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An expanded genetic linkage map of an intervarietal *Agaricus bisporus* var. *bisporus* × *A. bisporus* var. *burnettii* hybrid based on AFLP, SSR and CAPS markers sheds light on the recombination behaviour of the species

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ABSTRACT

A genetic linkage map for the edible basidiomycete *Agaricus bisporus* was constructed from 118 haploid homokaryons derived from an intervarietal *A. bisporus* var. *bisporus* × *A. bisporus* var. *burnettii* hybrid. Two hundred and thirty-one AFLP, 21 SSR, 68 CAPS markers together with the *MAT*, *BSN*, *PPC1* loci and one allozyme locus (*ADH*) were evenly spread over 13 linkage groups corresponding to the chromosomes of *A. bisporus*. The map covers 1156 cM, with an average marker spacing of 3.9 cM and encompasses nearly the whole genome. The average number of crossovers per chromosome per individual is 0.86. Normal recombination over the entire genome occurs in the heterothallic variety, *burnettii*, contrary to the homothallic variety, *bisporus*, which showed adaptive genome-wide suppressed recombination. This first comprehensive genetic linkage map for *A. bisporus* provides foundations for quantitative trait analyses and breeding programme monitoring, as well as genome organisation studies.

1. Introduction

Agaricus bisporus (Lange) Imbach is the major edible mushroom species cultivated throughout the world. In addition to its cultivation for food, the white button mushroom is a potential source of health-protecting and medicinal molecules (Adams et al., 2008; Savoie et al., 2008). Its bioremediation ability has also been demonstrated (Kameda et al., 2006). As a lignin decomposer fungus, the use of *A. bisporus* in the bioconversion of agricultural and industrial lignocellulose waste is promising (Stoknes et al., 2008). Despite its economic potential, only a few genetic studies have been carried out concerning *A. bisporus*, due to its particular life cycle. *A. bisporus* is an amphitallic species with a homothallic or heterothallic cycle depending on the ploidy level of the spores that can be heterokaryotic ($n + n$) or homokaryotic (n), respectively. Cultivated strains belong to *A. bisporus* var. *bisporus* with a predominantly pseudohomothallic life cycle. This type of sexual behaviour, equivalent to a pseudoclonal inbreeding system, hampers outcrossing and limits breeding success (Moquet et al., 1998). The discovery of the heterothallic *A. bisporus* var. *burnettii* among the wild genetic resources (Callac et al., 1993) makes it possible to overcome these limitations. In fact, this variety is predominantly heterothallic and produces mainly tetrasporic basidia necessary to homokaryotic spore production. Introducing the dominant tetrasporic allele (Kerrigan et al., 1994) into mushroom pedigrees greatly facilitates the breeding process. Thus, gathering genetic data on *A. bisporus* has become a key challenge for performing adequate selection and for managing breeding programmes. The development of molecular markers and the construction of genetic linkage maps are essential tools for such genetic studies. Genetic linkage maps for *A. bisporus* have already been described by Kerrigan et al. (1993), Callac et al. (1997) and Moquet et al. (1999). However, these maps were incomplete and thus of limited practical use. The map developed by Kerrigan et al. (1993) was based on the analysis of an *A. bisporus* var. *bisporus* intravarietal offspring, and appeared to be unusually compact with several genomic regions showing suppressed recombination. It was unsaturated with less linkage groups than the number of chromosomes ($n = 13$) and several unlinked markers. An intervarietal var. *bisporus* × var. *burnettii* offspring (Callac et al., 1993) was used to address recombination behaviour analysis on the chromosome *I*. Comparative mapping suggested that greater recombination rates occurred in the variety *burnettii* (Callac et al., 1997). The same intervarietal progeny was used by Moquet et al. (1999). Nevertheless, this latter linkage map comprised only five linkage groups and was far from saturation. Additional markers are needed to obtain a more complete and representative map that will serve as a foundation for genome analysis. In plants, recombination frequencies vary enormously between and within species, between individuals, accessions or populations, as well as between particular chromosomal regions (Esch et al., 2007; Mezard, 2006). Since

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recombination is a crucial component of evolution and breeding because it produces new genetic combinations, the knowledge of the recombination behaviour in *A. bisporus* will enhance the breeding process and facilitate analyses of wild populations.

The aims of this study were (1) to construct a complete linkage map for *A. bisporus*, and (2) to clarify the recombination behaviour over the whole genome *via* the analysis of crossover events. By using the available intervarietal mapping population obtained by Callac et al. (1993), we ensured consistency with previous mapping efforts. Amplified fragment length polymorphism (AFLP) markers were used to construct the frame of the map and to increase marker density. Microsatellite (SSR) markers and sequence tag sites mapped using the cleaved amplified polymorphism sequence (CAPS) technique were added in order to provide useful landmarks on the genome. Segregation data for the mating type (*MAT*), basidial spore number (*BSN*) and pilei-pellis colour (*PPC1*) loci were also included for our map construction. The usefulness of the present linkage map as a reference tool for genetic and genomic studies is discussed.

2. Materials and methods

2.1. Mapping population

The haploid progeny of 118 homokaryons (referred to hereafter as H_i) used for linkage analysis were obtained from the intervarietal hybrid JB3-83 × U1-7 (Callac et al., 1993; Kerrigan et al., 1994). JB3-83 and U1-7 were homokaryons from the wild strain of *A. bisporus* var. *burnettii* JB3 and the commercial hybrid U1, respectively (Callac et al., 1993).

2.2. DNA extraction

Total DNA was extracted from freeze-dried mycelium with the Nucleon Phytopure Genomic DNA Extraction kit (GE Healthcare Life Sciences) according to the manufacturer's instructions. A purification step with ethanol precipitation was systematically performed. DNA concentration was adjusted to 25 ng/μl and the samples were stored at −20 °C.

2.3. Development of CAPS markers

The cleaved amplified polymorphic sequence (CAPS) technique was used to reveal polymorphism in sequence tag sites developed on the basis of ESTs or genomic sequences available in public databases. PCR primer pairs were designed to amplify products between 300 and 800 bp using Primer3 software with default parameters (Rozen and Skaletsky, 2000). PCR were performed in 30 μl of reaction mixture containing 50 ng genomic DNA, 0.5 μM each of primer, 0.2 mM dNTPs, 2 μg BSA, 1 U *Taq* DNA Polymerase and 1x incubation buffer. Amplifications were carried out as follows: an initial denaturing step at 94 °C for 5 min; 35 cycles of 94 °C for 1 min, 55 °C for 90 s, 72 °C for 1 min; and a final extension at 72 °C for 5 min. Restriction polymorphisms between the two parental strains were screened using ten common enzymes (*Alu*I, *Hae*III, *Hha*I, *Hinf*I, *Hpa*II, *Hsp*92II, *Nde*II, *Rsa*I, *Taq*I, *Tru*9I). Five microlitre of the PCR product were digested with 8 U of the appropriate restriction enzyme for 120 min, at the optimal incubation temperature recommended by the manufacturer. CAPS products were resolved in 2% agarose gels, running at 90 V for 90 min. and stained with ethidium bromide. Primer sequences and the corresponding restriction enzyme for each CAPS marker are listed in Table 1. Other CAPS markers described by Callac et al. (1997) or by Moquet et al. (1999) were added to our mapping data set (Table 1).

2.4. Microsatellite markers

The SSR primers, PCR conditions, electrophoretic separation and visualisation were previously described in Foulongne-Oriol et al. (2009).

2.5. AFLP genotyping

AFLP markers were generated as described by Vos et al. (1995). A non-selective pre-amplification was performed with E + 0 (5′-GACT GCGTACCAATTC-3′) and /M + 0 (5′-GATGAGTCCTGAGTAA-3′) primers, followed by a selective amplification with two-base-extended primers. The sixteen primer combinations used in this study were as follows: E + AA/M + AA, E + AA/M + AG, E + AA/M + CA, E + AC/M + CA, E + AG/M + CA, E + AT/M + AC, E + AT/M + CA, E + AT/M + CC, E + CA/M + CA, E + CA/M + CT, E + CC/M + CA, E + CC/M + GA, E + CG/M + AC, E + CG/M + CC, E + GA/M + AA, E + GC/M + CG. AFLP products were separated on 6% denaturing polyacrylamide gel and stained with silver nitrate following the protocol of Budowle et al. (1991). Reliable AFLP markers were scored in a binary format according to the presence or absence of the amplified fragment.

2.6. Other markers

The phenotypic *MAT*, *BSN*, and *PPC1* loci and the alcohol dehydrogenase (ADH) allozyme marker, previously described (Callac et al., 1998; Imbernon et al., 1996; Moquet et al., 1999), were included in the mapping data.

2.7. Data analysis and mapping strategy

Genotypic data were independently scored by two people to minimise scoring and interpretation errors. Doubtful data remaining after re-verification were discarded. Deviations from the expected segregation 1:1 at each locus were tested by a χ^2 test. Linkage and mapping analysis were performed using Mapmaker/exp V3.0b software (Lander et al., 1987) with a backcross model for handling data. In a first round of mapping, markers showing more than 10% of missing data and/or skewed segregation ratios ($p < 0.05$) were omitted. Linkage groups were determined by pair-wise analysis ('group' command) with a likelihood of odds (LOD) score of four and a maximum recombination frequency of 0.3. For each group, markers were ordered using the 'order' command. Markers with multiple likelihood positions were discarded. In a second step, markers excluded from the first round of mapping were tested for linkage. The 'assign' command (LOD > 6) allowed the assignation of these markers to a linkage group defined in the first step. For each linkage group, additional markers were sequentially placed using the 'try' commands. Markers difficult to map or which lengthen the map by more than 10 cM were eliminated. Subsequent orders were tested with the 'ripple' command. AFLP markers with a distorted segregation ratio were cautiously tested for their contribution to the map in a third round of mapping. Instances of double-crossover were inspected and data were re-examined, corrected if necessary and reanalysed. The recombination rate was transformed into map distance using the Kosambi function. The linkage map was drawn using MapChart software (Voorrips, 2002).

Linkage groups (LGs) were assigned to chromosomes according to the literature and data available in public databases (Table 1); they were numbered with Roman numerals according to chromosome numbering.

2.8. Genome length estimate, map coverage and marker distribution

The genome length L_e was estimated as $\sum (L_{Gi} + 2s)$, where L_{Gi} is the length of linkage group i, and (s) the average marker spacing