The physiology of *Agaricus bisporus* in semi-commercial compost cultivation appears to be highly conserved among unrelated isolates

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Abstract

The white button mushroom *Agaricus bisporus* is the most widely produced edible fungus with a great economical value. Its commercial cultivation process is often performed on wheat straw and animal manure based compost that mainly contains lignocellulosic material as a source of carbon and nutrients for the mushroom production. As a large portion of compost carbohydrates are left unused in the current mushroom cultivation process, the aim of this work was to study wild-type *A. bisporus* strains for their potential to convert the components that are poorly utilized by the commercial strain A15. We therefore focused our analysis on the stages where the fungus is producing fruiting bodies. Growth profiling was used to identify *A. bisporus* strains with different abilities to use plant biomass derived polysaccharides, as well as to transport and metabolize the corresponding monomeric sugars. Six wild-type isolates with diverse growth profiles were compared for mushroom production to A15 strain in semi-commercial cultivation conditions. Transcriptome and proteome analyses of the three most interesting wild-type strains and A15 indicated that the unrelated *A. bisporus* strains degrade and convert plant biomass polymers in a highly similar manner. This was also supported by the chemical content of the compost during the mushroom production process. Our study therefore reveals a highly conserved physiology for unrelated strains of this species during growth in compost.

Keywords

Agaricus bisporus, transcriptomics, proteomics, carbohydrate active enzymes, carbon metabolism, commercial cultivation

Abbreviations

ABF, α-L-arabinofuranosidase; AGL, α-1,4-D-galactosidase; BGL, β-1,4-glucosidase; BXL, β-xylosidase; CAZymes, carbohydrate active enzymes; CBH, cellobiohydrolase; CDH, cellobiose dehydrogenase; GLA, glucoamylase; LAC, β-1,4-D-galactosidase; LPMO, lytic polysaccharide monooxygenase; MM, minimal medium; MND, β-1,4-mannosidase; *p*NP, *p*-nitrophenol; RHA, α-rhamnosidase.

Introduction

The basidiomycete litter-decomposing fungus *Agaricus bisporus*, also known as the white button mushroom, is the fourth most commonly produced edible mushroom worldwide (Royse et al., 2017). In addition to its significance as a commercially important agricultural product, *A. bisporus* is a plant biomass degrading fungus with a wide geographical distribution and it plays an ecologically crucial role in carbon cycling in terrestrial ecosystems (Morin et al., 2012). *A. bisporus* is commercially cultivated on compost, which is produced from wheat straw, horse and/or chicken manure and gypsum as the main raw materials (Gerrits, 1988). Thus, the majority of the organic matter in compost consists of lignocellulosic polymers originating from plant cell walls, i.e. polysaccharides cellulose and hemicellulose, and aromatic lignin (Gerrits et al., 1967; Iiyama et al., 1994; Jurak et al., 2014). The growth of *A. bisporus* in compost is a complex process consisting of a vegetative mycelial phase followed by a reproductive phase with the formation of fruiting bodies in several flushes of mushroom production (van Griensven, 1988). During vegetative growth and mushroom formation, *A. bisporus* secretes a range of extracellular enzymes, which convert the lignocellulosic fraction in compost (Gerrits, 1969; Fermor et al., 1991; Wood et al., 1991; Yague et al., 1997). Development of fruiting bodies is associated with increased rate of cellulose and hemicellulose degradation (Wood and Goodenough, 1977), while lignin is modified at the initial stage of growth in compost (Patyshakuliyeva et al., 2015). Gene expression analysis has suggested that *A. bisporus* consumes a variety of plant cell wall derived monosaccharides during the vegetative phase, but mainly hexose metabolism occurs in the fruiting bodies without accumulation of other sugars from lignocellulose (Patyshakuliyeva et al., 2013). This indicates that sugars other than hexoses likely provide energy for growth and

maintenance of the vegetative mycelium or are metabolically converted in the mycelium before transport to the fruiting body (Patyshakuliyeva et al., 2013).

Although the genome sequence of *A. bisporus* H97 homokaryon shows that this fungus has a potential to produce a full repertoire of carbohydrate active enzymes (CAZymes, http://www.cazy.org, Lombard et al., 2014) for plant biomass degradation in humic-rich environment (Morin et al., 2012), only a part of the plant cell wall polysaccharides present in compost are converted into fruiting bodies leaving a significant portion, 20-26%, of the compost carbohydrates unused (Jurak et al., 2014).

The main polysaccharides present in compost after the cultivation process of the commercially used *A. bisporus* heterokaryon A15 have been shown to consist of xylosyl and glucosyl residues (Jurak et al., 2014). Especially, arabinose and glucuronic acid substituted xylans are enriched in the compost during the cultivation (Jurak et al., 2015a). This has been suggested to be due to absence of α-glucuronidase activities in compost (Jurak et al., 2015a) as well as lack of α-arabinofuranosidases that are active on the double substituted xylan (Jurak et al., 2015b). Therefore, exploring new wild-type strains with different abilities to convert the polymers present in compost, e.g. substituted xylan, could provide valuable insights for the development of a new commercial strain with better abilities to degrade compost and utilize carbohydrates, leading to higher mushroom yields.

Current commercial strains of *A. bisporus* are genetically very similar (Sonnenberg et al., 2017). Therefore, in this work, our aim was to study, if unrelated wild-type *A. bisporus* strains have better abilities towards components that are poorly utilized by the commercially cultivated strain A15. First, we compared wild-type *A. bisporus* strains to A15 for their carbon utilization profiles and based on these results six wild-type strains with different carbon source preferences were

selected for semi-commercial scale compost cultivation experiment. Selected extracellular plant cell wall hydrolyzing enzyme activities were analyzed at different phases of the composting process together with the yield of the fruiting bodies. Based on this, three wild-type *A. bisporus* strains, together with the commercially cultivated A15 strain, were selected for transcriptome and proteome analyses to reveal possible molecular level differences in their potential to degrade and metabolize compost substrate. This data was further complemented with chemical analyses of the compost carbohydrates and lignin.

Materials and Methods

Fungal strains and their growth profiling on different carbon sources

A. bisporus wild-type strains 012 DD-1, 065 BP-8, 088 FS-44, 147 JB-41, 219 30P and 245 AMA-7 (Table S1)as well as the commercial strains A15 and U1 were all obtained from the company Sylvan Inc., USA. All chemicals were obtained from Sigma-Aldrich. For growth profiling, all strains were cultivated on minimal medium (MM) agar plates with monosaccharides D-glucose, D-mannose, D-xylose and L-arabinose, disaccharides cellobiose and maltose, polysaccharides starch, inulin, beechwood xylan, birchwood xylan, apple pectin and citrus pectin, and crude plant biomass wheat bran, citrus pulp, soybean hulls and alfalfa meal as carbon sources. MM consisted of 20.5 mM MOPS, 2 mM KH_2PO_4, 1 mM $MgSO_4$, 0.5 mM $CaCl_2$, 0.134 mM EDTA, 25 µM $FeSO_4$, 5 µM $ZnSO_4$, 5 µM $MnSO_4$, 4.8 µM H_3BO_3, 2.4 µM KI, 52 nM Na_2MoO_4, 4 nM $CuSO_4$, 4 nM $CoCl_2$, 0.5 µM thiamine HCl, 0.1 µM D(+)biotine and 20 mM NH_4Cl and was set at pH 6.8. A final concentration of 25 mM mono- and disaccharides, 1% polysaccharides and 3% crude carbon sources were added to MM. The MM without a carbon source was used as a control. The plates were performed in duplicate, and inoculated with a 1

mm mycelial plug from a freshly grown colony on 2% malt extract agar plates (2% (w/v) malt extract, 2% (w/v) agar agar) and incubated at 25°C. After 9 d incubation, clear differences between the carbon sources were detected with respect to colony diameter and density and the plates were photographed.

Compost cultures

The six *A. bisporus* wild-type strains, and the commercial strain A15 were cultivated in duplicate in semi-commercial conditions in crates containing 22 kg compost, which was based on wheat straw, horse and chicken manure, gypsum and water, according to commercial practice at CNC (Coöperatieve Nederlandse Champignonkwekersvereniging, Milsbeek, The Netherlands, http://www.cnc.nl/en/). The composts were inoculated with 176 mL of wheat kernels (spawns) colonized by the different strains. The crates were incubated in a commercial composting tunnel for 17 d after which they were moved to mushroom breeding farm and covered by 5 cm of casing layer. The incubation was continued in a breeding chamber similar to large scale commercial mushroom production. Approximately 1 L samples were taken from the middle of each crate after 16, 27, 30 and 39 d from the introduction of the spawns into the compost and corresponding to spawning, primordial and pinning stage, and the first flush, respectively (Table 1). The compost samples were immediately stored at -20°C.

Enzyme activity assays

Selected exo-acting plant biomass polysaccharide degrading enzyme activities were determined from compost extracts that were obtained according to Jurak et al. (2015a) at the different cultivation stages (Table 1) after 16, 27, 30 and 39 days of growth of the *A. bisporus* strains 012

DD-1, 065 BP-8, 088 FS-44, 147 JB-41, 219 30P, 245 AMA-7 and A15. Defrosted compost samples (10 g) were mixed (200 rpm) with 100 mL distilled water in 250 mL Erlenmeyer flasks for 1 h at 4°C. Samples were centrifuged (10 000 x g, 15 min, 4°C), and the supernatant was used for enzyme assays. The activity of α-L-arabinofuranosidase (ABF), cellobiohydrolase (CBH), glucoamylase (GLA), β-1,4-D-galactosidase (LAC), α-rhamnosidase (RHA), β-xylosidase (BXL), β-1,4-glucosidase (BGL), α-1,4-D-galactosidase (AGL) and β-1,4-mannosidase (MND) were assayed by using *p*-nitrophenol (*p*NP) -linked substrates (Sigma-Aldrich) as previously described (Benoit et al., 2015). Reaction mixtures were incubated at 30°C for 4 h and the reactions were terminated by adding 100 µl 0.5 M sodium carbonate. The amount of the released *p*NP was monitored at 405 nm (FLUOstar OPTIMA, BMG Labtech). The averages and standard deviations for two biological replicate compost cultures and three technical replicate reactions were calculated and the activities are expressed as nmol *p*NP/mL of sample/min.

RNA extraction, cDNA library preparation and RNA sequencing

Total RNA was extracted by using a CsCl gradient centrifugation (Patyshakuliyeva et al., 2014) from samples of the duplicate compost cultures of *A. bisporus* strains 065 BP-8, 219 30P, 245 AMA-7 and A15 collected at the primordial stage (30 d) and the first flush (39 d). RNA quantity and integrity were determined with RNA6000 Nano Assay (Agilent 2100 Bioanalyzer, Agilent Technologies, USA). Preparation of cDNA library and sequencing reactions were conducted in the BGI Tech Solutions Co., Ltd. (Hong Kong, China) as described previously (Patyshakuliyeva et al., 2015). On average, 51 bp sequenced reads were constituted, producing 460 MB raw yields for each sample.

Raw reads were produced from the original image data by base calling. After data filtering, the adaptor sequences, reads with unknown bases (N) >10% and low quality reads (more than 50% of the bases with quality value <5%) were removed. Clean reads were mapped to the genome sequence of *A. bisporus* var *bisporus* (H97) v2.0 (Morin et al., 2012) using BWA/Bowtie (Langmead et al., 2009; Li et al., 2009) with no more than two mismatches allowed in the alignment. On average, 78% of the clean reads mapped to the genome. The gene expression level as fragments per kilobase of exon per million fragments mapped (FPKM) was calculated by using RSEM tool (Li et al., 2009). Genes with FPKM value lower than 20 in all samples were considered as not expressed and filtered out. Differential expression was identified by Student's t-test. A fold change of >1.5 and P-value of <0.05 were used to identify differentially expressed genes between the strains and time points. The RNA-seq data were deposited to the Gene Expression Omnibus (GEO) database (Edgar et al., 2002) with accession number: GSE99928. Genome-wide principal component analysis (PCA) of the gene expression on duplicate samples was generated using FactoMineR package from Rcomander v.2.1-7 program in R statistical language and environment 3.1.2.

Protein extraction and proteomics analysis

Proteins were extracted from samples from duplicate compost cultures (10 g) colonized by the *A. bisporus* strains 065 BP-8, 219 30P, 245 AMA-7 and A15 at primordial stage and the first flush similarly as described for enzyme activity assays. The supernatants were concentrated 4x with vacuum concentrator (Speedvac, Savant Instruments, USA) according to Patyshakuliyeva et al. (2015). Protein separation by SDS-PAGE, trypsin digestion and mass spectrometry analysis were performed as previously described (Patyshakuliyeva et al., 2015). For data analysis, raw files

were processed using Proteome Discoverer 1.3 (version 1.3.0.339, Thermo Scientific), and data was normalized based on protein input (same amount of protein was loaded). Database search was performed using the genome of *A. bisporus* var. *bisporus* (H97) v2.0 (Morin et al., 2012) and Mascot (version 2.4.1, Matrix Science, UK) as the search engine according to Patyshakuliyeva et al. (2015). The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium (Vizcaíno et al., 2014) via the PRIDE (Martens et al., 2005) partner repository with the dataset identifier PXD007189.

Analysis of carbohydrates and lignin from *A. bisporus* grown compost samples

Carbohydrate and lignin composition and content of compost during cultivation of the *A. bisporus* strains 065 BP-8, 219 30P, 245 AMA-7 and A15 were analyzed at primordial stage, pinning stage and the first flush (Table 1). Dried compost samples were milled (<1 mm) using an MM 2000 mill (Retsch, Haan, Germany) prior to further analysis. Neutral carbohydrate and uronic acid content and composition was determined in technical duplicates from the biological duplicate samples, as described by Jurak et al. (2014). The composition of lignin was determined by analytical pyrolysis-GC/MS in triplicate, as described previously (Jurak et al., 2015c).

Results

Growth profiling reveals differences in physiology between *A. bisporus* strains

Initially, growth of 32 wild-type *A. bisporus* isolates were compared to the currently used commercial strain A15 and the strain U1 previously used in commercial production on 38 plant-biomass related carbon sources to select strains that were more likely to have different physiology with respect to consumption of carbohydrates (data not shown). Six wild-type strains, i.e. 147 JB-41, 245 AMA-7, 219 30P, 088 FS-44, 012 DD-1 and 065 BP-8, that showed

significant differences in growth on several carbon sources were selected for further experiments (Fig. S1). Interestingly, also U1 and A15 showed differences in growth on several carbon sources. No consistently improved or reduced growth was visible for any of the strains across the carbon sources tested. For instance, 012 DD-1 showed poor growth on D-glucose, while it was among the better growing strains on D-xylose. Strong growth differences were particularly observed on the crude plant biomass substrates. A15 and 088 FS-44 grew well on most crude plant biomass substrates (Fig. S1), while only selected crude carbon sources supported good growth of 245 AMA-7, 065 BP-8 and 012 DD-1. The variation in growth on these carbon sources suggests that the strains may have different abilities to degrade plant biomass derived polysaccharides, and transport and metabolize the resulting monomeric sugars.

Mushroom producing capacity of the *A. bisporus* strains differs in the semi-commercial cultivations

The mushroom producing capacity of the six wild-type strains were compared to A15 in semi-commercial cultivation conditions (Fig. S2). In addition, the activity of selected exo-acting extracellular plant cell wall hydrolysing enzymes was determined from the compost extracts after 16, 27, 30 and 39 days of growth using *p*NP-linked substrates. The wild-type strains 065 BP-8, 088 FS-44, 219 30P and 245 AMA-7, and A15 produced very similar enzyme activity patterns in compost (Fig. S3). Typically, the activities increased during the cultivation most likely due to the increase in fungal biomass in the compost. The highest activities were detected for ABF, LAC and AGL, whereas GLA activity was very low in all analyzed phases. Compared to A15, 065 BP-8 and 088 FS-44 secreted higher LAC activity throughout the compost cultures and produced higher BGL activity during the pinning stage (30 d) and the first flush (39 d), respectively. The

high enzyme activities correlated well with the highest mushroom yield (4.1 kg/crate) obtained with 065 BP-8. Also, higher RHA activity was detected in the compost samples of 088 FS-44 compared to A15. However, this strain produced only one large fruiting body that apparently repressed the growth of other fruiting bodies until it was removed, resulting in a mushroom yield of 3.2 kg/crate. The enzyme activity levels detected for 219 30P and A15 were highly similar (Fig. S3), but the mushroom yield of 219 30P (3.1 kg/crate) was slightly lower than that of the commercial strain A15 (3.8 kg/crate). While the BGL activity of 245 AMA-7 was lower than in the compost extracts of A15, it secreted higher LAC and AGL activity at the primordial (27 d) and pinning (30 d) stages, respectively, but showed moderate production of fruiting bodies with 2.4 kg/crate. Although the overall enzyme activity pattern of strain 012 DD-1 was similar, the activity levels of LAC, RHA, AGL and MND were markedly lower than those detected for A15. In line with the low activity levels, 012 DD-1 showed poor mushroom production (0.8 kg/crate). Strain 147 JB-41 grew poorly in compost, did not produce any fruiting bodies, and very low activity levels were detected in its compost extracts.

CAZy gene expression and enzyme production is largely conserved amongst the *A. bisporus* strains

Based on good production of the fruiting bodies and the extracellular enzyme activities in our tested semi-commercial composting conditions, the *A. bisporus* wild-type strains 065 BP-8, 219 30P and 245 AMA-7, and the commercial strain A15 were subjected for transcriptomic and proteomic analyses in order to study their potential to degrade and metabolize the wheat straw based compost substrate at the molecular level. The genetic relationship between the strains was determined by sequencing the commonly used housekeeping gene glucose-6-phosphate

dehydrogenase (G6PD) and performing a phylogenetic tree (Fig. S4). This revealed that the commercial isolate (H97, a monokaryon derived from A15) is most closely related to 219 30P that originates from Russia, but no clear correlation between phylogenetic and geographical distance was observed.

The PCA analysis showed good reproducibility for the biological duplicate RNA samples (Fig. S5). Overall, expression and production of plant cell wall degrading CAZy genes and enzymes were very similar when the significantly expressed genes and the highest produced extracellular enzymes in the wild-type strains were compared to A15 after 30 and 39 days of growth in compost (Fig. 1, Table 2). The CAZy expression was very similar especially between 065 BP-8 and A15 (Table S2). More CAZyme encoding genes were highly upregulated in 245 AMA-7 than in the other strains at the pinning stage after 30 days of growth (Fig. 1). Interestingly, these included five putative lytic polysaccharide monooxygenase (LPMO) encoding genes that were uniquely upregulated in 245 AMA-7 and one putative AA9 LPMO encoding gene that was upregulated in 245 AMA-7 and 065 BP-8. Also, an AA8-AA3_1 cellobiose dehydrogenase (CDH) encoding gene was highly upregulated in 245 AMA-7 and 219 30P after 30 days when compared to A15. However, after 39 days most of the LPMO encoding genes as well as the CDH encoding gene were upregulated in A15 compared to the other strains. As an indication of subtle differences in the utilization of compost substrate by the *A. bisporus* strains, a large set of putative CAZyme encoding genes were highly expressed in A15 during the first flush compared to the wild-type strains (Fig. 1). Markedly, the set of upregulated genes in A15 was different compared to each wild-type strain.

In line with the transcriptomics data, two AA1_1 laccases were the highest produced CAZymes by all strains at the pinning stage (Table 2), indicating their importance during the mycelial

growth of *A. bisporus* in compost. However, a lignin acting AA2 manganese peroxidase (MnP, protein ID 221245) was detected at lower and more constant level at both time points. Cellulose and xylan were the most abundant polysaccharides present in the studied composts (Table 3), and several enzymes degrading these polymers were detected in the proteomes (Table S3). The amount of cellulose, xylan and mannan acting enzymes increased during the first flush, which is largely in line with the gene expression data (Table 2, Table S2). In good agreement with the highest exo-acting extracellular plant cell wall hydrolyzing enzyme activities detected in the compost extracts (Fig. S3), a putatively mannan acting GH27 AGL (protein ID 70106), a GH35 LAC (protein ID 152299), which may act on several polysaccharides, and a putatively pectin acting GH51 ABF (protein ID 194576), were amongst the highest produced extracellular CAZymes (Table 2).

To evaluate the possible differences in the carbon source requirements and energy metabolism between the wild-type strains and A15, the expression of the genes encoding enzymes involved in central carbon metabolism was analyzed in the mycelium-grown compost samples. The transcriptome data indicated that the carbon metabolic pathways were active in all *A. bisporus* strains at the two studied time points (Table S4). In addition, the expression profiles of the carbon metabolic genes were very similar in all strains, suggesting that the strains have comparable abilities to utilize compost-derived sugars as carbon and energy source.

Temporal changes in chemical content of compost are similar between the *A. bisporus* strains

While no differences in the carbohydrate composition were detected, minor (not statistically significant) differences in total carbohydrate content were observed when the compost samples

of the three wild-type strains, 065 BP-8, 219 30P and 245 AMA-7, and the commercial strain A15 from primordial (27 d) and pinning stages (30 d) and from the first flush (39 d) were compared. The main carbohydrates in the composts were xylan, arabinose and uronic acids from xylan, glucose from cellulose, and microbial glucans (Table 3). While the total carbohydrate content of the composts slightly decreased over time for all strains, especially for A15, 065 BP-8 and 219 30P, the content of glucuronic acid substituted xylan increased from 27 to 39 d from 35-36 to 53-55 mol per 100 xylosyl residues (Table 3). This was observed to a lesser extent with 245 AMA-7 (from 35 to 45 mol per 100 xylosyl residues) and this strain seemed to be slowest in carbohydrate consumption. The accumulation of glucuronic acid substituted xylan was in line with low expression of putative α-glucuronidases encoding genes in the compost samples in all strains (Table S2).

For all four *A. bisporus* strains, minor differences in the composition of the aromatic polymer lignin were observed from the primordial stage to the first flush (Table 4). Relative abundances of each pyrolysis-GC/MS compounds analyzed and grouped based on their structural characteristics are given in Table S5. An increased ratio of pyrolysis-GS/MS analyzed unsubstituted over vinyl-substituted compounds was observed over time (Table 4). A minor decrease of pyrolysis GC/MS measured vinyl compounds was detected in the samples after pinning and before the first flush, suggesting cleavage of the ferulic and coumaric acids (Murciano Martínez et al., 2016). Changes were not observed in ratios of syringyl-like and guaiacyl-like lignin units (S/G-ratios), which remained constant (0.45-0.58).

Discussion

In this work, we studied the potential of the six wild-type *A. bisporus* strains for mushroom production in comparison with the commercially used A15 strain in semi-commercial composting conditions. After commercial production of *A. bisporus* mushrooms, compost still contains a significant amount of polysaccharides, which could be converted into fruiting bodies to increase mushroom yields and economical profitability of the process (Kabel et al., 2017). For example, recalcitrant xylan structures, substituted with glucuronic acid, and one and two arabinosyl residues, have been shown to accumulate in compost during *A. bisporus* cultivation (Jurak et al., 2015b). Therefore, exploring new wild-type strains with different abilities to degrade the polymers present in compost can provide valuable insights for the development of a new commercial strain with improved utilization of compost nutrients leading to higher mushroom production. We therefore selected two time points based on our previous study (Patyshakuliyeva et al., 2015) that allowed us to compare the strains at the moment they were still developing fruiting bodies and just after all fruiting bodies of the first flush were harvested. Overall, the strains had a similar timeline for mushroom formation, so sampling all strains at the same time most probably did not have a large effect on the results of our study.

Differences in the carbon utilization profiles of the wild-type *A. bisporus* strains and A15 were detected, using pure mono- and polysaccharides and several plant biomass feed stocks. The strain A15 has been selected for commercial cultivation due to its ability to produce mushrooms of good commercial quality in the highly-controlled composting process (Arce-Cervantes et al., 2015), whereas the wild-type *A. bisporus* strains are saprotrophic degraders of leaf and forest litter in nature (Kerrigan et al., 1998). This may suggest that these unrelated strains differ in their physiological abilities to use plant biomass based materials as a carbon and energy source and possibly have differences in adaptation to certain substrates. The physiological variation between

the studied strains was apparent, as strain 065 BP-8 produced slightly higher mushroom yield than the commercial strain A15 during the first flush, whereas strain 147 JB-41 was not able to grow in the compost. This indicates that screening of new wild-type isolates may result in candidate strains with improved mushroom production that can be further studied for the use in commercial composting conditions. It should be considered however, that the crate cultivation appears to produce a lower yield of mushrooms than normally observed in a full bed, and that only the first flush was measured, so it is not possible to reflect on the total mushroom producing capacity of the different strains at this stage.

A compost which is well-colonized with fungal mycelium is known to yield more fruiting bodies during flushes (Kabel et al., 2017). This is also supported with the results of our study that showed correlation between high activity of plant cell wall degrading enzymes and the highest mushroom production in the semi-commercial compost cultivations. However, the wild-type *A. bisporus* strains produced overall very similar activity patterns of extracellular polysaccharide degrading enzymes during growth in compost compared to A15. The highest exo-acting extracellular plant cell wall hydrolysing enzyme activities detected in the compost extracts were ABF, LAC and AGL. This in accordance with the previously reported high level activity of ABF by A15 throughout its growth in compost (Jurak et al., 2015a). ABFs are classified into GH43 and GH51 CAZy families and may have activity towards several polysaccharides. According to the phylogenetical analysis, and the recent subfamily system for GH43 (Mewis et al., 2015), only one of the four *A. bisporus* GH43 enzymes is a putative ABF, while the three other GH43 enzymes most likely encode endoarabinanases (Jurak et al., 2015b). However, this putative ABF has been suggested to act on single substituted, but not on double substituted xylo-oligomers (Jurak et al., 2015b), which is supported by the accumulation of highly substituted xylan in

373 compost during the growth of A15 (Jurak et al., 2015a). Concentration of arabinosyl and
374 glucuronic acid substituted xylan also increased in the compost samples of the wild-type strains
375 065 BP-8, 219 30P and 245 AMA-7. Thus, it is likely that similarly to A15, the studied wild-type
376 strains do not possess genes encoding enzymes that cleave arabinose from xylan, which is
377 substituted with two arabinosyl residues. In addition, the GH43 genes and GH115 α-
378 glucuronidases were lowly expressed in all strains. Surprisingly, laccase expression was still high
379 after 39 days in A15, as it has previously been shown to be highest during mycelial growth and
380 then decline at the start of fruiting (Ohga et al., 1999), which was also observed in this study for
381 the other strains. This may indicate a slight difference in the timing of the first flush, with A15
382 already being past fruiting at day 39, although this was not obvious from visual inspection. As all
383 samples were taken at the same depth in the compost, so the fact that depth affects the laccase
384 activity (Smith et al., 1989) is not likely to be a factor in our study. However, in accordance with
385 the previous studies (Wood and Goodenough, 1977; Wood, 1980) the level of extracellular
386 laccases decreased after 39 days also in A15.
387 Compost that is used for cultivation of *A. bisporus* contains mainly plant cell wall derived
388 components, which include cellulose and hemicellulose polysaccharides, and aromatic polymer
389 lignin (Gerrits et al., 1967; Iiyama et al., 1994; Jurak et al., 2014). Interestingly, the same CAZy
390 isoenzymes and genes encoding them were produced and expressed at the highest level both in
391 the A15 and the wild-type strains, showing the high level of conservation in conversion of
392 compost polymers between these unrelated *A. bisporus* strains. This is in contrast with the
393 reports demonstrating the large diversity in terms of enzyme and decay activity that has been
394 shown to exist within unrelated isolates of lignocellulose degrading saprotrophic basidiomycete
395 species, such as the white rot fungi *Phanerochaete chrysosporium* (Blanchette et al., 1988) and

Phlebiopsis gigantea (Żółciak et al., 2012). The expression of the ligninolytic genes, including one AA2 MnP (protein ID 221245), two AA1_1 laccases (protein IDs 146228 and 139148) and one AA5_1 copper radical oxidase (CRO, protein ID 193903), was delayed in all *A. bisporus* strains in comparison with the earlier study with A15 (Patyshakuliyeva et al., 2015), in which these genes were not highly expressed at the pinning stage. This may be due to differences between the large-scale composting process (Patyshakuliyeva et al., 2015) and the semi-commercial scale used in this study, or to slight differences in timing of the first flush between the isolates. However, the expression pattern of cellulase and xylanase encoding genes, which were upregulated during the first flush, was similar as reported by Patyshakuliyeva et al. (2015). Overall, no significant differences were detected in the expression of carbon metabolic genes between the strains, and in fact, the profiles of these genes were even more similar than those observed for the CAZyme encoding genes. Similarly, with the previous observations with A15 (Patyshakuliyeva et al., 2015), our results suggested that also the wild-type *A. bisporus* strains favour use of hexoses over pentoses.

The changes observed in the chemical composition of the composts were largely in agreement with the previous observations for the A15 strain (Patyshakuliyeva et al., 2015). Also, only minor differences were observed between lignin and carbohydrate content and composition as well as the degree of xylan substitution in the composts, indicating that the studied *A. bisporus* strains degrade commercial wheat straw based compost in a highly similar manner.

Despite clear differences between the tested strains regarding their physiology on defined saccharides and feed stocks, there was very little molecular level variation in the expression and production of the CAZymes as well as central carbon metabolic genes. Whether this implies that our initial growth-profiling based screening approach is not the best way to identify traits that

may improve mushroom production or whether a larger set of strains needs to be assessed is not yet clear. Furthermore, a more detailed understanding of factors that may affect the more efficient use of compost carbohydrates is needed, including the identification and functional characterization of secreted proteins with unknown function and sugar transporters. A more extensive dataset may then reveal traits (e.g. genes with diverse expression patterns across strains) that could possibly be used to further improve the commercial *A. bisporus* strains for mushroom production.

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Availability of data and materials

The data sets supporting the transcriptomic and proteomics results of this article are available in the GEO and PRIDE repository, respectively. The unique persistent identifiers and hyperlinks to

dataset(s) will be provided in the final version of the manuscript at the proof stage.

Figure captions

Figure 1. Venn diagrams depicting at least 4-fold changes in expression of CAZyme encoding genes detected in *A. bisporus* A15 in comparison with 065 BP-8, 219 30P and 245 AMA-7 strains after 30 and 39 days of growth in compost. For abbreviations, see Table S2.

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Supplementary material

Figure S1. Growth profiling of *A. bisporus* strains on different carbon sources.

Figure S2. The first flush and the mushroom yield of the six *A. bisporus* wild-type strains A) 012 DD-1, B) 065 BP-8, C) 088 FS-44, D) 147 JB-41, E) 219 30P, F) 245 AMA-7, and G) the commercial strain A15 grown in semi-commercial composting conditions.

Figure S3. Exo-acting plant cell wall polysaccharide hydrolyzing enzyme activities detected from the extracts obtained after 16, 27, 30 and 39 days of growth of the *A. bisporus* strains A) 012 DD-1, B) 065 BP-8, C) 088 FS-44, D) 147 JB-41, E) 219 30P, F) 245 AMA-7 and G) A15 on compost. ABF, α-L-arabinofuranosidase; CBH, cellobiohydrolase; GLA, glucoamylase; LAC, β-1,4-D-galactosidase; RHA, α-rhamnosidase; BXL, β-xylosidase; BGL, β-1,4-glucosidase; AGL, α-1,4-D-galactosidase; MND, β-1,4-mannosidase. The activities of two biological replicate compost cultivations are shown. The error bars indicate the standard deviation of three technical replicate reactions.

Figure S4. Phylogenetic relationship between the isolates selected in this study based on the glucose-6-phosphate dehydrogenase gene. *A. bisporus* var. *burnettii*, *L. gongylophorus* and *A. muscaria* were used to root the tree. The alignment was performed using MAFFT and the Maximum Likelihood tree was then build in MEGA7 using 1000 bootstraps.

Figure S5. Principal Component Analysis of the transcriptome data, demonstrating the high reproducibility of the replicates as well as the relationship between the samples of the different strains. Sample nomenclature: strainname_time (in days)_replicate.

Table S1. Origin of the wild-type *A. bisporus* strains used in this study.

Table S2. Fold changes in expression of plant biomass degradation related CAZyme encoding genes in *A. bisporus* strains.

Table S3. Proteomics data of plant biomass degradation related CAZymes detected in compost samples of *A. bisporus* strains.

Table S4. Fold changes in expression of carbon metabolism related genes in *A. bisporus* strains.

Table S5. Analytical pyrolysis GC/MS data of wheat straw, composts before inoculation, and composts grown with *A. bisporus* A15, 065 BP-8, 219 30P and 245 AMA-7 at primordial stage, pinning stage and the first flush.