

## BIODEGRADATION OF CARBOHYDRATES DURING THE FORMATION OF *AGARICUS BISPORUS* COMPOST

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### ABSTRACT

*Agaricus bisporus* is commonly grown on compost, which consists mainly of straw and horse manure. This means that the majority of the carbon source is present as plant-based polysaccharides, which themselves consist of many different monomeric components. This paper presents an overview of plant biomass degradation by fungi, with a special emphasis on the biochemical and molecular data available for *A. bisporus*.

**Keywords:** *Agaricus bisporus*, Compost, Polysaccharide degradation, Lignin degradation

### INTRODUCTION

*Agaricus bisporus* is a leaf-litter-degrading basidiomycete fungus that naturally grows in grasslands and forests. It is a nutritious edible fungus cultivated industrially all over the world. *A. bisporus* belongs to the family *Agaricaceae*, order *Agaricales* and subclass *Agaricomycetidae* [1]. Cultivation of *A. bisporus* for human consumption originated in France over 300 years ago. Growth of *A. bisporus* needs a substrate produced by the composting of animal manure (usually horse and chicken), wheat straw, gypsum, water and different additives [2-4]. Composting is an accelerated version of the natural recycling process for plant biomass (decomposition and humification) performed by microorganisms.

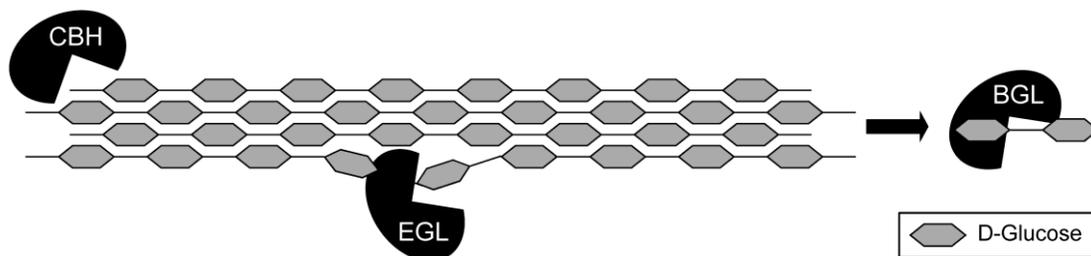
The procedure to generate the compost substrate for *A. bisporus* is usually done in two stages. Phase 1 involves regularly mixing, wetting and heating of compost ingredients [2-4]. During phase 2, which is an indoor process, pasteurization is accomplished for 3-6 hours (60 °C) to relieve the compost of mushroom pests and pathogens that may be present in the compost [2]. After phase 2 the compost is inoculated with millet or rye grains colonized with *A. bisporus* mycelium, a process called spawning [5]. The colonized substrate is covered with casing soil, which is usually a mixture of peat and limestone [4]. *A. bisporus* mycelium colonises the casing soil in about 10 days, after which the temperature is lowered to 16-18 °C and a relative high humidity with heavy watering promotes sporophore development [6]. The first harvestable mushrooms appear 18 to 21 days after addition of the casing layer and after that in repeating 7- to 8-day cycles known as flushes [7].

As a result of the composting process, the compost consists mainly of lignocellulose components together with microbial biomass [8]. A major part of this microbial biomass consists of the thermophilic fungus *Humicola insolens* var. *thermoidea* (*Scytalidium thermophilum*) which is important for increasing the growth rate of the mushrooms and reducing the ammonia concentration [9, 10].

## RESULTS AND DISCUSSION

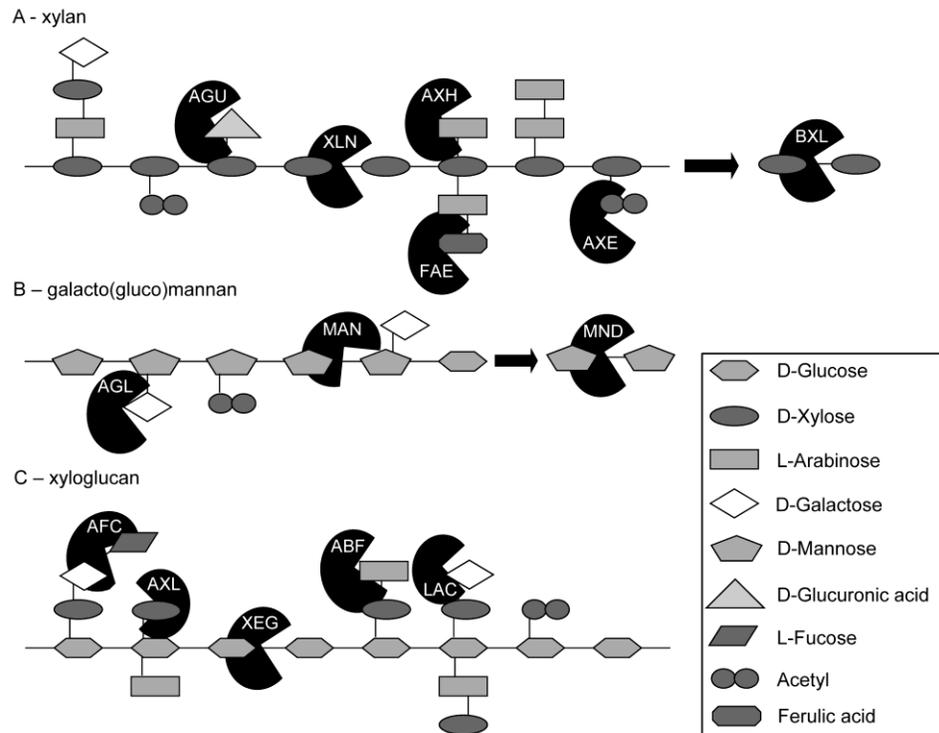
**Composition of plant biomass.** The main component of plant biomass is the plant cell wall, which itself consists mainly of polysaccharides, lignin and proteins. Depending on the plant species and tissue, as well as the season, the composition of plant cell walls varies. The different components interact with each other to provide the structural strength for plants and different fractions can be identified. One of these fractions is lignocellulose which consists of lignin, cellulose and hemicellulose (xyloglucan, xylan, galacto(gluco)mannan). The aromatic polymer lignin interacts with cellulose and forms covalent cross-links with hemicelluloses creating an intricate and hard-to-degrade network of polymers, which is a poor substrate for most microorganisms, but not for *A. bisporus* [8].

Cellulose consists of linear  $\beta$ -1,4-linked D-glucopyranose chains that are linked together by hydrogen bonds to form microfibrils. Degradation of this polymer involves endo- $\beta$ -1,4-glucanases (EC 3.2.1.4), cellobiohydrolases (EC 3.2.1.91) and  $\beta$ -glucosidases (EC 3.2.1.21) [11-14]. Endoglucanases hydrolyse cellulose chains randomly to  $\beta$ -1,4-gluco-oligosaccharides. Cellobiohydrolases remove cellobiose units from cellulose chains, which are degraded by  $\beta$ -glucosidases to D-glucose (see Fig. 1).



**Figure 1:** Schematic presentation of cellulose, its degradation and release of D-glucose.  
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One of the most complex components of hemicellulose present in straw-derived compost is xylan. It consists of a  $\beta$ -1,4-linked D-xylopyranose backbone which can be substituted with a large number of residues (e.g. L-arabinose, D-galactose, D-glucuronic acid, acetyl), depending on the origin [16]. The main enzymes involved in the conversion of xylan to monomeric sugars are endoxylanases (EC 3.2.1.8) and  $\beta$ -xylosidases (EC 3.2.1.37) (see Fig. 2). Endoxylanases cleave the xylan backbone to small xylo-oligosaccharides, which are converted to D-xylose by  $\beta$ -xylosidases [17, 18]. Other classes of enzymes involved in the removal of arabinosyl, glucuronosyl, acetyl and feruloyl residues from the xylan backbone are  $\alpha$ -L-arabinofuranosidases (EC 3.2.1.55), arabinoxylan arabinofuranohydrolases,  $\alpha$ -glucuronidases (EC 3.2.1.131), acetyl xylan esterases (EC 3.1.1.72) and feruloyl esterases (EC 3.1.1.73) (see Fig. 2) [19-20].



**Figure 2:** Schematic presentation of hemicellulose components (xylan, galacto(gluco)mannan, xyloglucan) and their degradation. Reprinted from [15] with permission from the publisher.

Other hemicellulose structures present in plant cell walls include galactoglucomannan and glucomannan. Both consist of a backbone of  $\beta$ -1,4-mannosidic linkages which can have  $\alpha$ -1,6-linked D-galactose residues. Endomannanases (EC 3.2.1.78) randomly hydrolyse  $\beta$ -1,4-mannosidic linkages in galacto(gluco)mannan, while  $\beta$ -mannosidases (EC 3.2.1.25) convert the resulting oligosaccharides to mannose monomers (see Fig. 2). The galactosyl residues attached to the mannan are removed by  $\alpha$ - and  $\beta$ - galactosidases (EC 3.2.1.21 and EC 3.1.2.23, respectively), while the acetyl residues are removed by galactomannan acetyl esterases [20, 21]. Xyloglucan is a component of hemicelluloses which consists of  $\beta$ -1,4-linked D-glucopyranose chains and is substituted with  $\alpha$ -linked xylose residues and other monosaccharides (see Fig. 2). Some enzymes which cleave the cellulose backbone are also involved in the biodegradation of xyloglucan.  $\alpha$ -D-xylosidases,  $\alpha$ -L-furanosidases,  $\alpha$ -L-arabinofuranosidases,  $\alpha$ -L-galactosidases,  $\beta$ -D-galactosidases and xyloglucan acetyl esterases remove xyloglucan side residues (see Fig. 2) [22].

Enzymes involved in the degradation and modification of carbohydrates, including plant polysaccharides, are classified in the Carbohydrate Active enzyme system (CAZy, [www.cazy.org](http://www.cazy.org)) [23].

Lignin is an essential part of plant cell walls. The main extracellular enzymes acting in lignin breakdown are manganese peroxidase (EC 1.11.1.13), heme-containing lignin peroxidase (EC 1.11.1.14) and Cu-containing laccase (EC 1.10.3.2) [24].

**Plant biomass degrading enzymes and their encoding genes from *A. bisporus*.** Some insight into the molecular basis of *A. bisporus* related to plant biomass degradation was obtained through the isolation of genes encoding the related enzymes and the detection of their

developmental regulation. Screening of a cDNA library, made using RNA isolated from cellulose-grown mycelium, with an endoglucanase antibody identified four cDNA clones assigned *cel1* to *cel4* [14, 25]. *Cel2* and *cel3* are similar to fungal cellobiohydrolase I and cellobiohydrolase II, and *cel4* shows similarity to fungal  $\beta$ -mannanases belonging to glycosyl hydrolase family 5. *Cel2* has homology to glycoside hydrolase family 7 (GH7) while *cel3* belongs to GH6. However, no homology to cellulose degrading enzymes was detected for *cel1* although it did have homology to fungal cellulose binding domains [14, 21, 25]. The transcript of *cel3* gene was detected about 13 times lower in glucose grown cultures than in cellulose grown cultures [11]. It was also shown that *cel3* expression increased during fruiting body development and the highest level comes at the veil break [26].

An endoxylanase encoding gene, *xlnA*, was isolated to analyze the hemicellulotic activities during cultivation of *A. bisporus* on compost [17]. It was shown that *xlnA* belongs to GH10 which includes fungal and bacterial enzymes hydrolyzing xylan, cellulose or both [17]. Expression of *xlnA* was detected in vegetative mycelium grown on axenic compost. However, no *xlnA* expression was detected in fruiting bodies. Also, no expression of *xlnA* was detected in mycelium of *A. bisporus* that was grown on compost supplemented with glucose, but a high level of *xlnA* expression was detected after transfer of the mycelium to medium containing cellulose, xylan or xylose [17]. An identical pattern of repression and expression was detected for the cellobiohydrolase II encoding gene *cel3* [17]. This suggests that *xlnA* and *cel3* are co-regulated and that their induction occurs by compost-specific factors.

Analysis of two laccase genes, *lcc1* and *lcc2*, demonstrated that high expression levels were detected in compost during colonization. No expression was detected during fruiting body development, but the expression level of laccase genes increased after harvesting and during the second flush [26].

Studies on the genes encoding cellulases, xylanases and laccases showed that they are developmentally regulated. Laccase increased during mycelia growth but declined at the onset of fruiting while cellulase and xylanase accumulated at the onset of fruiting.

**Plant biomass degradation during growth of *A. bisporus* on compost.** The major components of lignocellulose are cellulose and hemicellulose that are converted into microbial biomass during composting, while lignin is often complexed with proteins. The activity of endoxylanases degrading hemicellulose present in straw-derived compost (xylan) is slightly increased during the development towards fruiting bodies and the highest point in activity is after the veil break stage. However, increase in  $\beta$ -xylosidase activity was only observed after the button stage of the fruiting body development [18]. Increased xylanase activities during fruiting correlates well with the reduction in hemicellulose content of the compost [18].

The microflora of the compost only degraded lignin slightly whereas vegetative mycelium of *A. bisporus* was discovered to degrade lignin more efficiently, and that this degradation was mediated by the activity of manganese peroxidase and laccase [26, 27]. The activity of both laccase and manganese peroxidase has been observed in compost during the pinning stage of fruiting body formation and decreased at the onset of fruiting body formation. No lignin peroxidase activity was detected [28].

It was shown that lignin is degraded during the vegetative growth of *A. bisporus* in compost. In contrast, cellulose and hemicelluloses are already degraded after the addition of the casing soil. Low levels of cellulase activity have been found in the compost colonized mycelium but this level increases with the beginning of fruiting. In contrast, high levels of laccase activity have

been detected in the compost but after the beginning of the pinning stage these levels decline sharply [13, 29].

## CONCLUSIONS

Degradation of plant biomass is an important aspect of the cultivation process of edible mushrooms, but the mechanisms behind it are still largely a black box. The availability of genome sequences for *A. bisporus* as well as transcriptome analysis enables us to obtain a deeper understanding of the genes and enzymes involved in this process. This is likely to result in new strategies to improve mushroom cultivation.

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