Research from around the *World*

Oxidation-driven lignin removal by *Agaricus bisporus* from wheat straw-based compost at industrial scale (a)

Katharina Duran, Jeanne Miebach, Gijs van Erven, Johan J.P. Baars, Rob N.J. Comans, Thomas W. Kuyper, Mirjam A. Kabel. Int. J. Biological Macromolecules. 246:125575.

The secretome of *Agaricus bisporus*: Temporal dynamics of plant polysaccharides and lignin degradation (b)

Katharina Duran, Joris Magnin, Antoine HP America, Willem JH van Berkel, Thomas W Kuyper, Mirjam A Kabel. iScience 26, 107087.

WHAT'S IT ABOUT?

Once upon a time, *Agaricus* mushrooms were just another fungi, albeit widely distributed. They grew from Alaska to the Congo, and from coastal grasslands to mountain forests. This adaptivity was due in part to their natural diet, which was based on partially degraded leaf litter and other organic materials in soil.

Farmed *Agaricus* feeds primarily on wheat straw, degrading the tough celluloses, hemicelluloses (xylan) and lignin that together form plant cell walls. One key purpose of composting is to strip away straw's waxy cuticle and start to break down the linkages between carbohydrates and lignin, making these materials easier for the mycelium to digest – as would have occurred in the natural environment.

At the end of Phase II composting, 50-60% of celluloses and xylans in the starting material have already been degraded. However, lignin largely remains intact. Lignin interacts with cellulose and forms cross linkages with xylans, forming a complex, hard to break down network of polymers. The mycelium therefore need to at least partially break down lignin in order to access what have been assumed to be more energy-rich food sources (Patyshakuliyeva A. 2015).

Despite the abundance of lignin in the substrate, and its' likely importance in the *Agaricus* diet, it has been difficult to definitively characterise how it is broken down. Lignin in compost is not only structurally complex, but also insoluble, composed of aromatic rings and well integrated with other compounds. This makes it difficult for fungi to break down, as well as difficult to measure in the laboratory.

However, researchers at Wageningen University have developed a new, accurate method for measuring lignin. This combines pyrolysis (heating to high temperatures without oxygen) and GC-MS analysis, with carbon-13 labeled lignin used as an internal standard. Unlike other methods, measurements exclude lignin held within dead microbes, or which has already been partially degraded. This means it avoids overestimating the lignin remaining in substrate.

In 2023 PhD candidate Katherina Duran published two papers relating to her work (and that of the Wageningen team) on lignin. The aim was to determine whether there were biological bottlenecks during utilization of the substrate by *Agaricus*, and whether these could be addressed by changing composting technique or adding enzymic amendments.

The first (a) describes the different processes used by Agaricus to break down lignin. The team found that lignin remained stable during the first six days of mycelial growth. This is likely due to the young mycelium consuming the more easily accessible carbohydrates present – a bit like going straight to the cheese and biscuits at a dinner party. However, as the mycelium began to more fully colonise the substrate, delignification began in earnest.

Between days 6 and 10, 19% of total lignin was removed by the mycelia, with an additional 17% removed between days 10 to 13. Interestingly, after this there was little further change. This could indicate that the remaining lignin was in forms more difficult for the mycelia to break down, or that other biochemical bottlenecks limit continued extraction from the substrate.

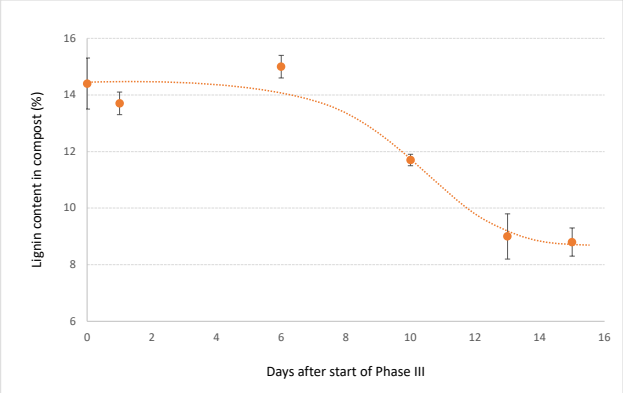

Figure 1. Total lignin (% dry weight) in substrate during phase III. Bars indicate the standard error of each mean. Dotted line approximates best fit to data. Derived from data presented in Duran et al, 2023a

In total, less than half (43%) of the total lignin present in the substrate was broken down, suggesting that availability of lignin is not the key factor limiting productivity.

This is consistent with results published in 2017; Vos et al. genetically engineered Agaricus to overexpress manganese peroxidase (MnP), with the aim of increasing lignin breakdown. It was hypothesised that this would improve total carbohydrate availability in compost. Although enzyme activity was tripled, total lignin content and yield were unaffected. This was thought due to lack of peroxidase, which is essential for MnP activity against lignin.

In the second paper, the Wageningen team explored the Agaricus secretome, and the effects of these compounds on lignin degradation. The "secretome" describes the set of proteins produced by the mycelia which are secreted into the surrounding substrate.

The paper confirms the earlier finding that lignin is an important food source for Agaricus; during Phase III the celluloses, xylans and lignin that make up wheat straw cell walls declined by 20%, 18% and 40% respectively.

This breakdown is catalysed by enzymes. Enzymes are types of proteins and consist of strings of amino acids linked together in polypeptide chains. It is therefore not surprising that degradation of materials in the straw was associated with a huge increase in protein expression.

At the start of phase III, the young hyphae were only secreting 11 proteins into the secretome. However, protein production exploded as the mycelia colonised the substrate. By day 10 the mycelia were expressing 116 different proteins, increasing to 187 identified proteins on day 13 (Figure 2).

Around 40% of these exudates were identified as enzymes associated with degradation of lignin (carbohydrate active enzymes or CAZymes). Another 15 to 19% were proteases. That is, enzymes that break down proteins, recycling nitrogen from organic sources. This reflects the substrate environment, where organic materials are already partially broken down. In contrast, only 2 to 7% of exudates in the secretomes of wood rot fungi (e.g. oyster mushrooms) are proteases.

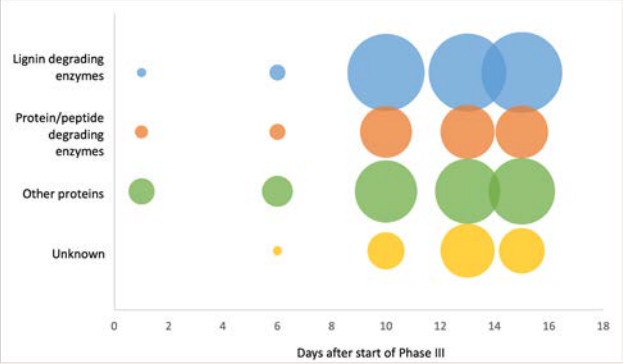

Figure 2. Number and types of proteins found in the secretome of *Agaricus bisporus* during Phase III. Size of circles indicates the number of proteins found. Derived from data presented in Duran et al., 2023b.

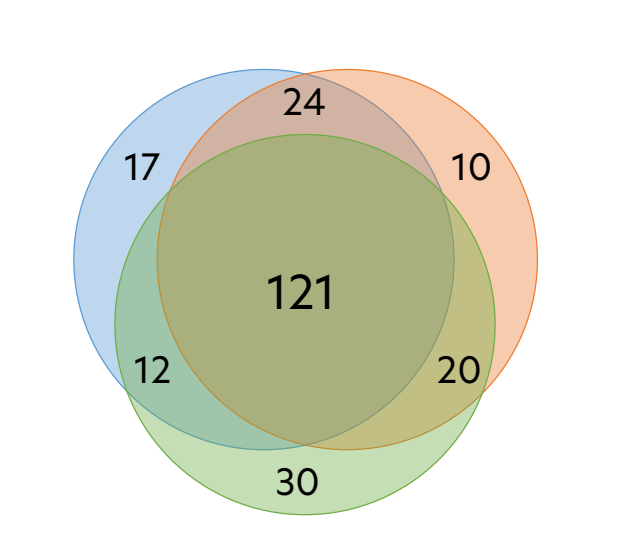

Figure 3. Venn diagram of proteins found in the secretomes of normally cultivated *Agaricus* mycelia 15 days after the start of Phase III (D15), compared to mycelia grown in sterilised media with straw particles included (CDF) or removed (CWS). Numbers indicate the total number of proteins found that are common to one, two or all three growing systems. From Duran et al, 2023b.

The researchers also compared the secretomes produced by Agaricus grown in normal compost with secretomes from Agaricus growing in a sterilised, boiled compost extract from which the solid straw particles had (CDF), or had not (CWS), been removed.

Only 16% of the proteins produced by Agaricus growing in normal compost were absent from either or both of the two sterile cultures. This confirmed that the proteins recorded were definitely produced by Agaricus and not by other microbes present in the compost (Figure 3).

This work has demonstrated the importance of lignin degrading enzymes in fuelling growth of the Agaricus mycelia. These enzymes, secreted into the surrounding substrate, are clearly essential for efficient extraction of nutrients from straw's tough cell walls.

Despite this, less than half of the nutrients available in the compost are transformed by Agaricus mycelia. Understanding the bottlenecks that limit further breakdown of lignin two weeks after the start of Phase III could help optimise extraction of energy held in the substrate, increasing productivity.

This work is only a small part of that submitted by Katherina Duran in defence of her thesis in late 2023. The remaining research is embargoed until the results have been published. MushroomLink will wait with interest to see what further light is shed on the complex process of feeding Agaricus.

Additional references:

Patyshakuliyeva, A. 2015. Unravelling the mystery of commercial cultivation of *Agaricus bisporus*: Plant biomass utilisation and its effect on mushroom production. Thesis submitted to Utrecht University. https://dspace.library.uu.nl/handle/1874/320601

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