# Relationship between Monokaryotic Growth Rate and Mating Type in the Edible Basidiomycete *Pleurotus ostreatus*

LUIS M. LARRAYA, GÚMER PÉREZ, IÑAKI IRIBARREN, JUAN A. BLANCO, MIKEL ALFONSO, ANTONIO G. PISABARRO, AND LUCÍA RAMÍREZ*

*Departamento de Producción Agraria, Universidad Pública de Navarra, E-31006 Pamplona, Spain*

The edible fungus *Pleurotus ostreatus* (oyster mushroom) is an industrially produced heterothallic homobasidiomycete whose mating is controlled by a bifactorial tetrapolar genetic system. Two mating loci (*matA* and *matB*) control different steps of hyphal fusion, nuclear migration, and nuclear sorting during the onset and progress of the dikaryotic growth. Previous studies have shown that the segregation of the alleles present at the *matB* locus differs from that expected for a single locus because (i) new nonparental *B* alleles appeared in the progeny and (ii) there was a distortion in the segregation of the genomic regions close to this mating locus. In this study, we pursued these observations by using a genetic approach based on the identification of molecular markers linked to the *matB* locus that allowed us to dissect it into two genetically linked subunits (*matBα* and *matBβ*) and to correlate the presence of specific *matBα* and *matA* alleles with differences in monokaryotic growth rate. The availability of these molecular markers and the mating type dependence of growth rate in monokaryons can be helpful for marker-assisted selection of fast-growing monokaryons to be used in the construction of dikaryons able to colonize the substrate faster than the competitors responsible for reductions in the industrial yield of this fungus.

Incompatibility systems are mechanisms for the creation of variability preventing selfing. The phytopathogenic fungus *Ustilago maydis* and the mushrooms *Coprinus cinereus* and *Schizophyllum commune* have been used as models to study mating incompatibility in basidiomycetes. In these species, mating is controlled by two unlinked multiallelic loci whose independent segregation generates four mating specificities in the progeny of a single individual (these fungi are then called tetrapolar) (for reviews, see references 2, 7, and 11). In tetrapolar basidiomycetes, a single basidiospore produces upon germination a hypha in which all nuclei are identical (homokaryon). Two hyphae with different mating alleles at the two incompatibility loci are able to fuse and give rise to a mycelium in which the two parental nuclei do not fuse throughout vegetative growth. This kind of mycelium is called dikaryotic, and the individual mycelium is called a dikaryon. Vegetative growth is maintained until a set of environmental conditions triggers fruit body formation. Karyogamy occurs within the basidia, and it is immediately followed by meiosis producing four uninucleate spores. The monokaryotic and dikaryotic conditions can be distinguished by the presence of clamp connections in dikaryons and their lack in monokaryons. Clamp connections are hook-shaped structures involved in equal nuclei sorting to the daughter cells produced by mitosis.

Genetic studies carried out in *C. cinereus* and *S. commune* have shown that the *A* incompatibility locus codes for homeodomain-containing transcription factors (2, 13, 14, 18, 21, 24, 27, 28). The *b* mating-type locus of *U. maydis* is homologous to the *A* locus and also codes for homeodomain proteins (6, 20). The *B* incompatibility locus of *C. cinereus* and *S. commune*, homologous to the *a* locus of *U. maydis*, codes for pheromones and pheromone receptors (1, 12, 29, 30). Two subloci (*Bα* and *Bβ*) form this locus in *S. commune*, and recombination between them is possible, giving rise to nonparental incompatibility alleles (2). The bipartite structure of the *B* locus has been described also for other basidiomycetes such as *Flammulina velutipes* and *Pleurotus ostreatus* (3, 5, 15).

Larraya et al. (15) previously reported a genetic analysis of the *A* incompatibility locus in *P. ostreatus* var. *florida* using molecular markers; parental and nonparental *B* genes with distorted segregation were described, but the reasons for their occurrence were not examined. In this study, we examined the genetic bases for this distorted segregation by analyzing the structure of the *mat B* incompatibility locus and found a relationship between the polygenic trait vegetative growth rate and the mating genes. Moreover, we have developed molecular markers genetically linked to the *mat B* locus that will provide information allowing one to select in a quick, certain, and easy way monokaryons with allelic combinations suitable to produce compatible crosses for use in breeding programs and to establish the basis for the isolation of genomic clones that either contain the *B* locus or are adjacent to it.

## MATERIALS AND METHODS

**Strains, culture conditions, and experimental protocols.** The strains of *P. ostreatus* used in this work have been previously described (15–17, 19) (Table 1). Strains N001 (Navarra 001, *P. ostreatus* var. *florida*), N002, N005, and N006 are commercial, while N003 is a wild isolate from Viana, Spain. The two nuclei present in the dikaryotic strain N001 have been previously separated by dedikaryotization (16), and the corresponding protoclones are deposited in the Spanish Type Culture Collection (PC9 [CECT20311] and PC15 [CECT20312]). For comparisons with other Agaricales, *Pleurotus quebecoise* and commercial strains of *Agaricus bisporus, Lentinus edodes*, and *Agrocybe aegerita* were used.

Molecular techniques, mating, and linkage analysis were performed as described by Larraya et al. (15–17), with the following modifications: (i) for the generation of rapidly amplified polymorphic DNA (RAPD) markers, oligonucleotides belonging to the L, P, R, and S Operon series (Operon Technologies

* Corresponding author. Mailing address: Departamento de Producción Agraria, Universidad Pública de Navarra, E-31006 Pamplona, Spain. Phone: (34) 948 169 130. Fax: (34) 948 169 732. E-mail: lramirez@unavarra.es.

TABLE 1. *P. ostreatus* strains studied in this work and *B* alleles identified in their progeny

| Strain (mating genotype) | Variety | Origin | *B* allele | Occurrence of *B* allele (%) | Recombination frequency (%) | $P^a$ |
|---|---|---|---|---|---|---|
| N001 (*A1A2 B1B2*) | *florida* | United States | *B1* | 63 | 15.8 | <0.05 |
| | | | *B2* | 38 | | |
| | | | *B3* | 11 | | |
| | | | *B4* | 8 | | |
| N017 (*A1A2 B3B4*) | *florida* | This study | *B3* | 45 | 15.7 | NS |
| | | | *B4* | 41 | | |
| | | | *B1* | 8 | | |
| | | | *B2* | 8 | | |
| N002 (*A5A6 B5B6*) | *ostreatus* | Germany | *B5* | 39 | 8.2 | NS |
| | | | *B6* | 51 | | |
| | | | *B15* | 6 | | |
| | | | *B16* | 2 | | |
| N018 (*A5A6 B15B16*) | *ostreatus* | This study | *B15* | 41 | 4.8 | NS |
| | | | *B16* | 59 | | |
| | | | *B5* | 3 | | |
| | | | *B6* | 2 | | |
| N003 (*A7A8 B7B8*) | *ostreatus* | Spain | *B7* | 86 | 0.6 | NS |
| | | | *B8* | 83 | | |
| | | | *B17* | 1 | | |
| N005 (*A11A12 B11B12*) | *colombinus* | Italy | *B11* | | | |
| | | | *B12* | | | |
| N006 (*A13A14 B13B14*) | *sajor-caju* | India | *B13* | | | |
| | | | *B14* | | | |

*a* The significance of any deviation from 1:1 in the segregation ratio of the two parental *B* alleles was determined by a chi-squared test. NS, not significant.

Inc., Alameda, Calif.) were used as primers; and (ii) the PCR amplification program used included a 4-min denaturation at 94°C followed by 39 cycles of 1-min denaturation at 94°C, 1-min annealing at 37°C, and 1.5-min extension at 72°C.

**Statistical analysis.** The quantitative trait vegetative mycelium growth rate was measured as the time elapsed from when a 16-mm$^2$ agar plug containing the monokaryon was placed at the center of the plate until it reached the edge of the petri dish (9-cm diameter). Three repetitions for each of the 120 monokaryons derived from strain N001 were performed. The data were subjected to a normality test, and subsequently significant differences in vegetative growth rate among the different mating types were determined following one-way variance analysis using SPSS version 8.0 (SPSS Inc.) with treatment effect fixed.

## RESULTS

**Determination of *B* incompatibility alleles present in *P. ostreatus.*** The mating genotype of *P. ostreatus* N001 (*A1A2 B1B2*), as well as those of the other strains, was determined by crossing spore-derived monokaryons against the corresponding testers. In a previous study, Larraya et al. (15) analyzed the segregation of the incompatibility genes present in *P. ostreatus* N001, examining progeny of 120 monokaryons, and found that in addition to the two expected *B* mating genotypes (*B1, B2*), new nonparental *B* alleles appeared. These new *B* types were identified because monokaryons harboring them were compatible with two different N001 mating testers having the same *A* but a different *B* allele. In all progeny, the frequencies of the four *B* mating types were 52.5% (*B1*), 31.6% (*B2*), 9.2% (*B3*), and 6.7% (*B4*) (Table 1). Two alternative hypothesis explaining the generation of these new *B* alleles were posited: they appeared as the result of an intralocus recombination event; alternatively, alleles *B3* and *B4* were produced by some kind of instability of the *B2* allele. The occurrence of nonparental alleles was studied in two other *P. ostreatus* strains, N002 and N003 (Table 1). In both cases, new *B* specificities appeared (*B15B16* in strain N002; *B17* in strain N003), albeit at a frequency lower than in *P. ostreatus* N001.

To test if nonparental *B* alleles were formed by an intralocus recombination event, we generated two hybrid strains, N017 (*A1A2 B3B4*) and N018 (*A5A6 B15B16*), carrying the nonparental *B* alleles appeared in the progeny of N001 and N002, respectively. The analysis of monokaryotic progenies derived from strains N017 and N018 showed that both parental and nonparental *B* alleles were obtained, which confirmed the complex nature of this locus in *P. ostreatus* and the recombinational nature of the new formed *B* alleles.

Recombination frequencies varied among different strains (Table 1). Strains N001 and N017 (both belonging to variety *florida*) showed recombination frequencies higher than 15%, whereas the strains belonging to variety *ostreatus* (N002, N018, and N003) had values of 8.2% or lower. This result suggest that the recombination frequency at the *B* locus is variable.

Additionally, we carried out a statistical analysis to determine if the frequency of parental and recombinant *B* alleles appeared in monokaryotic progenies of each strain was that expected as a consequence of a single recombinational event. In every case but one, the observed frequencies were as expected. The only exception was strain N001, where a significant bias was detected in the progeny of the *B* alleles observed (Table 1).

The *B* alleles differed among the *P. ostreatus* strains used in this work (Table 1). Nevertheless, it could be possible that the new *B* mating-type genes that appeared by intralocus recombination in N001 or N002 were functionally identical to those present in another *P. ostreatus* strain. To test this, monokaryons carrying nonparental *B* alleles (*B3, B4, B15*, and *B16*) were crossed with the mating testers corresponding to strains N001, N002, N003, N005, and N006 to search for incompatibilities revealing common *B* alleles. No common *B* genes were found among the strains analyzed in this study. Thus, the allelic compositions of *B* types *B1, B2, B3*, and *B4* were defined as
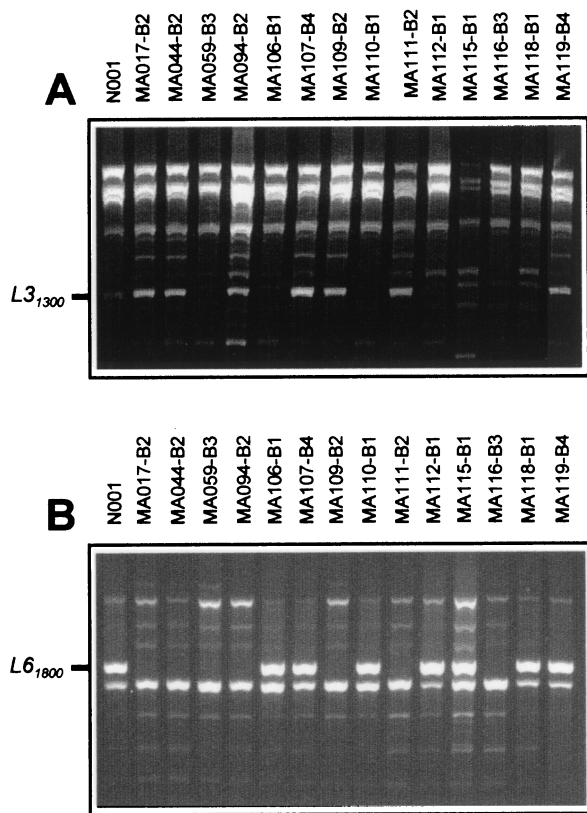
FIG. 1. RAPD markers found in dikaryon N001 of *P. ostreatus* and in different monokaryons derived from it, using primers L3 (A) and L6 (B). Markers $L3_{1300}$ and $L6_{1800}$, genetically linked to *matB*α and *matB*β, respectively, are indicated. The incompatibility type of each monokaryon is indicated.

TABLE 2. Correspondence between RAPD markers, RFLP alleles, and *matB* alleles[a]

| RAPD marker | | RFLP allele | | *matB* allele |
|---|---|---|---|---|
| Name | Size (bp) | Name | Size (bp) | |
| $L3_{1300}$ | 1,300 | $rL3_{1300}2$ | 6,800 | *matB*α2 |
| | | $rL3_{1300}1$ | 8,600 | *matB*α1 |
| $L6_{1800}$ | 1,800 | $rL6_{1800}3$ | 3,900 | *matB*β1 |
| | | $rL6_{1800}2$ | 4,300 | *matB*β2 |

[a] Alleles in the same row are in coupling phase.

them corresponded to nonrepetitive DNA sequences (Fig. 2). Two different *Pst*I restriction alleles were found using marker $L3_{1300}$ (Fig. 2A): $rL3_{1300}1$ (8,600 bp), present in monokaryons bearing *B1* or *B3*, and $rL3_{1300}2$ (6,800 bp), present in monokaryons carrying *B2* or *B4*. Considering the *matB*α alleles present in each of the *B* genes, alleles $rL3_{1300}1$ and $rL3_{1300}2$ are linked in coupling phase to *matB*α1 and *matB*α2, respectively, with no recombinants between RFLP alleles and the corresponding *matB*α alleles. On the other hand, when the cloned RAPD marker $L6_{1800}$ was used as probe, three *Pst*I DNA fragments were identified (Fig. 2B): $rL6_{1800}1$, which was a monomorphic 4,800-bp band; $rL6_{1800}2$, a 4,300-bp-long band present in *B2* or *B3* monokaryons; and $rL6_{1800}3$, which was 3,900 bp long and detected in monokaryons carrying incompatibility types *B1* or *B4*. Segregation of bands $rL6_{1800}2$ and $rL6_{1800}3$ indicated that they were alleles of the same locus. Taking into account the allelic composition of the *B* incompat-

*matB*α1 *matB*β1, *matB*α2 *matB*β2, *matB*α1 *matB*β2, and *matB*α2 *matB*β1, respectively.

**Molecular markers to tag the *B* incompatibility locus and verification of its bipartite structure.** The identification of molecular markers genetically linked to characters of interest was the strategy of choice to facilitate their cloning. To generate molecular markers linked to the *B* incompatibility locus, an approach combining RAPD and bulk segregant analysis was used in a population of 80 monokaryons derived from dikaryotic strain N001. Two RAPD markers linked to the *B* locus were found. Marker $L3_{1300}$ (obtained by using as a primer Operon oligonucleotide L3, 1,300 bp long) was present in all monokaryons bearing either *B2* or *B4*, while it was absent in monokaryons carrying *B1* or *B3* (Fig. 1A). On the other hand, marker $L6_{1800}$ was present in monokaryons with *B1* or *B4*, while it was absent in those with *B2* and *B3* (Fig. 1B). No recombinants between marker $L3_{1300}$ and *B2* or *B4* and a single recombinant between $L6_{1800}$ and *B1* or *B4* were found in the analyzed population. These results indicate that RAPD markers $L3_{1300}$ and $L6_{1800}$ were genetically linked in coupling phase to *matB*α2 and *matB*β1 alleles, respectively (Table 2).

The RAPD markers genetically linked to the *B* mating-type locus were converted into restriction fragment length polymorphic (RFLP) markers. RFLP analysis using the cloned $L3_{1300}$ and $L6_{1800}$ RAPD markers as probes revealed that both of
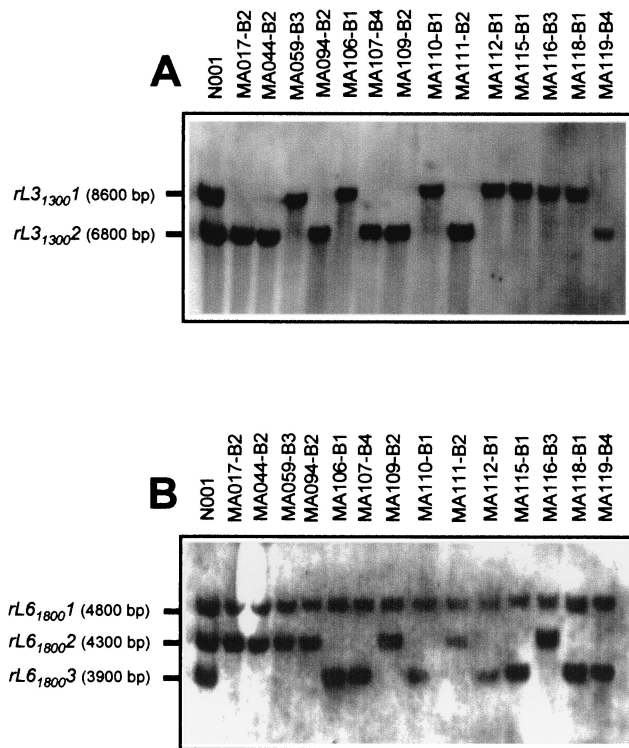


FIG. 2. RFLP patterns detected in *Pst*I genomic DNA digestions of *P. ostreatus* dikaryon N001 and of different monokaryons derived from it, using the $L3_{1300}$ (A) and $L6_{1800}$ (B) RAPD markers as probes. The incompatibility type of each monokaryon is indicated.
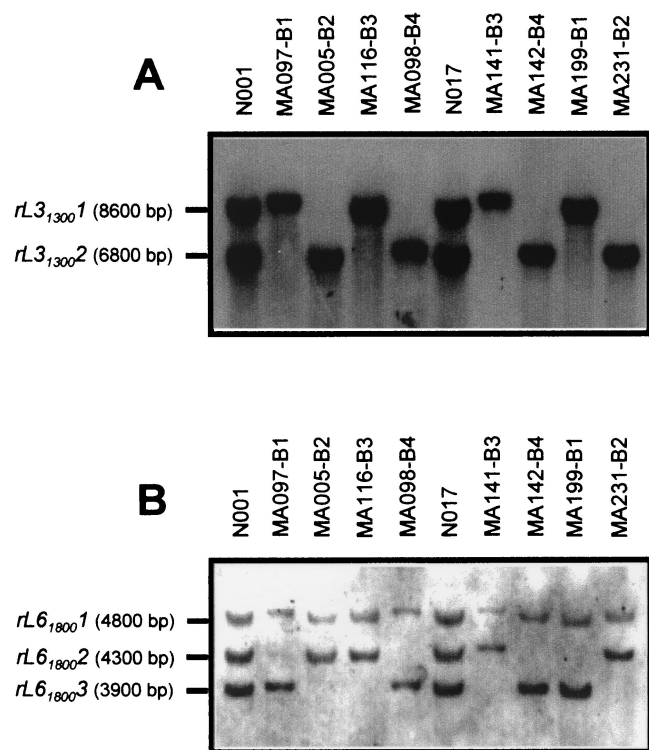
FIG. 3. RFLP patterns detected in *Pst*I genomic DNA digestions of *P. ostreatus* dikaryons N001 and N017 and of four monokaryons (bearing different *B* alleles) derived from each of them, using the $L3_{1300}$ (A) and $L6_{1800}$ (B) RAPD markers as probes. The incompatibility type of each monokaryon is indicated.

ibility locus, RFLP alleles $rL6_{1800}2$ and $rL6_{1800}3$ appeared to be genetically linked in coupling phase to *matBβ2* and *matBβ1*, respectively. Finally, RAPD markers $L3_{1300}$ and $L6_{1800}$ cosegregated with RFLP alleles $rL3_{1300}2$ and $rL6_{1800}3$, respectively (Table 2).

The consistency of the linkage phases found in strain N001 was tested using a monokaryotic progeny derived from dikaryon N017 (*A1A2 B3B4*). DNA samples from each of the four testers corresponding to N017 were digested using *Pst*I, and the RFLP alleles $rL3_{1300}$ and $rL6_{1800}$ were studied. Figure 3 shows that markers $rL3_{1300}1$ and $L3_{1300}2$ cosegregated with

*matBα* alleles, and markers $rL6_{1800}2$ and $rL6_{1800}3$ cosegregated with *matBβ* alleles, as expected (RFLP markers in monokaryons MA097, MA005, MA116, and MA098). Additionally, the RFLP markers linked to the different mating type genes present in the progeny of dikaryon N017 were also those expected from the previous analysis (markers in monokaryons MA141, MA142, MA199, and MA231). These results indicate that the intralocus recombination event that recovered *B1* and *B2* alleles in the progeny of N017 also recovered their corresponding $rL3_{1300}$ and $rL6_{1800}$ genotypes, corroborating molecularly the recombinational nature of the newly formed *B* alleles after meiosis.

**Molecular markers linked to the *B* mating-type locus are species specific.** To determine whether loci $rL3_{1300}$ and $rL6_{1800}$ were also associated with the *B* locus in other *P. ostreatus* strains, the corresponding probes were hybridized to membranes containing genomic DNA from dikaryon N001, N002, N003, N005, or N006 and from some monokaryons derived from them, digested with *Eco*RI, *Pst*I, or *Xho*I. Both probes gave clear signals in each case and revealed a high level of polymorphism (Table 3). To test the presence of RFLP markers homologous to those revealed by $L3_{1300}$ and $L6_{1800}$ in other mushrooms, *Pst*I digestions of genomic DNA purified from *P. quebecoise*, *A. bisporus*, *Agrocybe aegerita*, and *L. edodes* were probed. *P. quebecoise* gave a weak hybridization signal when $L3_{1300}$ was used as probe, whereas no homologous sequences could be detected in the other species (data not shown).

**Analysis of distorted segregation in parental *B* alleles.** The monokaryotic progeny derived from strain N001 carried four different *B* alleles, two parental (*B1* and *B2*) and two nonparental *B* types (*B3* and *B4*), as a result of recombination between the two subunits (*matBα* and *matBβ*) on the *B* locus. Because the parental *B* alleles did not segregate 1:1 as expected (Table 1), we investigated the reason for such a discrepancy. To determine whether this bias was explained by differences in either growth rate of the vegetative mycelium or spore germination, we measured the vegetative growth rate of each of the 80 monokaryons forming the sample population. A one-way variance analysis test was applied to look for differences in the quantitative trait vegetative mycelium growth rate for the different mating genotypes. No significant differences in growth rate were found between *matBα1* and *matBα2* alleles ($P = 0.8$) or between *matBβ1* and *matBβ2* alleles ($P = 0.9$) in

TABLE 3. Restriction length polymorphism of loci $L3_{1300}$ and $L6_{1800}$ in different *P. ostreatus* strains

| RFLP probe | Enzyme | Size (kbp) of corresponding restriction fragments | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | B1 | B2 | B5 | B6 | B7 | B8 | B11 | B12 | B13 | B14 |
| $L3_{1300}$ | *Eco*RI | 4.5 | 11.5 | 17.0 | 17.0 | 17.0 | 17.0 | 17.0 | 17.0 | 11.5 | 11.5 |
| | *Pst*I | 8.6 | 6.8 | 8.6 | 8.6 | 11.2 | 8.6 | 8.6 | 8.6 | 8.6 | 8.6 |
| | *Xho*I | 3.4 | 9.0 | 12.5 | 4.2 | 3.7 | 9.0 | 4.2 | 4.2 | 14.0 | 3.2 |
| $L6_{1800}$ | *Eco*RI | 13.0 | 13.0 | 20.0 | ND[a] | 21.5 | 21.5 | 15.3 | 15.3 | 15.3 | 18.5 |
| | | 11.0 | 11.0 | 4.5 | | 10.0 | 14.0 | | | 3.7 | 3.1 |
| | | | | | | | | | | 3.5 | |
| | *Pst*I | 4.8 | 4.8 | 8.6 | 8.6 | 8.6 | 8.6 | 8.6 | 8.6 | 7.4 | 7.4 |
| | | 3.9 | 4.3 | | | | | | | | |
| | *Xho*I | 12.0 | 4.5 | 10.2 | 12.5 | 12.5 | 7.8 | 12.0 | 14.3 | 16.5 | 16.5 |
| | | 4.5 | 4.2 | 3.3 | 10.2 | | 3.8 | | | | 4.2 |
| | | | | | 7.8 | | | | | | |

[a] ND, not determined.

monokaryons bearing the *A1* mating allele, whereas significant differences ($P = 0.04$) were observed between *matBα1* and *matBα2* alleles in monokaryons whose genomes bore the *A2* mating allele. Interestingly, no significant differences appeared between *matBβ1* and *matBβ2* alleles ($P = 0.5$) in an *A2* genome context. Monokaryons with mating genotype *B1* or *B3* (*matA2 matBα1matBβ−*) grew faster than those with the genotype *B2* or *B4* (*matA2 matBα2matBβ−*). Finally, significant differences ($P = 0.01$) in growth rate were observed for both alleles of the *A* locus. Monokaryons carrying the *A2* allele grew faster than those with *A1* specificity.

**Mapping of the *B* incompatibility locus.** The progeny of 80 monokaryons described above were used to map the *B* locus. Fourteen RAPD markers ($L14_{1525}$, $P16_{1525}$, $P12_{950}$, $P3_{1375}$, $P1_{1600}$, $P2_{2650}$, $R2_{1600}$, $L3_{1300}$, $P2_{2100}$, $L6_{1800}$, $R15_{675}$, $P2_{725}$, $P19_{525}$, and $R3_{2275}$) were assigned to the linkage group to which the *B* locus belongs (17). The *matBα* and *matBβ* subunits were 19.0 centimorgans (Kosambi units [10]) apart, easily tagged by the tightly linked markers $L3_{1300}$ and $L6_{1800}$. The linkage group to which the *B* locus maps corresponds to the physically separated chromosome IX (16). Four molecular markers which were linked to the *matBα* sublocus ($P1_{1600}$, $P2_{2650}$, $R2_{1600}$, and $L3_{1300}$) showed distorted segregation. This was not the case for markers $P2_{2100}$, $L6_{1800}$, $R15_{675}$, $P2_{725}$, $P19_{525}$, and $R3_{2275}$, which are close to the *matBβ* sublocus at the end of the chromosome.

## DISCUSSION

The control of hyphal fusion and dikaryon formation is essential for filamentous fungi, as their mycelia form intricate mats in which the chance for contacts between sister branches is high. In *P. ostreatus*, as in other higher basidiomycetes, this control is based on two unlinked loci (*A* and *B*) responsible for different steps involved in the fusion process and in sorting of nuclei during dikaryotic hyphal growth. These two genes have been called either incompatibility loci or mating genes throughout the literature, and these two terms were considered here to be synonyms. In a previous paper, Larraya et al. (15) analyzed the *A* locus in five different *P. ostreatus* strains, isolated molecular markers genetically linked to it, and concluded that the *A* gene is controlled by a multiallelic single locus for which nine functionally different members were identified. In the present study, genetic experiments have allowed the identification of molecular markers genetically linked to the *B* mating-type gene, which confirmed that new *B* alleles can be formed as a consequence of intralocus recombination between the two subloci (*matBα* and *matBβ*) of the *B* gene. Considering the *B* incompatibility locus as a complex unit, an allelic series similar to that described for the *A* gene (15) has been found. Fifteen functionally different *B* mating alleles were distinguished, some of which resulted from intralocus recombination (Table 1).

The frequency of intralocus recombination yielding new (i.e., nonparental) *B* types is an estimate of the intergenic linkage distances between loci *matBα* and *matBβ*. However, it is known that the recombination frequency in *S. commune* does not depend exclusively on the physical distances between the two subunits of the *B* locus but also is under genetic control of a different locus where alleles for low recombination frequency are dominant over those for high recombination rate (9, 22, 26). Mating genes and recombination-controlling genes are genetically linked, although they can be physically separated by recombination (25, 26). It could be possible that similar mechanisms account for differences in recombination frequencies in the different *P. ostreatus* strains.

In a previous study (17), a distorted segregation was observed for all molecular markers surrounding *matA* and *matBα* genes, whereas no bias in the segregation was found in molecular markers surrounding the *matBβ* gene. Three hypotheses were put forward to explain this observation: (i) a nonrandom segregation of mating types that would drive a skewed segregation of markers linked to them, (ii) differences in viability, germination, or vegetative growth rate associated with different mating haplotypes that may cause preferred selection for some phenotypes in the population, and (iii) the occurrence of balancing selection on mating types that could counteract some negative selection on loci linked to the mating type. The results presented here indicate that there exists a relationship (linkage) between mating genes *matA* and *matBα* and the quantitative trait vegetative mycelium growth rate which could explain the distortion observed. The statistical analysis carried out here shows that monokaryons bearing the *A2* mating allele grew faster than those bearing the *A1* allele. The same is true for monokaryons with the *matBα1* allele (*B1* and *B3*) with respect to those carrying the *matBα2* allele (*B2* and *B4*). It is conceivable that slow-growing monokaryons need more time than fast-growing ones to develop a colony after germination, and these differences could have promoted a preferred selection for some genotypes when the population analyzed here was established. This skewed selection produced an increase in the frequency of alleles derived from the leading genotype in relation to those derived from the lagging one. The effect of negative selection against slowly germinating spores has been previously discussed by Eger (4) with respect to *P. ostreatus* var. *florida* and by Kerrigan et al. (8) with respect to *A. bisporus*. When the monokaryotic growth rate was studied in crossing programs using *S. commune* as the model system, it was also seen that faster-growing monokaryons belong to a certain mating type (23). Taken together, these data suggest that evolution has conserved genome regions in Agaricales, where genetic determinants affecting growth rate and mating type genes are kept together. In this way, those monokaryons whose mating alleles and polygenic traits related to growth rate display a *cis* configuration would be preferentially selected over those with a *trans* genetic organization.

The molecular markers isolated and described in this report constitute a first step toward the cloning and characterization by chromosome walking of the *B* mating-type genes and their flanking regions and useful tools for identifying in a quick and easy way monokaryons used as parentals in breeding programs. The RFLP profiles revealed by probe $L6_{1800}$ and restriction enzymes *Eco*RI and *Xho*I allow the identification of each *B* allele present in our collection. The genomic sequences detected by these RFLP probes are present in all of the *P. ostreatus* strains tested, although they bear different mating alleles, but they cannot be detected in *P. quebecoise* or in other Agaricales, suggesting that these sequences can be considered bonafide species-specific molecular markers. This was also the case of molecular markers $S11_{900}$ and $S18_{1300}$, linked to the *A*

locus (15). The availability of RFLP markers linked to the mating-type genes can be useful in marker-assisted selection for fast-growing monokaryons eligible for construction of dikaryons presumably able to colonize the substrate quicker than other competitors responsible for the reduction of yield in the industrial production of oyster mushrooms.

## REFERENCES

1. **Bölker, M., and R. Kahmann.** 1993. Sexual pheromones and mating responses in fungi. Plant Cell **5:**1461–1469.
2. **Casselton, L. A., and N. S. Olesnicky.** 1998. Molecular genetics of mating recognition in basidiomycete fungi. Microbiol. Mol. Biol. Rev. **62:**55–70.
3. **Chang, S.-T., and P. G. Miles.** 1989. Edible mushrooms and their cultivation, p. 96–111. CRC Press, Inc., Boca Raton, Fla.
4. **Eger, G.** 1976. *Pleurotus ostreatus*-breeding potential of a new cultivated mushroom. Theor. Appl. Genet. **47:**155–163.
5. **Eugenio, C. P., and N. A. Anderson.** 1968. The genetics and cultivation of *Pleurotus ostreatus*. Mycology **60:**627–634.
6. **Gillissen, B., J. Bergemann, C. Sandmann, B. Schroeer, M. Bölker, and R. Kahmann.** 1992. A two-component regulatory system for self/non-self recognition in *Ustilago maydis*. Cell **68:**647–657.
7. **Hiscock, S. J., and U. Kües.** 1999. Cellular and molecular mechanisms of sexual incompatibility in plants and fungi. Int. Rev. Cytol. **193:**165–295.
8. **Kerrigan, R. W., J. C. Royer, L. M. Baller, Y. Kohli, P. A. Horgen, and J. B. Anderson.** 1993. Meiotic behavior and linkage relationships in the secondarily homothallic fungus *Agaricus bisporus*. Genetics **133:**225–236.
9. **Koltin, Y., J. R. Raper, and G. Simchen.** 1967. The genetic structure of the incompatibility factors of *Schizophyllum commune*: the B factor. Proc. Natl. Acad. Sci. USA **57:**55–62.
10. **Kosambi, D. D.** 1944. The estimation of map distance from recombination values. Ann. Eugen. **12:**172–175.
11. **Kothe, E.** 1999. Mating types and pheromone recognition in the Homobasidiomycete *Schizophyllum commune*. Fungal Genet. Biol. **27:**146–152.
12. **Kronstad, J. V., and C. Staben.** 1997. Mating type in filamentous fungi. Annu. Rev. Genet. **31:**245–276.
13. **Kües, U., and L. A. Casselton.** 1993. The origin of multiple mating types in mushrooms. J. Cell Sci. **104:**227–230.
14. **Kües, U., W. V. Richardson, A. M. Tymon, E. S. Mutasa, B. Gottgens, S. Gaubatz, A. Gregoriades, and L. A. Casselton.** 1992. The combination of dissimilar alleles of the A alpha and A beta gene complexes, whose proteins contain homeo domain motifs, determines sexual development in the mushroom *Coprinus cinereus*. Genes Dev. **6:**568–577.
15. **Larraya, L., M. M. Peñas, G. Pérez, C. Santos, E. Ritter, A. G. Pisabarro, and L. Ramírez.** 1999. Identification of incompatibility alleles and characterisation of molecular markers genetically linked to the *A* incompatibility locus in the white rot fungus *Pleurotus ostreatus*. Curr. Genet. **34:**486–493.
16. **Larraya, L. M., G. Pérez, M. M. Peñas, J. P. Baars, T. S. P. Mikosch, A. G. Pisabarro, and L. Ramirez.** 1999. Molecular karyotype of the white rot fungus *Pleurotus ostreatus*. Appl. Environ. Microbiol. **65:**3413–3417.
17. **Larraya, L. M., G. Pérez, E. Ritter, A. G. Pisabarro, and L. Ramírez.** 2000. A genetic linkage map of the edible basidiomycete *Pleurotus ostreatus*. Appl. Environ. Microbiol. **66:**5290–5300.
18. **Magae, Y., C. Novotny, and R. Ullrich.** 1995. Interaction of the Aα Y and Z mating-type homeodomain proteins of *Schizophyllum commune* detected by the two-hybrid system. Biochem. Biophys. Res. Commun. **211:**1071–1076.
19. **Peñas, M. M., S. A. Asgeirsdóttir, I. Lasa, F. A. Culiañez-Macià, A. G. Pisabarro, J. G. H. Wessels, and L. Ramírez.** 1998. Identification, characterization, and in situ detection of a fruit-body-specific hydrophobin of *Pleurotus ostreatus*. Appl. Environ. Microbiol. **64:**4028–4034.
20. **Schulz, B., F. Banuett, M. Dahl, R. Schlesinger, W. Schäfer, T. Martin, I. Herskowitz, and R. Kahmann.** 1990. The *b* alleles of *U. maydis*, whose combinations program pathogenic development, code for polypeptides containing a homeodomain-related motif. Cell **60:**295–306.
21. **Shen, G. P., D. C. Park, R. C. Ullrich, and C. P. Novotny.** 1996. Cloning and characterization of a *Schizophyllum* gene with A beta 6 mating-type activity. Curr. Genet. **29:**136–142.
22. **Simchen, G.** 1967. Genetic control of recombination and the incompatibility system in *Schizophyllum commune*. Genet. Res. (Cambridge) **9:**195–210.
23. **Simchen, G.** 1966. Monokaryotic variation and haploid selection in *Schizophyllum commune*. Heredity **21:**241–263.
24. **Specht, C., M. M. Stankis, L. Giasson, C. P. Novotny, and R. C. Ullrich.** 1992. Functional analysis of the homeodomain-related proteins of the *A*α locus of *Schizophyllum commune*. Proc. Natl. Acad. Sci. **89:**7174–7178.
25. **Stamberg, J.** 1969. Genetic control of recombination in *Schizophyllum commune*: separation of the controlled and controlling loci. Heredity **24:**306–309.
26. **Stamberg, J.** 1968. Two independent gene systems controlling recombination in *Schizophyllum commune*. Mol. Gen. Genet. **102:**221–228.
27. **Stankis, M. M., C. P. Specht, H. Yang, L. Giasson, U. R. C., and C. P. Novotny.** 1992. The *A*α mating locus of *Schizophyllum commune* encodes two dissimilar multiallelic homeodomain proteins. Proc. Natl. Acad. Sci. USA **89:**7169–7173.
28. **Tymon, A. M., U. Kües, W. V. J. Richarson, and L. A. Casselton.** 1992. A fungal mating type protein that regulates sexual and asexual development contains a POU-related domain. EMBO J. **11:**1805–1813.
29. **Vaillancourt, L., and C. A. Raper.** 1996. Pheromones and pheromone receptors as mating-type determinants in basidiomycetes, p. 219–247. *In* J. K. Setlow (ed.), Genetic engineering, principles and methods, vol. 18. Plenum, New York, N.Y.
30. **Wendland, J., and E. Kothe.** 1996. Allelic divergence at the *B*α pheromone receptor genes of *Schizophyllum commune*. FEMS Microbiol. Lett. **145:**451–455.