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Regular Articles

A detailed analysis of the recombination landscape of the button mushroom *Agaricus bisporus* var. *bisporus*

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ABSTRACT

The button mushroom (*Agaricus bisporus*) is one of the world's most cultivated mushroom species, but in spite of its economic importance generation of new cultivars by outbreeding is exceptional. Previous genetic analyses of the white *bisporus* variety, including all cultivars and most wild isolates revealed that crossing over frequencies are low, which might explain the lack of introducing novel traits into existing cultivars. By generating two high quality whole genome sequence assemblies (one *de novo* and the other by improving the existing reference genome) of the first commercial white hybrid Horst U1, a detailed study of the crossover (CO) landscape was initiated. Using a set of 626 SNPs in a haploid offspring of 139 single spore isolates and whole genome sequencing on a limited number of homo- and heterokaryotic single spore isolates, we precisely mapped all COs showing that they are almost exclusively restricted to regions of about 100 kb at the chromosome ends. Most basidia of *A. bisporus* var. *bisporus* produce two spores and pair preferentially via non-sister nuclei. Combined with the COs restricted to the chromosome ends, these spores retain most of the heterozygosity of the parent thus explaining how present-day white cultivars are genetically so close to the first hybrid marketed in 1980. To our knowledge this is the first example of an organism which displays such specific CO landscape.

1. Introduction

The button mushroom *Agaricus bisporus* has a long history of cultivation of more than 300 years, and the first commercial cultivation has been reported in France in the 18th century (Pardo et al., 2010; Tournefort, 1707). Button mushrooms are grown worldwide with a product volume of 3.9 million tonnes in 2009 (Sonnenberg et al., 2011) and a fast increasing production in China since the last few years (Zhang et al., 2014). The productivity and quality of the crop has been improved considerably during the last 30 years mainly by improving cultivation techniques, i.e., composting, casing, growing conditions, and mechanical spawning and harvesting. With a few exceptions, breeding has played a very minor role in the improvements. Genetic studies and breeding trials for advanced cultivars have been conducted meanwhile for cap colour (Callac et al., 1998), resistance to pathogens (Moquet et al., 1998; Foulongne-Oriol et al., 2012b), yield (Foulongne-Oriol et al., 2012a), earliness (Foulongne-Oriol et al., 2012a), and resistance to mechanical bruising (Gao et al., 2013, 2015). This has, however, not yet resulted in commercial cultivars that are substantially superior to the first hybrid strain Horst U1, which was marketed in 1980 (Fritsche, 1982). The cultivars released since 1980 were either genetically identical or very similar to the first hybrid (Sonnenberg et al., 2011). The main reason for the lack of introduction of new cultivars lies in the typical life cycle of the button mushroom. We have carried out a more detailed analysis of its meiosis as has been done so far to make clear what the obstacles are in breeding and also to explain the very narrow genetic base of almost all cultivars of the white button mushroom grown worldwide.

Sexual reproduction in fungi is mainly divided in homothallic (inbreeding/selfing) or heterothallic (outbreeding) sexuality (as reviewed recently by Ni et al., 2011). Compatibility between partners is determined by one (bifactorial) or two (tetrafactorial) mating loci where different mating types are needed for a successful

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mating. Homothallism has been defined historically in fungi as the ability of a single spore to produce a sexually reproducing colony when propagated in complete isolation (Blakeslee, 1904), since they have both mating type genes in a single genome, whereas in heterothallic organisms mating always takes place between individuals with different mating types. The species *A. bisporus* is mainly represented by two varieties, var. *bisporus* and the var. *burnettii* (Callac et al., 2003), both with a unifactorial mating system and displaying both types of sexuality and which can thus be designated as amphithallic (Kühner, 1977). Each variety, however, differs in ploidy level for the majority of its offspring, n + n in the *bisporus* and n in the *burnettii* variety (Kerrigan et al., 1994). As in all basidiomycetes, meiosis takes place in *A. bisporus* in basidia where, after fusion of the two different constituent haploid nuclei, meiosis I and II result in four haploid nuclei. In the majority of the basidia of *A. bisporus* var. *bisporus*, these nuclei are distributed over two spores with a preference of pairing non-sister nuclei (Callac et al., 1993; Summerbell et al., 1989; Kamzolkina et al., 2006). Germination of these spores results in heterokaryons able to produce fruiting bodies. Around 10–15% of the basidia produce three or four spores, the majority of this receiving one haploid nucleus which will germinate into homokaryons that need to be mated with a compatible partner to produce fruiting bodies. In *A. bisporus* var. *burnettii* approximately 90% of the basidia generate four spores (Kerrigan et al., 1994) where each spore receives one haploid nucleus and germinates into a homokaryon. There are indications that the heterokaryotic offspring of the var. *burnettii* also arises by pairing of non-sister nuclei (Kerrigan et al., 1994) but direct evidence for this has not been obtained yet by genotyping recovered constituent nuclei. The variety *bisporus* has thus mainly a homothallic sexuality whereas the variety *burnettii* is predominantly heterothallic. The meiotic behaviour of *A. bisporus* var. *bisporus* has been studied in the past using molecular markers (Royse and May, 1982; Allen et al., 1992; Summerbell et al., 1989). Genotyping of large numbers of random selections of single spore isolates (SSI) with a limited number of markers revealed that 95–100% retained complete heterozygosity. This indicates a low recombination frequency and pairing of non-sister nuclei. Kerrigan et al. (1993) studied segregation of a substantial number of markers in a set of homokaryotic offspring and showed that *A. bisporus* var. *bisporus* has a normal meiosis but here also a low recombination frequency was seen. Foulongne-Oriol et al. (2010, 2011) performed an extended linkage analysis using crosses between *bisporus* and *burnettii* varieties and showed a crossover landscape comparable to what is seen in other fungi and observed a strong impact of the genetic background on recombination ability, indicating a low recombination frequency in the *bisporus* and normal frequency in the *burnettii* variety. A thorough study of the meiosis of the variety *bisporus*, however, using large populations and many molecular markers has not been done. It is still unclear where and how frequent recombinations take place in the genome of the variety *bisporus*. Are crossovers (CO) indeed infrequent or do many CO result in lethal offspring? The utilisation, so far, of a limited number of offspring and markers also raises the question if all recombinations have been detected. Crossovers at extreme chromosome ends may not always be detected due to the presence of repetitive elements (Fulcher et al., 2014). It has been suggested that CO in *A. bisporus* var. *bisporus* are mainly localised at chromosome ends (Kerrigan et al., 1993; Foulongne-Oriol et al., 2009) which raises the question whether recombination is indeed suppressed in the variety *bisporus* or not always detected because they occur mainly at chromosome ends. The variety *bisporus* represents all commercial cultivars and most wild isolates. This variety is thus an important base for breeding and a more detailed understanding of its meiotic behaviour is important. By generating two high quality whole genome sequences (one *de novo* and the other by improving the existing reference sequence) of the first commercial white hybrid Horst U1, a thorough study was made of the CO landscape in offspring of this cultivar. A high number of SNPs in large haploid offspring and a detailed analysis with whole genome sequencing on a limited number of homo- and heterokaryotic single spore isolates was done to precisely map the CO landscape of the var. *bisporus* revealing a remarkable CO landscape which clearly demonstrates the origin of most of the present-day commercial white cultivars of the button mushroom.

2. Materials and methods

An overview of the genetic analyses and the strains involved is given in the supplementary data (Fig. S1).

2.1. Strains

All heterokaryons used in this study were obtained from the strain collection of Wageningen UR Plant Breeding (Table 1). Cultures of commercial cultivars have been obtained in the past via spawn ordered from companies and stored as vegetative cultures in the collection. SSI isolation and confirmation of ploidy level was done as described elsewhere (Gao et al., 2013). The recovery of constituent nuclei as homokaryons from heterokaryons was done by protoplasting as described previously (Sonnenberg et al., 1988).

2.2. DNA isolation

For sequencing, high molecular weight DNA of good quality and in high quantity is required. To obtain this, mycelium was harvested after growing for two weeks on agar plates covered with cellophane, subsequently lyophilized and grinded to a fine powder in an Eppendorf tube. One ml of DNA extraction buffer (200 mM Tris/HCl pH 8.0; 25 mM EDTA; 250 mM NaCl) was added and mixed thoroughly. Subsequently, 700 µl phenol and 300 µl SEVAG (Chloroform:isoamyl alcohol, 24:1 v/v) were added and mixed. After centrifugation for 1 h at 14,000 rpm (4 °C) the supernatant was transferred to a new tube, 12 µl RNase (10 mg/ml) was added and the mixture incubated at 37 °C for 30 min. One vol. of SEVAG was added, mixed gently and centrifuged for 30 min at 14,000 rpm (4 °C). The supernatant was transferred to a new tube and 0.55 vol. isopropanol (−20 °C) was added and gently mixed. After centrifugation for 10 min at max rpm (4 °C), the pellet was washed with 70% ethanol (−20 °C), dried and dissolved in 30 µl $T_{10}E_{0.1}$. For genotyping, genomic DNA was extracted from lyophilized mycelium using the Wizard® Magnetic 96 DNA Plant System (Promega) according to the manufacturer's protocol.

2.3. De novo sequencing and genome comparison

High molecular DNA of one of the constituent nuclei of Horst U1 (homokaryon H39) was *de novo* sequenced with the PacBio RSII technology using and p6-c4 chemistry (Pacific Biosciences, Menlo Park, California, USA). The sequence data (1,147,175 reads with a N50 length of 9461 nt, total 6,446,332,774 nt representing a coverage of 200×) were assembled into scaffolds using HGAP2 and polished with the Quiver protocol; both are part of SMRT® Analysis version 2.3.0.140936.p1.142411 algorithm (SMRT® portal, Pacific Biosciences, Menlo Park, California, USA). Resulting scaffolds were further assembled based on SMRT® View BridgeMapper (SMRT® portal, Pacific Biosciences, Menlo Park, California, USA), linkage map information (see Section 2 on linkage analysis) and the results of a BLAST search (CLC Genomics Workbench version 7.5.1, Qiagen, Aarhus, Denmark) of scaffold ends against all scaffolds.