (2.5 ± 0.6) | 3 | Outcross |
| | D16 | 87 | 44 | 14.7 ± 7.2 | (2.8 ± 0.8) × (2.8 ± 0.7) | 3 | Selfing |
| B16 | A2 | 82 | 20 | 20 | 5.0 ± 2.3 | 1 | Selfing |
| | A9 | 72 | 44 | 5.5 ± 1.0 | (2.0 ± 0.2) × (7.9 ± 3.9) | 9 | |
| B13 | E16 | 64 | 8 | 8 | 3.1 ± 2.2 | 1 | Outcross |
| D11 | E12 | 84 | 22 | 3.7 ± 1.6 | (2.0 ± 0.2) × (7.9 ± 3.9) | 8 | Selfing |

[a]Cultivation lasted for the third round harvest.
[b]Weight of individual fruiting body.
[c]Number of total harvested fruiting bodies.

prerequisite to increase the efficiency of development of new mushroom strains.

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