Whole Genome Sequence of the Commercially Relevant Mushroom Strain Agaricus bisporus var. bisporus ARP23

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ABSTRACT Agaricus bisporus is an extensively cultivated edible mushroom. Demand for cultivation is continuously growing and difficulties associated with breeding programs now means strains are effectively considered monoculture. While commercial growing practices are highly efficient and tightly controlled, the over-use of a single strain has led to a variety of disease outbreaks from a range of pathogens including bacteria, fungi and viruses. To address this, the Agaricus Resource Program (ARP) was set up to collect wild isolates from diverse geographical locations through a bounty-driven scheme to create a repository of wild Agaricus germplasm. One of the strains collected, Agaricus bisporus var. bisporus ARP23, has been crossed extensively with white commercial varieties leading to the generation of a novel hybrid with a dark brown pileus commonly referred to as ‘Heirloom’. Heirloom has been successfully implemented into commercial mushroom cultivation. In this study the whole genome of Agaricus bisporus var. bisporus ARP23 was sequenced and assembled with Illumina and PacBio sequencing technology. The final genome was found to be 33.49 Mb in length and have significant levels of synteny to other sequenced Agaricus bisporus strains. Overall, 13,030 putative protein coding genes were located and annotated. Relative to the other A. bisporus genomes that are currently available, Agaricus bisporus var. bisporus ARP23 is the largest A. bisporus strain in terms of gene number and genetic content sequenced to date. Comparative genomic analysis shows that the A. bisporus mating loci in unifactorial and unsurprisingly highly conserved between strains. The lignocellulolytic gene content of all A. bisporus strains compared is also very similar. Our results show that the pangenome structure of A. bisporus is quite diverse with between 60–70% of the total protein coding genes per strain considered as being orthologous and syntenically conserved. These analyses and the genome sequence described herein are the starting point for more detailed molecular analyses into the growth and phenotypical responses of Agaricus bisporus var. bisporus ARP23 when challenged with economically important mycoviruses.

KEYWORDS Button mushroom Agaricus bisporus Genome report Agaricus Resource Program Agaricus bisporus mating locus Agaricus pangenome
this genome report is a wild isolate from the ARP collection referred to as *A. bisporus* var. *bisporus* ARP23. Crosses of homokaryons of U1 and old-fashioned brown led to an intermediate hybrid that was subsequently crossed with ARP23 to produce a novel commercially productive hybrid referred to as 'Heirloom'. Wild strains are also a promising resource for the introduction of disease-resistant traits for common commercial mushroom diseases. It has been shown that the wild tetrasporic *A. bisporus* var. *burnetii* has heightened resistance to the pathogen that causes bacterial blotch (*Pseudomonas tioalasia*) through genetic markers linked to the Ppc1 allele (Moquet et al. 1999). Polygenic inheritance of resistance attributes has been described (Kerrigan 2000) and so the consideration of the introduction of wild *A. bisporus* germplasm into breeding novel strains must be considered on the basis of careful selection of screened wild strains with distinct mechanisms pertaining to disease resistance (Foulonoge-Oriol et al. 2011).

To date, two genomes of the constituent homokaryons of Horst U1 have been sequenced, H97 (Morin et al. 2012) and H39 (Sonnenberg et al. 2016). This represents the genome of the first commercially cultivated white hybrid strain. The manuscript that reported the H97 genome sequence also described the genome of *Agaricus bisporus* var. *burnetii* (IB137–58), a strain exclusively native to the Sonoran Desert of California. That study uncovered the genetic and enzymatic mechanisms that favor *A. bisporus* to a humic-rich environment by primary degradation of plant material. A gene arsenal of compost-induced carbohydrate enzymes (heme-thiolate peroxidase, β-etherases, multicopper oxidase) and CYP450 oxidoreductases for example, together with high protein degradation and nitrogen-scavenging abilities were determined to be crucial to the challenges posed by complex composts (Morin et al. 2012).

The genome presented herein, represents the first commercially relevant genome for a wild cultivar of *Agaricus bisporus* var. *bisporus*, and is an invaluable tool for future efforts in mushroom breeding. Furthermore the genome sequence described will act as the starting point for more detailed OMIC based studies into the growth and phenotypical responses of *Agaricus bisporus* var. *bisporus* ARP23 when challenged with economically important mycoviruses.

**METHODS**

**Strain, culture conditions and homokaryon genotyping**

*A. bisporus* var. *bisporus* ARP23 cultures were grown on compost extract agar (aqueous extract of phase II mushroom compost, double-autoclaved for sterility) for three weeks at 25°C in the dark. 10 ml of protoplanting medium (50 mM maleic acid, 0.6 M saccharose, 1 M NaOH pH 5.8) containing 10 g Glucanex (Sigma Aldrich cat. no. L1412) (Kerrigan 1994) was added to established mycelium and incubated at 25°C for two weeks at 25°C in the dark, at 25°C. 7 mm agar plugs were excised from the growing hyphal edges of homokaryons of MAT2 genotype (the MAT1 genotype was not recovered) and shaken at 30 Hz for 7 min in 500 μL malt extract and the resulting homogenate added to 50 ml malt extract liquid medium (10 g malt extract in 600 ml of dH2O) in a 500 ml Erlenmeyer flask. Liquid cultures were grown for 12 days at 25°C in the dark at 150 rpm.

**DNA isolation and libraries**

Fungal mycelium was isolated in Miracloth and washed with sterile PBS. The mycelium was flash frozen and ground in liquid nitrogen using a mortar and pestle. DNA isolation was carried out immediately on ground material with the Wizard Genomic DNA Purification Kit (Promega) following the plant tissue method with minor modifications. Nuclei lysis buffer was supplemented with 0.5 M EDTA and 0.1 mg/mL Proteinase K and cell lysis was carried out at 37°C for 30 min. The remainder of the DNA isolation was as per manufacturer’s guidelines. An Illumina paired-end sequencing library with insert size of 270 bp (80 X coverage) and a Pacbio RSII mate pair library of 20 Kb insert size were generated for a hybrid assembly approach. Sequencing on HiSeq 4000 and Pacbio RSII generated 5.90 GB and 57.64 GB of raw sequence data, respectively. DNA library construction and sequencing on the Pacbio (RSII) and Illumina (HiSeq 4000) platforms was carried out by BGI Tech Solutions Co., Ltd. (Hong Kong, China).

**Fruit-body material, RNA isolation and sequencing**

ARP23 mycelia was added to mushroom compost in crates (n = 3) and incubated at 25°C, 90–95% relative humidity (spawn-run phase) for 17 days. A layer of peat was added to the surfaces of the colonized compost (case-run phase) and incubated for another 7 days. Temperatures and relative humidity were lowered to 18°C and 85–90% and fruit-bodies were harvested after 7 days of development. All cropping procedures were as per standard mushroom growing practices. Fruit-bodies were flash-frozen, freeze-dried and material was crushed in liquid N2. RNA was isolated using the RNaseq plant minikit (Qiagen) as per manufacturers guidelines. DNA digestion was done with DNase I (Invitrogen). RNA quantity and quality was assessed with an RNA6000 Nano Assay (Agilent 2100 Bioanalyzer, Agilent Technologies, USA). High-quality RNA was sent to BGI Tech Solutions Co., Ltd. (Hong Kong, China) for RNA sequencing (RNA-seq).

**Genome assembly and gene calling**

Short-read libraries had adaptor removal (Martin 2011) and quality trimming performed using Trim Galore! (https://github.com/FelixKrueger/TrimmGalore). A minimum phred score cut-off of 25 and a minimum read length of 90 nt was applied to short-read libraries. Long-read libraries were corrected using short reads using Proovread (Hackl et al. 2014). Corrected long reads were then self-corrected with additional
Genome functional annotation and characterization
Putative open reading frames (ORFs) were assigned protein family (PFam) domains using funannotate (https://github.com/nextgenusfs/funannotate) using default settings of Interproscan 5 (Jones et al. 2014). Gene ontology (GO) IDs (Ashburner et al. 2000) were assigned where available and a corresponding GO term map was obtained using YeastMine (Balakrishnan et al. 2012). Information on the pathways associated with different genes was analyzed with KEGG (Kyoto Encyclopedia of Genes and Genomes) (Ogata et al. 1999) by assigning KO (KEGG ontology) through BlastKOALA (Kanehisa et al. 2016). A search for repetitive elements was done by identifying tandem repeats (TR) and transposable elements (TE). TRs were identified over the entire assembly with Tandem Repeats Finder (TRF 4.07) (Benson 1999). Classification of the different categories of TE in the genome were conducted using RepeatMasker 4.06 with the modified version of NCBI Blast for RepeatMasker, RMBLAST (http://www.repeatmasker.org/RMBlast.html). Simple single repeats were also determined using RepeatMasker with the default settings. tRNAs were identified across all scaffolds using tRNAscan-SE v 2.0 (Lowe and Chan 2016) and tRNAs were also identified with RNAmmer 1.2 (Lagesen et al. 2007).

Carbohydrate-active enzymes
Translated ORFs were used to search dbCAN2 (Zhang et al. 2018) for presence of Carbohydrate Active Enzymes (CAZys) (Lombard et al. 2014). For comparative purposes 32 fungal genomes consisting of a variety of Ascomycota and Basidiomycota species (Table S1) were also searched to catalog their CAZy content. As well as including brown rot and white rot fungi, genomes for the top 10 most highly cultivated mushrooms are also included in this dataset (Table S1).

Mating locus
The locus coding for homeodomain proteins typical for the A mating type in Coprinopsis cinerea was used to locate the unifactorial A. bisporus mating-type locus. The homeodomain proteins as well as flanking proteins were individually searched using BLASTp (Altschul et al. 1997) (evalue 10^-2) against the predicted proteomes of A. bisporus AR23, H97 and JB137-S8 respectively. Top hits from these gene sets were then searched back against the C. cinerea gene set to locate reciprocal best BLAST hits which were then considered orthologs.

Phylogenomic reconstruction
Orthologous gene families were identified with OrthoFinder2 (Emms and Kelly 2015), using BLASTp (Altschul et al. 1997) as the search algorithm, an inflation value of 2.0 for MCL clustering (Enright et al. 2002) and the command-line parameter ”-msa”. 71 gene families were ubiquitously present and single copy and used for phylogenomic analysis. Each family was individually aligned using MUSCLE (Edgar 2004) and trimmed using trimAl (Capella-Gutierrez et al. 2009) with the parameter ”-automated” to remove poorly aligned regions. Trimmed alignments were concatenated together resulting in a final supermatrix alignment of 27,861 amino acids. Phylogenomic analyses were performed using both Maximum likelihood and Bayesian inference. IQ-TREE (Nguyen et al. 2015) was used to perform maximum likelihood analysis under the LG+F+R5 model, which was the best fit model according to ModelFinder (Kalyaanamoorthy et al. 2017), and 1,000 ultrafast bootstrap replicates (Hoang et al. 2018). Bayesian analyses were carried out using PhyloBayes with the CAT model (Lartillot et al. 2009). Two independent chains were run for 8,000 cycles and convergence was assessed using bcpomp and tracecomp. A consensus Bayesian phylogeny was generated with a burn-in of 10%. Support values represent posterior probabilities. The phylogeny was visualized and annotated using the Interactive Tree of Life (iTOl) (Letunic and Bork 2007).

Agaricus bisporus pangenome dataset assembly
Genome assembly data for three Agaricus bisporus strains (H97, JB137 and H39) were obtained from NCBI. Gene sequence and genomic location datasets were generated for each of the three strains through the pangenome analysis pipeline Pangloss: a gene prediction strategy using a combination of HMM-dependent gene prediction with GeneMark-ES and PWM-dependent long ORF prediction was conducted with the Basidiomycota (odb9) lineage. A summary of the genome statistics is shown in Table 1.

Table 1 Genome statistics for A. bisporus strains AR23, H97 and JB137-S8

| Feature                     | AR23       | H97        | JB137-S8   |
|-----------------------------|------------|------------|------------|
| Number of Scaffolds         | 169        | 29         | 206        |
| Largest Contig              | 1,506,893  | 3,343,696  | 2,973,556  |
| Total Size of Scaffolds (Mb)| 33.49      | 30.23      | 31.20      |
| N50                         | 350,711    | 2,334,609  | 1,225,131  |
| L50                         | 26         | 6          | 8          |
| GC content (%)              | 46.33      | 46.48      | 46.59      |
| Number of introns           | 70,261     | 50,356     | 53,337     |
| Complete BUSCOs (G)         | 87.6%      | 87.8%      | 88.3%      |
| Number of protein coding    | 13,030     | 10,863     | 11,289     |
| genes                       |            |            |            |
| Proteins with a signal peptide | 750     | 717        | 734        |
| Number of tRNA              | 200        | 160        | 215        |
| Number of rRNA              | 10         | 22         | 3          |
| Number of sRNA              | 93         | 90         | 87         |
| Repetitive regions (%)      | 0.79       | 0.79       | 0.75       |
| Number of tandem repeats    | 2,287      | 2,353      | 2,480      |
| Simple repeat sequences (%) | 0.54       | 0.54       | 0.53       |
| Low complexity regions (%)  | 0.13       | 0.13       | 0.12       |
| Non-LTR transposons         | 250        | 240        | 134        |
| LTR transposons             | 1          | 2          | 2          |

BUSCO analysis conducted with the Basidiomycota (odb9) lineage.
chosen (McCarthy and Fitzpatrick 2019b). Combined with data
from *A. bisporus* ARP23, a total of 42,264 *A. bisporus* gene sequences
and their corresponding genomic locations were predicted. An all-vs.-
all BLASTp search was performed on the *A. bisporus* dataset using an
e-value cutoff of 1e-4 (Camacho et al. 2009).

Data availability
The Bioproject designation for this project is PRJNA544931. This Whole
Genome Shotgun project has been deposited at DDBJ/ENA/GenBank
under the accession VCNO00000000. The version described in this
paper is version VCNO01000000.

Table S1 shows the genomes, taxonomy and download links for the
32 genomes used in the phylogenomic and CAZy studies. Table S2
shows the presence of lignocellulolytic genes in the 32 fungal genomes.
Figure S1: Macrosynteny between *A. bisporus* H97 chromosomes and
all *A. bisporus* ARP23 scaffolds. Only regions larger than 10,000bp are
connected with links. Macrosynteny visualized with Jupiter Plot. Sup-
plemental material available at FigShare: https://doi.org/10.25387/
g3.9076991.

RESULTS
Whole-genome assembly
The genome of the monokaryotic *A. bisporus* var. *bisporus* strain ARP23
ARP23 herein) was sequenced using a hybrid approach of short (Illumi-
mina Hiseq 4000) paired-end reads and long-reads (Pacbio RSII). A
total of 8,861,726 reads representing a cumulative size of 4.074 GB were
generated including 8,424,105 and 437,621 reads from Illumina and
PacBio sequencing platforms respectively. Upon trimming adaptors,
error-correction and hybrid-assembly of both short and long-read li-
braries, a 33.49Mb genome with a GC content of 46.33% was generated.
The assembly is comprised of 169 contigs, the longest being 1.5Mb with

Figure 1 Macrosynteny between *A. bisporus* H97 chromosomes and *A. bisporus* ARP23 scaffolds. Only regions larger than 10,000bp are
connected with links. Macrosynteny visualized with Jupiter Plot. For display purposes only the largest scaffolds that correspond to 75% of the
ARP23 assembly are incorporated. When all scaffolds are included higher levels of coverage are observed particularly with respect to H97
chromosomes 1 and 13 (Figure S1).
an N50 of 350,711 and an L50 of 26 (Table 1). The average length of contigs is 198,204 bp. Kmer-analyses conducted with GenomeScope (Vurture et al. 2017) suggest a genome size of 34.02Mb indicating that the 169 scaffolds of this assembly cover 98.44% of the entire genome. The completeness of the assembly was quantified by determining the presence/absence of the 1,315 fungal orthologs found in the Basidiomycete BUSCO set. A total of 1,170 (87.6%) complete BUSCO genes were located in the ARP23 assembly, this is comparable to what is observed in A. bisporus H97 (1,172 or 87.8%) and A. bisporus JB137-S8 (1,179 or 88.3%) strains (H97 and JB137-S8 respectively herin) (Table 1). Macrosynteny between the ARP23 assembly and the 13 complete H97 chromosomes was visualized with Jupiter Plot. Overall high levels of synteny are observed with the vast majority of ARP23 scaffolds mapping directly to individual H97 chromosome (Figure 1 & Figure S1). There are a number of scaffolds that have hits to multiple chromosomes indicating low levels of genome rearrangements have occurred (Figure 1 & Figure S1). There are no scaffolds in the ARP23 that do not map to the H97 assembly. Scaffold 74 was found to be 137,116 nucleotides in length and contains all 17 mitochondrial genes previously described in the mitochondrial of H97 (Férandon et al. 2013). Furthermore, it is also of a comparable length to the H97 mitochondrial genome (135,005 bp), therefore scaffold 74 corresponds to the full length ARP23 mitochondrial genome.

Genome annotation

The total length of repetitive elements in A. bisporus strain ARP23 amounted to 264,404 bp (0.79% of the total assembly) this is similar to what is observed for both H97 and JB137-S8 (Table 1). The number of tandem repeat regions across the 169 scaffolds of the assembled genome was 2,287, with ‘scaffold00003’ containing the greatest number at 321 repeat regions. Simple sequence repeats (SSRs) amounted to 180,995 bp (0.54%) and low complexity regions covered 44,108 bp (0.13%) (Table 1). We searched for the two sub-types of Non-LTR retrotransposons; long interspersed nuclear elements (LINEs) and short interspersed nuclear elements (SINEs). Of the 241 LINE-type elements; 17 belonged to L1, 69 to L2 and 78 to L3. For the 30 regions designated to SINES; 9 belonged to the MIRs and none were classified as ALUs. A single LTR retrotransposon was found for the endogenous retroviruses (ERV) class I. A single hAT-Charlie family DNA transposon and 7 DNA/TcMar-Tiggers were found out of a total of 55 DNA elements. Overall the ARP23 assembly contains 200 tRNAs, 10 rRNAs and 93 sRNAs (Table 1). In total 13,030 putative protein-coding genes were called for ARP23. Of these 7,725 (59.3%) were annotated with PFam domains. An analysis of the presence of the BUSCO set of orthologous genes for Basidiomycetes in the ARP23 gene set revealed that is missing 40 (3%) of the BUSCO genes. This is comparable to the predicted proteomes of H97 and JB137-S8 which are both missing 39 (2.9%) of the BUSCO genes. ARP23 gene calling incorporated RNAseq data from fruitbodies, the final gene set showed evidence that 775 (5.95%) of protein coding genes are alternatively spliced. Overall the number of genes predicted for ARP23 is larger than that for H97 (10,863) and JB137-S8 (11,289) (Table 1). The average number of introns per ORF for the ARP gene set is 5.4, this number is larger than that observed in H97 (4.6) and JB137-S8 (4.7) respectively (Table 1). High-level functional annotations were assigned for predicted genes using BlastKOALA (Kanehisa et al. 2016). Of the 13,030 predicted ARP23 genes, KO assignments were made for 3,808 (29.2%). General functions and protein families relating to genetic information processing accounted for the majority of KEGG annotation with 1,693 (45.46%) of proteins falling into these categories. Other pathways highly represented were carbohydrate metabolism with 313 (8%) and environmental information processing with 176 (5%). A total of 750 secreted proteins were predicted by assigning signal peptides.
Putatively secreted proteins with a transmembrane domain downstream of the N-terminus signal peptide were excluded (Sonnhammer et al. 1998), with the subsequent prediction of 606 proteins. Secreted proteins involved in hydrolysis of glycoside (n = 54, GO:0004553), oxidation-reduction processes (n = 82, GO:0055114), and fungal hydrophobins (n = 20, PF01185) were highly represented.

Genome phylogeny
The availability of whole genomes permits the reconstruction of phylogenomic trees. From our dataset of 32 fungal genomes we located 71 ubiquitously distributed gene families. These were individually aligned and concatenated to give a supermatrix of 27,861 amino acids. Using this supermatrix, phylogenomic reconstruction analyses were performed using both Maximum likelihood and Bayesian inference (Figure 2). The resultant phylogeny successfully resolved strongly supported monophyletic clades for the Ascomycota and Basidiomycota phyla. It also resolved monophyletic clades for the Agaricales, Boletales and Polyporales orders within the Basidiomycota clade (Figure 2).

Within the Agaricales order a strongly supported monophyletic Marasmioid clade containing Schizophyllum commune, Moniliophthora roreri, Gymnopus luxurians and Lentinula species is present. A monophyletic Agaricoid clade containing Laccaria bicolor, Coprinopsis cinerea and Agaricus bisporus is also present (Figure 2). The single Tricholomatoid clade species, Hypsizygus marmoreus is grouped beside the Agaricoid clade in agreement with previous studies (Matheny et al. 2006). However the two Pluteoid clade species, Pleurotus ostreatus and Volvariella volvacea are not grouped together, however it has been suggested that the Pluteoid clade may not be monophyletic (Matheny et al. 2006).

With respect to the phylogenetic relationships between the A. bisporus strains, our phylogeny groups all three strains in a monophyletic clade with maximum Bayesian posterior probability (BPP) and bootstrap support (BP). Furthermore, H97 and ARP23 are grouped as sister taxa with relatively strong BPP and BP (Figure 2). This phylogenetic relationship infers that ARP23 is more closely related to H97 that it is to JB137-S8.

Carbohydrate-Active Enzymes (CAZys)
A. bisporus is adapted to growth in a humic-rich, leaf-litter environment. The genome of H97 has a carbohydrate-active enzyme gene (CAZyme) (Lombard et al. 2014) repertoire more similar to that of white- and brown-rot basidiomycetes as opposed to closer taxonomically-related species such as Coprinopsis cinerea and L. bicolor (Figure 2) (Morin et al. 2012). Similar results were replicated in our analyses of the genomes of A. bisporus H97, JB137-S8 and ARP23 (Table S2). A total of 411 putative CAZymes were found in the genome of ARP23 including 176 glycoside hydrolases, 60 glycosyl transferases and 14 carbohydrate-binding modules. In terms of lignocellulolytic genes the three strains of A. bisporus have very similar repertoires with ARP23, JB137-S8 and H39 having 149, 142 and 136 genes respectively. Specifically, ARP23 was found to contain 48 cellulases, 19 hemicellulases, 12 pectinases, 17 lignin oxidases and 53 lignocellulolytic auxiliary enzymes (Table S2).

Mating locus
Agaricus bisporus has a pseudo-homothallic life cycle with a unifactorial mating system (Miller 1971; Raper et al. 1972). Pseudo-homothallism is a particular system in which automixis is forced, as two haploid nuclei from one meiotic tetrad are packaged together into one spore, therefore self-fertility is the result of the packaging of two independent and opposite mating type nuclei within a single spore (Wilson et al. 2015).
This lifestyle not only enables the fungus to reproduce without finding a compatible partner, but also to cross with any compatible mate it may encounter (Grognet and Silar 2015). To date this type of reproduction in Basidiomycetes has only been observed in Agaricomycetes (Nieuwenhuis et al. 2013). It is possible that pseudo-homothallics benefit from both homothalism, which allows the possibility to self-cross when no compatible partner is present and heterothallism, which favors the creation of genetic variation through recombination during outbreeding (Grognet and Silar 2015).

The locus encoding the homeodomain proteins has previously been located on Chromosome 1 of A. bisporus H97 (Morin et al. 2012). Our analysis of the mating-type locus of A. bisporus ARP23, H97 and JB137-S8 shows they are all very similar to the A mating-type locus of the model species Coprinopsis cinerea and are located on scaffold 16, chromosome 1 and scaffold 1 respectively. The locus contains a pair of homeodomain transcription factor genes orthologous to b1-2 and a1-2 from C. cinerea (Figure 3). The mitochondrial intermediate peptidase (MIP) gene and a Beta-flanking gene which typically accompany the mating A locus are also found in the genomic vicinity (Figure 3). Interestingly, all three A. bisporus strains have an additionally copy of the Beta-flanking gene relative to C. cinerea. Levels of synteny with respect to other flanking genes are also very high (Figure 3). The JB137-S8 assembly contains six ORFs between the homeodomain proteins, sequence analysis leads
us to believe that this is a misassembly artifact, furthermore all ORFs have homologs in H97 and ARP23 but are located on different scaffolds. Relative to the other mating loci of the other two assemblies, H97 has an additional putative ORF (Figure 3). A homolog for this gene is absent for the assemblies of both ARP23 and JB137-SB, furthermore it does not contain any known Pfam domains and a BLASTP search against GenBank retrieves a single significant hit to another Agaricales species (*Leucoagaricus* sp) therefore it may be dubious.

**Pan genome analysis of Agaricus bisporus**

Individual reference genomes do not and cannot contain all genetic information for a species due to genetic and genomic variation between individuals within a species. To account for such variation, it has become increasingly common to refer to species with multiple genomes sequenced in terms of their pan genome, which is defined as the union of all genes observed across all isolates/strains of a species. A species pan genome for *A. bisporus* was constructed using the synteny-dependent PanOCT method implemented in Pangloss with the default parameters (Fouts et al. 2012; McCarthy and Fitzpatrick 2019a, 2019b). PanOCT clusters homologous sequences into synteny ortholog clusters (SOCs) based on BLAST score ratio (BSR) assessment of sequence similarity and on proportions of relative synteny (conserved gene neighborhood, CGN) between potential orthologs (Rasko et al. 2005; Fouts et al. 2012). SOCs with synteny orthologs from all four *A. bisporus* strain genomes in our dataset were classified as "core" SOCs, and clusters missing an ortholog from ≥ 1 strain genome were classified as "accessory" SOCs. After initial construction with PanOCT, the *A. bisporus* pan genome was refined by merging accessory SOCs based on reciprocal strain best hits between all members of a given pair of accessory SOCs (McCarthy and Fitzpatrick 2019a, 2019b). In total, we identified 7,732 core SOCs and 8,478 accessory SOCs within our *A. bisporus* dataset (16,120 in total) (Figure 4). The proportion of core SOCs relative to the total number of protein coding genes per genome ranged from a low of ~60% in ARP23 to a high of 71% in H97. This proportion of core to accessory genes is lower than we have previously observed in a number of model fungal species (McCarthy and Fitzpatrick 2019a). Analysis of the distribution of SOCs within the *A. bisporus* accessory genome was performed within Pangloss using UpSetR, which is an R implementation of the UpSet method for visualization of set intersections and occurrences within a dataset using matrix representation. The UpSetR plot in Figure 4 shows that singleton SOCs (i.e., singleton genes) are the most common within the accessory genome, with 2,161 singleton SOCs from ARP23 alone and 6,574 in total (~78% of all syntenic SOCs in the accessory genome). The distribution of the remaining 1,904 non-singleton SOCs within the *A. bisporus* accessory genome appears to follow evolutionary history (Figure 2), for example H97 and ARP23 share 727 accessory SOCs either exclusively or along with one other strain, while JB137 and ARP23 only share 615 accessory SOCs exclusively or with another strain (Figure 4).

Selection analysis of the core and accessory genomes was performed using the Yang & Nielsen method as implemented in yn00 with the default parameters for yn00. 680 of 7,732 core SOCs (~9% of core SOCs) and 172 of 1,904 non-singleton accessory SOCs (~9% of non-singleton accessory SOCs, ~2% of all accessory SOCs) showed evidence of at least 1 pairwise alignment under positive selection where $d_{ns}/d_{s} \cong 1$ and $d_{ns}/d_{s} \neq \infty$ (Yang and Nielsen 2000).

**Conclusion**

In this analysis, we have presented the high quality genome sequence of *A. bisporus* var. *bisporus* ARP23, a commercially relevant genome. In total the genome was found to be 33.49 Mb in length, have high levels of synteny to H97 and contain 13,030 putative protein coding genes. Relative to the other two *A. bisporus* genomes that are currently available, ARP23 is the largest *A. bisporus* strain sequenced to date. Our analyses show that phylogenetically speaking ARP23 is more closely related to H97 than JB137-SB. Furthermore, all three strains have highly conserved mating loci. The lignocellulolytic gene content of all three *A. bisporus* strains is very similar. The pangenome of *A. bisporus* is quite diverse with between 60–70% of genes considered as core SOCs depending on isolate under consideration. The above analyses and genome sequence are the starting point for more detailed molecular analyses into the growth and phenotypical responses of ARP23 when challenged with economically important mycoviruses.

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