Linkage analysis of incompatibility factor constitutions in various *Pleurotus* mushrooms

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Abstract

The genetic constitution of *A* and *B* incompatibility factors in five species of *Pleurotus* mushrooms was surveyed by linkage analysis. The *B* incompatibility factor recombinants from monospore isolates of *P. citrinopileatus*, *P. cornucopiae*, *P. eryngii*, *P. ostreatus* and *P. sajor-caju* were found. It was also demonstrated that the genetic distances between two subunits of the *B* incompatibility factor in these mushrooms, *Bα* and *Bβ*, were widely varied between 0 and 18.2 cM among the test strains. On the other hand, we could not isolate any *A* recombinants among about three hundred monospore isolates from either one of the six test strains of *Pleurotus* mushrooms. There was no linkage between the *A* and *B* incompatibility factors in the test fungi as similar to various tetrapolar basidiomycetous mushrooms.

Introduction

Tetrapolar basidiomycetous mushrooms are regulated by the tetrapolar mating system consisting of two sets of multiple alleles that control the formation of dikaryons between two monokaryons carrying different mating factors, which are called the *A* and *B* incompatibility factors. Mushrooms such as *Coprinus cinereus* (Schaeff. : Fr.) S. F. Gray, *Flammulina velutipes* (Curt. : Fr.) Singer, *Schizophyllum commune* Fr. and *Lentinus edodes* (Berk.) Singer ( = *Lentinula edodes* (Berk.) Pegler) were demonstrated to carry tetrapolar incompatibility factors. Many researchers have described that the *A* and *B* incompatibility factor genes of tetrapolar mushrooms do not show any linkage between them. On the other hand, in the case of a tetrapolar mushroom, *P. ostreatus* (Jacq. ex Fr.) Quél., Larraya et al. reported the production of the *B* factor recombinants by sexual reproduction. However, there were no reports on the constitution of the *A* incompatibility factor in this mushroom.

The mushrooms belonging to *Pleurotus* species appear in various ecological environments from the temperate zone to the tropical regions, and distribute throughout Asia, North America, Europe and Australia. *P. ostreatus* is the type species of the genus *Pleurotus* and this genus has been known to have more than thirty-eight species.

The *Pleurotus* species are excellent decomposers of agricultural wastes that can be used as the substrates of these edible mushrooms. Recently, the yields of *Pleurotus* cultivation dramatically increased and accounted for about twenty-five percents of the total world mushroom production.
Therefore, a better understanding of the genetic characteristics is increasingly important for the research and development of breeding of *Pleurotus* mushrooms. In the present study, we surveyed the tetrapolar incompatibility factor constitutions in five species of *Pleurotus* mushrooms.

**Materials and Methods**

**Test organisms**

Six strains of *Pleurotus* mushrooms; *P. citrinopileatus* Sing. (0577), *P. cornucopiae* (Paulet) Rolland (MH-00301), *P. eryngii* (D.C. ex Fr.) Quéél. (TD-202), *P. ostreatus* (Jacq. ex. Fr.) Quéél. (TD-33 and MH-006057) and *P. sajor-caju* (Fr.) Sing. (TD-991) were used as the test organisms in the present study. The strains MH-00301, MH-006057 were kindly furnished by Dr. S. Inatomi of the mushroom laboratory, Hokuto Sangyo Co. Ltd.

**Cultivation of fruit bodies**

The cultivation of fruit-bodies of the test mushrooms was carried out by using a sawdust substrate. The substrate was prepared by mixing sawdust from beech (*Fagus crenata* Blume) and rice bran at a volumetric ratio of 3:1, and adjusting the moisture content with tap water to about 65 % before autoclaving. About 200 g of the substrate was placed in a 300 ml polypropylene bottle, plugged with a plastic cap, and autoclaved at 120 °C for 20 min. After cooling the bottle in air, the culture was inoculated with the dikaryotic mycelium of the test stocks grown on 10 ml of a PDA plate in a Petri dish, 9 cm in diameter, and incubated for 20-25 days at 25°C in the dark. After completing the spawn running, the surface of the mycelia in the culture bottle was scratched with a sterile spatula and then the old mycelia were removed. The culture bottle was then filled up with sterilized water, and the culture bottle was kept at 10°C for 24 h. After removing the water in the bottle by decantation, the culture was then incubated at 25°C under continuous light at about 200 lx provided by fluorescent light tubes for the initiation and development of fruit-bodies.

**Preparation of monospore derived isolates**

The mature pilei of fruit-bodies were cut off with a sterile knife, and put into the sterile Petri dish. The dish was then kept at 10°C for 2-3 days to obtain spore prints. For preparing the basidiospore derived monokaryotic isolates, 2 ml of sterilized water was poured onto the spore prints in the Petri dishes, and vigorously shaken to prepare spore suspension. The basidiospore suspension was transferred into a test tube and diluted with water to make $1 \times 10^3 - 1 \times 10^4$ spores/ml. The spore concentration was determined by counting the number of spores with a hemocytometer (Kayagaki Erika Kogyo Co., Ltd.) under a microscope. The spore suspension (0.1 ml) was put into 2 ml of the melted warm PDA soft agar, mixed thoroughly and poured on a PDA plate to prepare a bi-layer agar plate. Spore cultures were incubated at 25°C in the dark. The spore cells usually germinated after 3-4 days, and formed monokaryotic mycelium colonies. When the colony exceeded 2 mm in diameter, it was excised from the agar plate and planted onto PDA slants to prepare spore isolate stocks. These slants were incubated for about 7 days at 25°C before being used in the crossing experiments.

**Linkage analysis of incompatibility factor constitutions**

The linkage analysis on the *A* and *B* incompatibility factor constitutions of the test mushroom was performed by crossing experiments in the spore isolates with either one of the four tester stocks derived from parental dikaryon. These two
monokaryons were inoculated on a PDA plate at 3-5 mm apart. After incubating at 25°C for 7 days, the formation of clamp connections in the mycelium fragment taken from the contact zone between the paired mycelial colonies was first examined under a light microscope. Then the fragments of mycelia from either side of the parental monokaryons were observed. If the cross of the test monokaryon produced clamp connections with either two of four tester monokaryons, for example with A1B1 and A1B2 or A2B1 and A2B2 testers, the monokaryon was identified to carry a new B incompatibility factor. A similar crossing experiment was done to detect a new A incompatibility factor. The recombinants of the A and B factors were designated to be Ar and Br respectively, with numerical suffixes to distinguish among different recombination products. The genetic distance between the subunits of the A or B incompatibility factor was shown as centi-Morgan (cM) which was equal to the percentage of the recombination rate.

Results and Discussion

In order to determine the genetic constitution of tetrapolar A and B incompatibility factors in five species of Pleurotus mushrooms, the existence of subunits of the A and/or B factors in the six test strains was surveyed by linkage analysis.

Among 296 monospore isolates of P. citrinopileatus 0577, one isolate carrying A1Br in addition to four mating types of isolates; 90 of A1B1, 84 of A1B2, 61 of A2B1 and 60 of A2B2 were found (Table 1a). Almost equal ratio appearances in the four mating types among the spore derived monokaryons of these strains showed that there was no linkage between the A and B incompatibility factors in the test fungus which is similar to various tetrapolar basidiomycetous mushrooms24,18. The new B incompatibility factors of monospore isolates might be the product of the recombination between two subunits of the B incompatibility factor, Ba and Bβ, in the process of basidiospore formation. The genetic distance between two subunits of the B factor in the test strains was only 0.34 cM. On the other hand, we did not find the A incompatibility recombinants (Ar) from the monospore isolates of this test strain.

Table 1b shows the results of the linkage analysis of incompatibility factors of P. cornucopiae MHi-00301. One monospore isolate carrying the B incompatibility factor recombinant gene was found from 196 monospore isolates. However, we did not detect the Ar recombinant from this test stock. The apparent genetic distance between two subunits of the B incompatibility factor was 0.51 cM in this strain.

Table 1c shows the results of the linkage analysis of incompatibility factors of P. eryngii. Four of the A1Br and five of the A2Br with no Ar recombinant were found from the monospore isolates of the strain TD-202. This strain showed a relatively wide genetic distance between Ba and Bβ (4.19 cM).

Table 1d and 1e show the results of linkage analysis on incompatibility factor constitutions of two strains of P. ostreatus. A large number of Br recombinants were found from the monospore isolates from the strain MHi-006057, although no Ar recombinant was detected among 286 monospore isolates. The apparent genetic distance between the two subunits of B incompatibility factor of the test strain was estimated to be 18.2 cM. On the other hand, neither Ar nor Br recombinants from 371 monospore isolates of the strain TD-33 were found. Larraya et al. also reported a few number of the B factor recombinants from five test strains of this species, but they could not isolate Ar factors in these strains10). The recombination frequency for producing Br factors in their test strain was reported to be variable between 0.6 and 15.8 cM.

Table 1f shows the results of linkage analysis
Table 1. Linkage analysis of the incompatibility factor constitutions of six strains in the genus Pleurotus.

(a) P. citrinopileatus 0577

<table>
<thead>
<tr>
<th></th>
<th>B1</th>
<th>B2</th>
<th>Br *</th>
</tr>
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<tbody>
<tr>
<td>A1</td>
<td>90</td>
<td>84</td>
<td>1</td>
</tr>
<tr>
<td>A2</td>
<td>61</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td>Ar*</td>
<td>0</td>
<td>0</td>
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Recombination rate between B factor subunits 1/296×100 = 0.34%

Total of 296 isolates

(b) P. cornucopiae MH-00301

<table>
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<th>B1</th>
<th>B2</th>
<th>Br *</th>
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<tbody>
<tr>
<td>A1</td>
<td>53</td>
<td>45</td>
<td>1</td>
</tr>
<tr>
<td>A2</td>
<td>57</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>Ar*</td>
<td>0</td>
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</table>

Recombination rate between B factor subunits 1/196×100 = 0.51%

Total of 196 isolates

(c) P. eryngii TD-202

<table>
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<th>B1</th>
<th>B2</th>
<th>Br *</th>
</tr>
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<tbody>
<tr>
<td>A1</td>
<td>56</td>
<td>46</td>
<td>4</td>
</tr>
<tr>
<td>A2</td>
<td>51</td>
<td>53</td>
<td>5</td>
</tr>
<tr>
<td>Ar*</td>
<td>0</td>
<td>0</td>
<td>0</td>
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Recombination rate between B factor subunits 9/215×100 = 4.19%

Total of 215 isolates

(d) P. ostreatus MH-006057

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<th>B2</th>
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<tbody>
<tr>
<td>A1</td>
<td>59</td>
<td>49</td>
<td>4</td>
</tr>
<tr>
<td>A2</td>
<td>63</td>
<td>63</td>
<td>5</td>
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<tr>
<td>Ar*</td>
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Recombination rate between B factor subunits 52/286×100 = 18.2%

Total of 286 isolates

(e) P. ostreatus TD-33

<table>
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<tr>
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<th>B2</th>
<th>Br *</th>
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<tr>
<td>A1</td>
<td>102</td>
<td>88</td>
<td>0</td>
</tr>
<tr>
<td>A2</td>
<td>98</td>
<td>83</td>
<td>0</td>
</tr>
<tr>
<td>Ar*</td>
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Recombination rate between B factor subunits 0/371×100 = 0%

Total of 371 isolates

(f) P. sajor-caju TD-991

<table>
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<tr>
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<th>B1</th>
<th>B2</th>
<th>Br *</th>
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<tbody>
<tr>
<td>A1</td>
<td>66</td>
<td>110</td>
<td>2</td>
</tr>
<tr>
<td>A2</td>
<td>61</td>
<td>62</td>
<td>1</td>
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<tr>
<td>Ar*</td>
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<td>0</td>
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</table>

Recombination rate between B factor subunits 3/302×100 = 0.99%

Total of 302 isolates

Ar* and Br* show the A and B incompatibility recombinants, respectively.

on incompatibility factor constitutions of P. sajor-caju TD-991. Two A1Br and one A2Br were found among 302 monospore isolates of this strain. However, any Ar recombinants were not detected in the present experiment.

Linkage analysis of the incompatibility factors of tetrapolar mushrooms such as Coprinus lagopus (= C. cinereus)\textsuperscript{31}, F. velutipes\textsuperscript{28}, L. edodes\textsuperscript{19} and S. commune\textsuperscript{8} demonstrated that both A and B factors were consisted of two subunits, respectively. Further, it is apparent that the genetic distances between Aα and Aβ were somewhat longer than those of Bα and Bβ. In the case for Pleurotus species, we also found the Br recombinants in five strains in the present study. The genetic distances between two subunits of the B incompatibility factor of these mushrooms were widely varied between 0 and 18.2 cM among the test strains. Although the B recombinant was not detected among 371 monospore isolates from TD-33 of P. ostreatus, this strain might also have two subunits of the B incompatibility factor as that of the strain MH-006057 of the same species. On the other hand, we could not isolate any Ar recombinants from six test strains of Pleurotus mushrooms. However, we assume the possibility of a subunit constitution of the A incompatibility factor, which is similar to those of the typical tetrapolar mushrooms\textsuperscript{24,25,31}. Further study is underway to elucidate the existence of A and B factor subunits in...
ヒラタケ属のこの不和合性因子構成の連鎖分析

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ヒラタケ属のこの5種のAおよびB不和合性因子の遺伝学的構成を連鎖分析により調べた。P. citrinopileatus, P. cornucopiae, P. eryngii, P. ostreatusおよびP. sajor-cajuの単胞子分離株からB因子組換株が見出された。これらの中このB因子のαおよびβサブニュートの遺伝的距離は0〜18.2 cMと供試菌株により多様であった。一方、供試した6菌株のそれぞれ約300株の単胞子分離株からはA不和合性因子の組換株は見出されなかった。また、本属の中このAおよびB因子は、既報の4極性きのこのように、連鎖していないことが確認された。

References

6) Mittwoch, U.: J. Genet. 50, 202-205 (1951)

(2001年11月5日受理)