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Regular Articles

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 one of 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.

1. 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*

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

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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.

2. Materials and methods

2.1. 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) 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.

2.2. 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.

2.3. 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*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

Table 1
Description of the compost samples used in this study.

Compost sample	Description of composting stage	Days after introducing spawns into compost
Spawning stage	Compost at the end of the spawning stage	16 d
Primordial stage	Just before the transition to pinning	27 d
Pinning stage	Pinning was clearly started, the first pinheads were visible	30 d
First flush	Compost just before harvesting the mushrooms of the first flush	39 d