「森林総合研究所研究報告」(Bulletin of FFPRI), Vol.4, No.3 (No.396), 217 - 223, Mar. 2005

研究資料（Research material）

# Tetrad analyses of mating types in shiitake (*Lentinula edodes*)

MIYAZAKI Kazuhiro[1)]*, NEDA Hitoshi[2)]
and SHIRAISHI Susumu[3)]

**Abstract**

Shiitake (*Lentinula edodes* (Berk.) Pegler) is a tetrapolic fungus having two mating factors, *A* mating factor and *B* mating factor. Four kinds of mating pattern, $A_1B_1$, $A_1B_2$, $A_2B_1$, and $A_2B_2$, were caused from a parental strain with a mating factor type of $A_1A_2B_1B_2$. In this study, we isolated 33 tetrads, 132 basidiospores, from the outbred strains, MCR14/MCR15, and then carried out the intra-tetrad mating tests. Fourteen of tetrapolic tetrads and nineteen of dipolic tetrads were detected. The mating tests with the tester strains were performed for the detection of mating types on basidiosporic strains. In two tetrads, MCR14B-121 and MCR14B-130, the strains having new *B* mating factor were detected. In dipolic tetrads, the tetrads with parental mating types ($A_1B_1$ or $A_2B_2$) and non-parental mating types ($A_1B_2$ or $A_2B_1$) were occurred, and the ratio was 10:7. The distances of the *A* mating factor and the *B* mating factor from each centromere were expected 31.8 units (the *A* mating factor) and 26.0 units (the *B* mating factor).

**Key words :** *Lentinula edodes*, Shiitake, tetrad analyses, mating type

## Introduction

*Lentinula edodes* (Berk.) Pegler popularly known as shiitake, the black oak mushroom or xiang-gu, is extensively cultivated in Japan, China, Europe and USA. In the wild forest, *L. edodes* distributes widely in Asia and Australiella (Kobayashi and Shimizu, 1951; Kobayashi, 1966; Aoshima and Furukawa, 1980).

*L. edodes* has two unlinked mating factors (*A* and *B*), and is a tetrapolic fungus (Takemaru, 1961; Murakami and Takemaru, 1975). The mating factors are incompatible, and determine the compatibility between two monokaryotic strains from the same sporephorma or others. However, since there are multiallelic mating factors in *L. edodes* (Tokimoto et al., 1973; Fox et al., 1994), most combinations between strains collected from different sites are compatible.

Tetrad analysis is a procedure of genetic analysis using four daughter cells having the different chromosomes as a result of meiosis from one mother cell (Mather and Beale, 1942). Tetrad analysis has several advantages, e.g. all the genetic information of the parental strain is analyzed avoiding a bias of genes for rate of germination and growing rate after germination. In addition, by using the tetrads a dominant marker such as random amplified polymorphic DNA (RAPD) marker can be used as a co-dominant marker confirming the segregation to 2 : 2 in a tetrad, and on the low reliability genetic markers like RAPD (Miyazaki et al., 2000). Tetrad analysis is useful for detecting of linkage (Perkins, 1949, 1953; Whitehouse, 1949, 1950, 1958). Genes are mapped from chromosome centromere by tetrad analysis using the frequency of tetratypes (Perkins, 1949; Whitehouse, 1949, 1950).

We isolated tetrads for genetic analyses of *L. edodes* without the genes omitted by the random isolating. The sequence characterized amplified region (SCAR) markers tightly linking to mating factors have already been developed by tetrad analyses (Tanaka et al., 2004). In this study, we determined the mating types of tetrads isolated and observed the segregation of the mating type factors inside a basidium during meiosis.

## Materials and Methods

### Strains

原稿受付：平成 16 年 12 月 6 日 Received Dec. 26, 2004　原稿受理：平成 17 年 6 月 9 日 Accepted Jun. 9, 2005
* Forest Microbiology Group, Kyushu Research Center, Forestry and Forest Products Research Institute (FFPRI), 4-11-16 Kurokami, Kumamoto, 860-0862, Kumamoto, Japan; e-mail: miyazaki@affrc.go.jp
1) Forest Microbiology Group, Kyushu Research Center, Forestry and Forest Products Research Institute (FFPRI)
2) Department of Applied Microbiology, Forestry and Forest Products Research Institute (FFPRI)
3) Faculty of Agriculture, Kyushu University

Parental heterokryons, MCR14 and MCR15, were produced from homokaryons D703PP-9 (mating-type: $A_1B_1$) obtained from D703, New Zealand strain, and G408PP-4 ($A_2B_2$) obatained from G408, Japanese wild strain (Miyazaki and Neda, 2004). Two parental strains, MCR14 and MCR15, have the same nuclei, but MCR14 has a mitochondrial genome derived from G408, and MCR15 has a mitochondrial genome derived from D703. In this study, 948 basidiospores isolated from MCR14/MCR15 were used.

### The measurement of matured spore germination rate

Matured spores were collected from the sporephores of dikaryotic strain, MCR14. Basidiospores that fell down onto sterilized aluminum foil were regarded as matured spores. After the aluminum foil was dried in the air, basidiospores were suspended by sterilized distilled water. This suspension was spread on potato dextrose agar (PDA) medium, and then incubated at 25℃ for two days. Basidiospores were observed by the microscope, and the number of germinated and non-germinated spores was counted. Spore germination rate was calculated from No.-germinated-spores/No.-counted-spores x 100 (%).

### Tetrad isolation

Parental strains, MCR14 and MCR15, were inoculated into sawdust media (water content; 65%) containing beech-sawdust : rice-bran = 4 : 1 (by volume), and then cultured for 2 － 3 months at 25℃. After removal from its bag, mycelial blocks were transferred to the growing room (humidity; 85%, temperature condition; 10℃, 5h, to gradient 23℃, 3h in a day). From fruiting bodies a piece of gill was picked up with tweezers, and used to inoculate the PDA plate by lightly touching it. Four basidiospores close to each other and derived from a single basidium were isolated with a micromanipulator and cultured on PDA media in a 24-well plate (4 x 6 holes) (Coster, USA). The obtained strains were confirmed to be monokaryons from the absence of clamp-connections by microscopy, and maintained on PDA slants.

### Mating tests

The pieces of agar from PDA slants of the two monokaryons for the pair-wise test on PDA medium in a Petri dish were allowed to grow until two fronts of the advancing mycelia from the agar pieces met and developed a conspicuous contact zone. From the contact zone on each plate a piece of mycelium was taken and examined under a microscope (x400) for clamp-connections. If clamp-connections were observed in the contact zone, mycelia in the outer edges of paired colonies were also examined. When clamp-connections were also seen in the edge of paired colonies, sexual compatibility of the mated pair was scored positive.

### Molecular analyses of tetrads showing the irregular mating patterns

In the case of tetrads showing the irregular mating patterns, to check which four spores originated from a basidium, random amplified polymorphic DNA (RAPD) and sequence characterized amplified region (SCAR) analyses were carried out. Condition of RAPD analysis (Miyazaki et al, 2000) and SCAR analysis (Tanaka et al, 2004) were as described methods.

### Mapping the mating type factors from centromeres

It was reported that the relationship in the latter case had been shown to be :

$$p = x + y - 3xy/2$$

where $p$ was the proportion of tetrapolic tetrads and $x$ and $y$ were the proportions of second-division segregation at each locus. The segregation data of a SCAR, *M17*, unlinking *A* and *B* mating type factors was used to calculate the distances for *A* and *B* mating factors from each centromere (Hisaeda et al., unpublished data).

## Results

We isolated 237 sets of four spores on PDA with a micromanipulator (190 sets from MCR14 and 47 sets from MCR15; 948 basidiospores in all). In 33 of the 237 sets, all four spores germinated. Twenty nine sets were isolated from MCR14, and four sets were isolated from MCR15. The success rate for obtaining tetrads of the outbred lines, MCR14 and MCR15, was 13.9% (=33/237 x 100).

We performed mating tests by the paired culture of intra-tetrads. In mating tests using intra-tetrads, both the tetrapolic mating pattern and dipolic mating pattern were observed (Table 1). The number of tetrapolic tetrads was 14, and number of dipolic tetrads was 19.

The matured spore germination rate of MCR14 was 84.5% (=552/653 x 100, on PDA plate, 25℃, 48h).

Table1. Results of mating test on intra-tetrad

(a)

| | MCR14B-7 | | | |
|---|---|---|---|---|
| | -1 | -2 | -3 | -4 |
| MCR14B-7-1 | - | + | - | - |
| MCR14B-7-2 | + | - | - | - |
| MCR14B-7-3 | - | - | - | + |
| MCR14B-7-4 | - | - | + | - |

(b)

| | MCR14B-5 | | | |
|---|---|---|---|---|
| | -1 | -2 | -3 | -4 |
| MCR14B-5-1 | - | - | + | + |
| MCR14B-5-2 | - | - | + | + |
| MCR14B-5-3 | + | + | - | - |
| MCR14B-5-4 | + | + | - | - |

"+" and "-" indicate the presence and the absense of clamp-connections.

(a) In case of MCR14B-7 (tetrapolic type).

(b) In case of MCR14B-5 (dipolic type).

A tetrad of MCR14B-30 that showed a tetrapolic pattern was subjected to the mating test versus D703PP-9 ($A_1B_1$) and G408PP-4 ($A_2B_2$), the original monokaryotic strains of parental strains, for use as the tester strains to determine mating types of other meiotic strains. D703PP-9 was compatible in MCR14B-30-3, and G408PP-4 was compatible in MCR14B-30-2. Therefore, a mating type of MCR14B-30-3 was concluded to be $A_2B_2$, and it of MCR14B-30-2 was concluded to be $A_1B_1$. In pairs of D703PP-9 vs. MCR14B-30-1 and G408PP-4 vs. MCR14B-30-4, 'barrage' phenomena, which were observed in *A*-different/*B*-common broke out. Therefore, the mating type of MCR14B-30-1 was concluded to be $A_2B_1$, and that of MCR14B-30-4 was concluded to be $A_1B_2$. Four strains of MCR14B-30 were adopted as tester strains to determine the mating types of other meiotic strains.

Of the sets in which all four spores germinated, MCR14B-90 and MCR14B-91 showed irregular types in the mating tests within four strains of one set, though they were assumed to be tetrads from successful of germination. Then, we analyzed these strains using molecular markers. In the tests with SCARs, sOPP19-560, and sOPOH09-590 did not show a 2:2 segregation within the four strains of MCR14B-91, and sOPH09-590 did not show a 2:2 segregation within the four strains of MCR14B-90 (data not shown). In addition, several RAPD markers also confirmed to be heterozygous markers did not show a 2:2 segregation, within the four strains of MCR14B-90 and MCR14B-91 (data not shown). We concluded that these sets were not tetrads.

In the mating tests using the tester strains, 31 of 33 tetrads gave results similar results to those obtained using intra-tetrads. In dipolic tetrads, the tetrads with parental mating types ($A_1B_1$ or $A_2B_2$) and non-parental mating types ($A_1B_2$ or $A_2B_1$) were occurred (Table 2). The parental to non-parental ratio was 10 : 7. However, two tetrads, MCR14B-121 and MCR14B-130, showed the irregular mating patterns in the tests by the tester strains (Miyazaki et al, unpublished data). Strains of MCR14B-121 and MCR14B-130 were checked by molecular markers also. In case of these sets, all of molecular markers segregated 2:2 (Miyazaki et al, unpublished data), thus MCR14B-121 and MCR14B-130 were concluded as tetrads. Table 3 shows the results of crossing and Table 4 shows the mating types of each meiotic strains.

The ratio of $A_1B_1$:$A_1B_2$:$A_2B_1$:$A_2B_2$ was 32:30:30:32. When we carried out the linkage analysis of two mating factors with the results obtained from tetrad analyses, the linkage between mating factors was not allowed ($\chi^2$=0.13, n=3, P=0.98).

When the genotype of *M17* was treated as *M17n* (D703 type) or *M17p* (G408 type), $M17nA_1$:$M17nA_2$:$M17pA_1$:$M17pA_2$ was 30:32:32:30, and $M17nB_1$:$M17nB_2$:$M17pB_1$:$M17pB_2$ was 28:34:34:28. Tetratype frequency on *M17* and the *A* mating type factor was 0.484, and tetratype frequency on *M17* and the *B* mating type factor was 0.452. The distances of the *A* mating factor and the *B* mating

Table 2. Results of mating pattern of MCR14B-7 (tetrapolic type), MCR14B-5(parental dipolic type) and MCR14B-101(non-parental dipolic type).

| | MCR14B-7 | | | | MCR14B-5 | | | | MCR14B-101 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| strain (mating type) | -1 | -2 | -3 | -4 | -1 | -2 | -3 | -4 | -1 | -2 | -3 | -4 |
| MCR14B-30-1 (*A2B1*) | - | - | - | + | - | - | - | - | + | - | - | + |
| MCR14B-30-2 (*A1B1*) | - | + | - | - | - | - | + | + | - | - | - | - |
| MCR14B-30-3 (*A2B2*) | + | - | - | - | + | + | - | - | - | - | - | - |
| MCR14B-30-4 (*A1B2*) | - | - | + | - | - | - | - | - | - | + | + | - |
| mating type | *A1B1* | *A2B2* | *A2B1* | *A1B2* | *A1B1* | *A1B1* | *A2B2* | *A2B2* | *A1B2* | *A2B1* | *A2B1* | *A1B2* |

"+" and "-" indicate the presence and the absense of clamp-connections.

factor from each centromere were expected 31.8 units (the *A* mating factor) and 26.0 units (the *B* mating factor).

## Discussion

The success rate for obtaining a tetrad expected from the value of spore germination rate (84.5%) is 0.510 (=$0.845^4$). However, the actual value obtained in our survey (13.9%) was lower than the expected value. This was considered to be due to the contamination with immature spores torn from basidia when the agar medium was inoculated with a piece of gill. This phenomenon needs to be taken into consideration when isolating tetrads with a micromanipulator.

*L. edodes* has been already reported as a tetrapolic fungus (Takemaru, 1961). In this study, tetrapolic, parental dipolic, and non-parental dipolic tetrads, were observed by the intra-tetrad mating analyses and the mating tests with the tester strains. When random basidiospore isolates of *L. edodes* are used, a mixture of these three pattern would be produced.

We concluded by the molecular analyses MCR14B-90 and MCR14B-91 were not tetrads. Contamination of spores from other basidia may have occured during the isolation with a micromanipulator. On the other hand, in the case of MCR14B-121 and MCR14B-130 showing the irregular mating pattern, these sets were concluded as tetrads by the similar molecular analyses. Thus, it is essential to confirm that the four strains isolated are the correct tetrad by mating tests and molecular analyses

Table 3. Results of the mating pattern of tetrads by each mating test.

| No. | origin | No. basidium | intra-corossing* | crossing by testers* | types |
|---|---|---|---|---|---|
| 1 | MCR14 | B-5 | di | di | parental |
| 2 | MCR14 | B-7 | tetra | tetra | - |
| 3 | MCR14 | B-8 | tetra | tetra | - |
| 4 | MCR14 | B-10 | tetra | tetra | - |
| 5 | MCR14 | B-16 | di | di | parental |
| 6 | MCR14 | B-23 | tetra | tetra | - |
| 7 | MCR14 | B-30 | tetra | tetra | - |
| 8 | MCR14 | B-31 | tetra | tetra | - |
| 9 | MCR14 | B-55 | di | di | parental |
| 10 | MCR14 | B-81 | di | di | parental |
| 11 | MCR14 | B-92 | tetra | tetra | - |
| 12 | MCR14 | B-101 | di | di | non-parental |
| 13 | MCR14 | B-109 | tetra | tetra | - |
| 14 | MCR14 | B-110 | tetra | tetra | - |
| 15 | MCR14 | B-111 | di | di | non-parental |
| 16 | MCR14 | B-121 | di | irre. | - |
| 17 | MCR14 | B-128 | di | di | non-parental |
| 18 | MCR14 | B-130 | di | irre. | - |
| 19 | MCR14 | B-138 | di | di | parental |
| 20 | MCR14 | B-140 | di | di | non-parental |
| 21 | MCR14 | B-142 | di | di | non-parental |
| 22 | MCR14 | B-143 | di | di | non-parental |
| 23 | MCR14 | B-146 | di | di | parental |
| 24 | MCR14 | B-147 | tetra | tetra | - |
| 25 | MCR14 | B-149 | tetra | tetra | - |
| 26 | MCR14 | B-150 | di | di | non-parental |
| 27 | MCR14 | B-154 | di | di | parental |
| 28 | MCR14 | B-180 | di | di | parental |
| 29 | MCR14 | B-182 | tetra | tetra | - |
| 30 | MCR15 | B-4 | di | di | parental |
| 31 | MCR15 | B-6 | tetra | tetra | - |
| 32 | MCR15 | B-33 | tetra | tetra | - |
| 33 | MCR15 | B-47 | di | di | parental |

di: a dipolic type
tetra: a tetrapolic type
irre.: an irregular type

before use for the construction of linkage maps. On the MCR14B-121-3, MCR14B-121-4, MCR14B-130-1, and MCR14B-130-4 having new *B* mating type factors, it was expected that crossing over between *Bα* and *Bβ* was occurred. In this study, the recombination value between *Bα* and *Bβ* of *L. edodes* was 3.03% (= 4/132 x 100). Takemaru (1961) reported that the recombination value between *Bα* and *Bβ* of *L. edodes* calculated 7.5% (3/40 x 100). Recombenation analysis by larger number of basidiospores was desired to determine the recombination

Table 4. Mating types of the tetrad strains

| No. | strain | mating type | No. | strain | mating type | No. | strain | mating type |
|---|---|---|---|---|---|---|---|---|
| 1 | MCR14B-5-1 | *A1B1* | 49 | MCR14B-109-1 | *A2B2* | 97 | MCR14B-149-1 | *A2B2* |
| 2 | MCR14B-5-2 | *A1B1* | 50 | MCR14B-109-2 | *A1B1* | 98 | MCR14B-149-2 | *A1B2* |
| 3 | MCR14B-5-3 | *A2B2* | 51 | MCR14B-109-3 | *A1B2* | 99 | MCR14B-149-3 | *A1B1* |
| 4 | MCR14B-5-4 | *A2B2* | 52 | MCR14B-109-4 | *A2B1* | 100 | MCR14B-149-4 | *A2B1* |
| 5 | MCR14B-7-1 | *A1B1* | 53 | MCR14B-110-1 | *A1B1* | 101 | MCR14B-150-1 | *A1B2* |
| 6 | MCR14B-7-2 | *A2B2* | 54 | MCR14B-110-2 | *A1B2* | 102 | MCR14B-150-2 | *A2B1* |
| 7 | MCR14B-7-3 | *A2B1* | 55 | MCR14B-110-3 | *A2B2* | 103 | MCR14B-150-3 | *A1B2* |
| 8 | MCR14B-7-4 | *A1B2* | 56 | MCR14B-110-4 | *A2B1* | 104 | MCR14B-150-4 | *A2B1* |
| 9 | MCR14B-8-1 | *A1B2* | 57 | MCR14B-111-1 | *A2B1* | 105 | MCR14B-154-1 | *A2B2* |
| 10 | MCR14B-8-2 | *A1B1* | 58 | MCR14B-111-2 | *A2B1* | 106 | MCR14B-154-2 | *A1B1* |
| 11 | MCR14B-8-3 | *A2B2* | 59 | MCR14B-111-3 | *A1B2* | 107 | MCR14B-154-3 | *A2B2* |
| 12 | MCR14B-8-4 | *A2B1* | 60 | MCR14B-111-4 | *A1B2* | 108 | MCR14B-154-4 | *A1B1* |
| 13 | MCR14B-10-1 | *A2B2* | 61 | MCR14B-121-1 | *A1B2* | 109 | MCR14B-180-1 | *A1B1* |
| 14 | MCR14B-10-2 | *A1B1* | 62 | MCR14B-121-2 | *A2B1* | 110 | MCR14B-180-2 | *A2B2* |
| 15 | MCR14B-10-3 | *A2B1* | 63 | MCR14B-121-3 | *A2B3* | 111 | MCR14B-180-3 | *A1B1* |
| 16 | MCR14B-10-4 | *A1B2* | 64 | MCR14B-121-4 | *A1B4* | 112 | MCR14B-180-4 | *A2B2* |
| 17 | MCR14B-16-1 | *A1B1* | 65 | MCR14B-128-1 | *A1B2* | 113 | MCR14B-182-1 | *A2B1* |
| 18 | MCR14B-16-2 | *A2B2* | 66 | MCR14B-128-2 | *A2B1* | 114 | MCR14B-182-2 | *A1B2* |
| 19 | MCR14B-16-3 | *A1B1* | 67 | MCR14B-128-3 | *A1B2* | 115 | MCR14B-182-3 | *A2B2* |
| 20 | MCR14B-16-4 | *A2B2* | 68 | MCR14B-128-4 | *A2B1* | 116 | MCR14B-182-4 | *A1B1* |
| 21 | MCR14B-23-1 | *A1B1* | 69 | MCR14B-130-1 | *A2B5* | 117 | MCR15B-4-1 | *A2B2* |
| 22 | MCR14B-23-2 | *A2B1* | 70 | MCR14B-130-2 | *A1B1* | 118 | MCR15B-4-2 | *A2B2* |
| 23 | MCR14B-23-3 | *A2B2* | 71 | MCR14B-130-3 | *A2B2* | 119 | MCR15B-4-3 | *A1B1* |
| 24 | MCR14B-23-4 | *A1B2* | 72 | MCR14B-130-4 | *A1B6* | 120 | MCR15B-4-4 | *A1B1* |
| 25 | MCR14B-30-1 | *A2B1* | 73 | MCR14B-138-1 | *A1B1* | 121 | MCR15B-6-1 | *A2B1* |
| 26 | MCR14B-30-2 | *A1B1* | 74 | MCR14B-138-2 | *A2B2* | 122 | MCR15B-6-2 | *A1B2* |
| 27 | MCR14B-30-3 | *A2B2* | 75 | MCR14B-138-3 | *A1B1* | 123 | MCR15B-6-3 | *A2B2* |
| 28 | MCR14B-30-4 | *A1B2* | 76 | MCR14B-138-4 | *A2B2* | 124 | MCR15B-6-4 | *A1B1* |
| 29 | MCR14B-31-1 | *A1B1* | 77 | MCR14B-140-1 | *A2B1* | 125 | MCR15B-33-1 | *A2B2* |
| 30 | MCR14B-31-2 | *A1B2* | 78 | MCR14B-140-2 | *A1B2* | 126 | MCR15B-33-2 | *A1B2* |
| 31 | MCR14B-31-3 | *A2B1* | 79 | MCR14B-140-3 | *A1B2* | 127 | MCR15B-33-3 | *A2B1* |
| 32 | MCR14B-31-4 | *A2B2* | 80 | MCR14B-140-4 | *A2B1* | 128 | MCR15B-33-4 | *A1B1* |
| 33 | MCR14B-55-1 | *A2B2* | 81 | MCR14B-142-1 | *A1B2* | 129 | MCR15B-47-1 | *A2B2* |
| 34 | MCR14B-55-2 | *A1B1* | 82 | MCR14B-142-2 | *A2B1* | 130 | MCR15B-47-2 | *A2B2* |
| 35 | MCR14B-55-3 | *A1B1* | 83 | MCR14B-142-3 | *A2B1* | 131 | MCR15B-47-3 | *A1B1* |
| 36 | MCR14B-55-4 | *A2B2* | 84 | MCR14B-142-4 | *A1B2* | 132 | MCR15B-47-4 | *A1B1* |
| 37 | MCR14B-81-1 | *A2B1* | 85 | MCR14B-143-1 | *A1B2* | | | |
| 38 | MCR14B-81-2 | *A1B2* | 86 | MCR14B-143-2 | *A2B1* | | | |
| 39 | MCR14B-81-3 | *A2B1* | 87 | MCR14B-143-3 | *A2B1* | | | |
| 40 | MCR14B-81-4 | *A1B2* | 88 | MCR14B-143-4 | *A1B2* | | | |
| 41 | MCR14B-92-1 | *A1B1* | 89 | MCR14B-146-1 | *A2B2* | | | |
| 42 | MCR14B-92-2 | *A1B2* | 90 | MCR14B-146-2 | *A2B2* | | | |
| 43 | MCR14B-92-3 | *A2B2* | 91 | MCR14B-146-3 | *A1B1* | | | |
| 44 | MCR14B-92-4 | *A2B1* | 92 | MCR14B-146-4 | *A1B1* | | | |
| 45 | MCR14B-101-1 | *A1B2* | 93 | MCR14B-147-1 | *A2B1* | | | |
| 46 | MCR14B-101-2 | *A2B1* | 94 | MCR14B-147-2 | *A2B2* | | | |
| 47 | MCR14B-101-3 | *A2B1* | 95 | MCR14B-147-3 | *A1B2* | | | |
| 48 | MCR14B-101-4 | *A1B2* | 96 | MCR14B-147-4 | *A1B1* | | | |

value between *Bα* and *Bβ* of *L. edodes*.

On the linkage maps constructed by Kwan and Xu (2002) and Terashima et al. (2002), two mating factors were divided into different linkage groups. When we carried out the linkage analysis of two mating factors with the results obtained from tetrad analyses, the ratio of $A_1B_1$:$A_1B_2$:$A_2B_1$:$A_2B_2$ = 32:30:30:32 clearly fitted the 1:1:1:1 ratio ($\chi^2$=0.13, n=3, P=0.98). The results showed that the tetrad strains isolated had no distortion for the division of mating factors. We consider that the tetrads we isolated are appropriate for use in the linkage analyses and the quantitative trait loci (QTL) analyses.

## References

Aoshima, K. and Furukawa, H. (1980) Morphological and cultural characteristics of tropical Shiitake, *Lentinus edodes*, Res. J. Food Agric., **3**, 35-39. (In Japanese)

Fox, H. M., Burden, J., Chang, S. T. and Peberdy, J. F. (1994) Mating-type incompatibility between commercial strains of *Lentinula edodes*, Exp. Mycol., **18**, 95-102.

Kobayashi, Y. (1966) Tropical distribution of *Lentinus edodes*, J. Jpn. Bot., **41**, 257-258. (In Japanese)

Kobayashi, Y. and Shimizu, D. (1951) Nomenclature distribution and deformation of "Shiitake", J. Jpn. Bot., **26**, 29-31. (In Japanese)

Kwan, H. S. and Xu, H. L. (2002) Construction of a genetic linkage map of shiitake mushroom *Lentinula edodes* strain L54, J. Biochem. Mol. Biol., **35**, 465-471.

Mather, K. and Beale, G. H. (1942) The calculation and precision of linkage values from tetrad analysis, J. Genet., 43, 1-30.

Miyazaki, K., Maeda, H., Sunagawa, M., Tamai, Y. and Shiraishi, S (2000) Screening of heterozygous DNA markers in shiitake (*Lentinula edodes*) using de-dikaryotic via preparation of protoplasts and isolation of four meiotic monokaryons from one basidium, J. Wood Sci., **46**, 395-400.

Miyazaki, K. and Neda, H. (2004) Evaluation of the use of outbred lines for Screening of genetic markers in Shiitake (*Lentinula edodes*), Breeding Sci., **54**, 75-78.

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

Takemaru, T. (1961) Genetic studies on fungi. IX. The mating system in *Lentinus edodes* (Berk.) Sing., Rept. Tottori Mycol. Inst., **1**, 61-68.

Tanaka, A., Miyazaki, K., Murakami, H. and Shiraishi, S. (2004) Sequence characterized amplified region markers tightly linked to the mating factors of *Lentinula edodes,* Genome, **47**, 156-162.

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

Tokimoto, K., Komatsu, M. and Takemaru, T. (1973) Incompatibility factors in the natural population of *Lentinus edodes* in Japan, Rept. Tottori Mycol. Inst., **10**, 371-376. (In Japanese)

Perkins, D. D. (1949) Biochemical mutants in the smut fungus *Ustilago maydis*, Genetics, **34**, 607-626.

Perkins, D. D. (1953) The detection of linkage in tetrad analysis, Genetics, **38**, 187-197.

Whitehouse, H. L. K. (1949) Multiple-allelomorph heterothalism in the fungi, New Phytol., **48**, 212-244.

Whitehouse, H. L. K. (1950) Mapping chromosome centromeres by the analysis of unordered tetrads, Nature, **165**, 893-893.

Whitehouse, H. L. K. (1958) Use of loosely linked genes to estimate chromatid interference by tetrad analysis, Natune,**182**, 1173-1174

# シイタケ (*Lentinula edodes*) の交配型のテトラッド分析

宮崎 和弘 [1)*]・根田 仁 [2)]・白石 進 [3)]

**要旨**

シイタケ (*Lentinula edodes*(Berk.)Pegler) は、２つの交配因子 (*A* 因子および *B* 因子 ) を持つ 4 極性の菌である。$A_1A_2B_1B_2$ の交配因子型を持つ親株からは、 4 種類の交配型 ($A_1B_1$, $A_1B_2$, $A_2B_1$, および $A_2B_2$) が生じる。今回の研究で、我々は遠縁交配株 (MCR14 および MCR15) から、合計 33 のテトラッド（四分子 )(132 担子胞子菌株 ) を分離し、テトラッド内の交配試験を実施した。その結果、14 の 4 極性型テトラッドと 19 の 2 極性型テトラッドが認められた。次に、担子胞子菌株の交配型決定のためにテスター菌株との交配試験を実施した。2 つのテトラッド (MCR14B-121 および MCR14B-130) では、異なる B 交配因子を有する菌株が検出された。2 極性型テトラッドでは、親と同型の交配型 ($A_1B_1$ もしくは $A_2B_2$) をもつテトラッドと、親と異なる交配型 ($A_1B_2$ もしくは $A_2B_1$,) をもつテトラッドが生じ、その比率は 10:7 だった。また、動原体から *A* 交配因子までの距離は 31.8 単位、動原体から *B* 交配因子までの距離は 26.0 単位と予想された。最終的に、我々は、33 テトラッドの 132 担子胞子菌株の交配型を決定した。

**キーワード**：*Lentinula edodes*、シイタケ、テトラッド ( 四分子 ) 分析、交配型

* 森林総合研究所九州支所　〒860-0862　熊本県熊本市黒髪四丁目 11 番 16 号　e-mail: miyazaki@ffpri.affrc.go.jp
1) 森林総合研究所九州支所森林微生物管理研究グループ
2) 森林総合研究所きのこ・微生物研究領域
3) 九州大学大学院農学研究院