BIOLOGICAL EFFICIENCY OF WILD TYPE AND COMMERCIAL *AGARICUS BISPORUS* STRAINS

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ABSTRACT

In the cultivation of the button mushroom (*Agaricus bisporus*), the cost of substrate production (compost) represents a large part of the total production cost. In Europe, the button mushroom uses only 25% of the organic matter in the compost in two flushes. Most white button strains used worldwide today are closely related to the first hybrid strains developed in the Netherlands over 30 years ago. Wild strains may provide possibilities to improve the efficiency in utilization of the compost (biological efficiency). Our research group has a large collection of mushrooms strains, many of which are derived from the ARP collection. Analysis of this collection has shown a large genetic variation. A selection, representing the genetic variation of the collection, has been cultivated in the Dutch shelf-system on a small scale. A large variation in biological efficiency has been found. The present commercial strains are among the ones with the highest biological efficiency. Next to this we noticed a clear correlation between the dry weight of mushrooms produced and the drop in pH in the compost. Loss in dry matter in compost as a result of mushroom production can be attributed for 95% to loss in hemicellulose and cellulose. Total amounts of lignin and undefined organic matter do not decrease much during the cultivation of mushrooms. Furthermore, there is a clear correlation between mushroom production and consumption of hemicellulose from the substrate. This correlation is less clear for cellulose.

Keywords: *Agaricus bisporus*, compost, hemicellulose, genetic diversity

INTRODUCTION

In the Netherlands, yields of button mushroom (*Agaricus bisporus*) range up to 350 kg of mushrooms per tonne of compost. Nevertheless, only 16% of the dry matter (25% of the organic matter) present in the compost is degraded to produce 2 flushes of mushrooms. Increasing the efficiency with which the substrate is converted into mushrooms will therefore have a considerable impact on the economics of production. It will also impact the environmental burden of the crop; a higher biological efficiency would result in less transport of compost and also in a smaller quantify of spent compost. Currently, mushrooms strains are only able to use a relatively small portion of the organic matter present in compost.Wild strains may provide possibilities to improve the biological efficiency with which compost can be converted into mushrooms but might also offer opportunities to use other ingredients to generate a good yielding substrate.

The Department of Plant Breeding of Wageningen University & Research Centre houses a large collection of button mushroom strains. This collection was started and expanded mainly by the Mushroom Experimental Station in Horst, the Netherlands, starting in 1954. It consist of old commercial strains (<1980), the currently available commercial strains and wild collected strains (mainly from the ARP collection) [1].

Research on biological efficiency of button mushroom may also improve our understanding of which nutrients may be limiting mushroom production. Basically, compost consists for a large part of an inert carrier material which provides a number of important physical characteristics such as water-holding capacity and structure / low bulk density allowing gas exchange. Cellulose and hemicellulose are the major source of carbon and represent only 32% of the dry matter in the compost. Sonnenberg and Blok [2] have shown that during production of flushes of mushrooms, strain Sylvan A15 only 16% of the dry matter in phase III compost is consumed, representing a loss of 25% of the total organic matter. While 83 % of the hemicellulose present is consumed, only 41% of the cellulose present is consumed. So, even though there is still quite a lot of cellulose present in the compost, a third flush of mushrooms usually has a much lower yield than the first two flushes.

Results described above were obtained, using *A. bisporus* strain Sylvan A15. In this article we present results of substrate consumption by a variety of genetically dissimilar button mushroom strains.

MATERIALS AND METHODS

Strains

Strains are maintained in liquid nitrogen according to the method published by Homolka and coworkers [3]. After revival from liquid nitrogen the strains were grown on malt extract agar, set at pH 7.0 using 10 mM MES/KOH. When needed, agar plates were covered with cellophane discs to facilitate collection of the mycelial colonies.

Molecular techniques

DNA isolation was performed using the Promega magnetic particle Plant kit (Promega Corporation, Madison, WI, USA). DNA samples were subsequently used for SNP analysis. All publically available genomes of *A. bisporus* (H97 & JB137-S8 [4]) and 4 in house generated sequences were used to detect single nucleotide polymorphisms. This resulted in a list of 120 SNP markers. For each sequence we selected about 2 markers per scaffold. SNP analysis were performed by Van Haeringen Lab in Wageningen (https://www.vhlgenetics.com/en-us/home.aspx). After analysis, a dataset of 117 SNP markers was obtained. After modification towards an input file for the program NTSY Spc 2.1 [5] in which the UPGMA clustering method was used to analyse genetic relatedness of the strains.

Cultivation techniques

For a selection of 66 *A. bisporus* strains, taken from the full width of genetic diversity, spawn was made. Strains were inoculated in triplicate into 8 kg portions of freshly prepared phase 2 compost (CNC Grondstoffen, Milsbeek, the Netherlands) and filled in plastic tubs with 0.1 m^2 growing surface (approx 80 kg compost/m^2). After 16 days of spawn run at 24 ^oC, a casing layer was applied. After 11 days of colonization, the casing soil was ruffled. After an additional 3-4 days of recovery growth, the trays were vented for a period of 4 days to reach an air temperature of 18 ^oC, 90% RH and 1000 ppm CO_2. Subsequently two flushes of mushrooms were harvested of quality I (closed mushrooms varying in size between 15 and 45 mm). Total yield per tray was recorded in grams wet weight. Dry weight of samples of the mushrooms was determined after drying at 105 ^oC.

Compost analysis

Compost was sampled at spawning and after the second flush of mushrooms. Proximate composition of the compost samples was determined by Havens (Maashees, the Netherlands) using Near Infra-Red Spectroscopy (NIR). To determine substrate utilization during spawn-run, a duplicate experiment was performed. Trays in this experiment were sacrificed at venting and compost composition was determined using NIR Havens (Maashees, the Netherlands).

RESULTS AND DISCUSSION

Genomic analysis

In total, 235 strains of *A. bisporus* were analysed for genetic variability, using 120 SNP markers. Results are shown in Fig 1. Names of the different strains are lacking, as the format used makes them unreadable. Strains of *Pleurotus ostreatus*, *A. arvensis* and *A. blazei* have been used as an outgroup. Strains of the outgroup have been indicated in black. As can be seen two strains from the outgroup (*A. arvensis* and *A. blazei*) appear in the group of four-spored *A. bisporus* isolates from the Californian desert.

The analysis clearly discriminates large groups of strains. The traditional commercial white strains and the traditional off-white strains, both widely used in the pre-hybrid era, can easily be discriminated. Given the considerable variety of the traditional white strains, it seems unlikely that these varieties all originate from a single white strain isolated in 1926 on a bed of cream mushrooms as has been suggested in the past [6]. For comparison, the strains indicated in red represent the

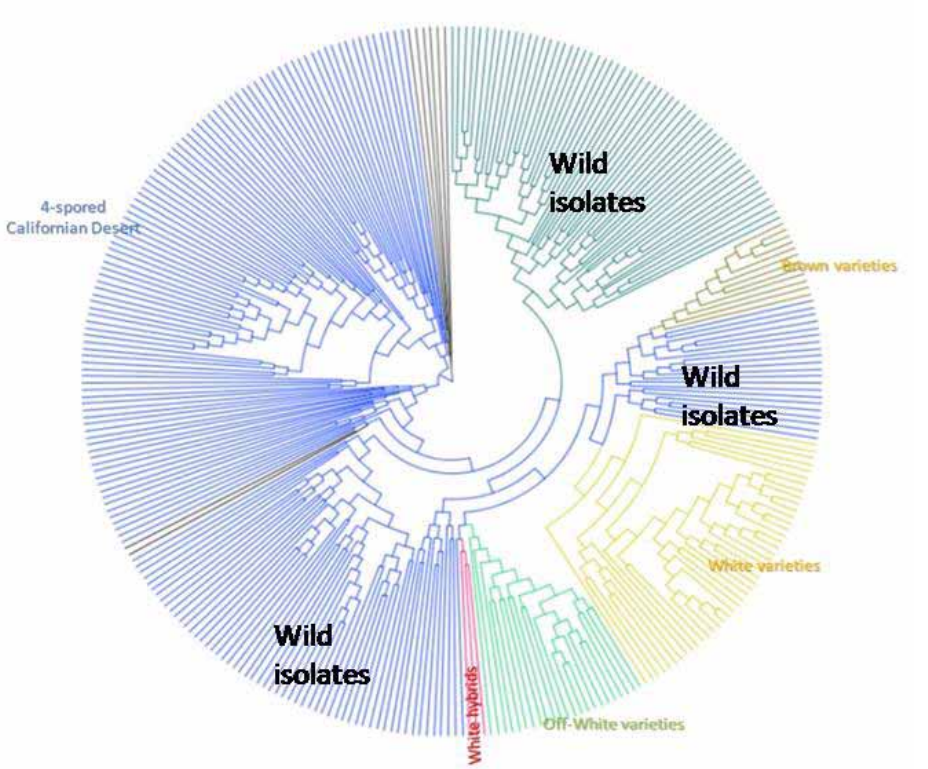

Figure 1. Analysis of genetic relatedness of 235 strains of *A. bisporus*. Analysis clearly identified the traditional commercial white and off-white varieties from the pre-hybrid era. It also shows the very limited genetic base of the currently commercially used white hybrids. Among the non-commercial strains, the large genetic diversity of the 4 spored *A. bisporus* var. *burnettii* strains from the Californian desert (blue) can be seen, as well as the genetic diversity of the wild type bisporic strains. The black lines represent the out-group.

currently used white hybrid strains. These strains are known to have all been derived directly or indirectly from the first white hybrids released to the market in 1980 and represent thus a very narrow genetic base. Also the traditional commercial brown varieties (used before the market introduction of Heirloom and Brawn by Amycel) can easily be identified as a group. Dispersed between these groups, wild collected bisporic *A. bisporus* strains are found (Fig. 1). The large blue group between the outliers represents four-spored varieties originating from the Californian desert. This genetic analysis clearly shows that only a very small part of the genetic diversity among *A. bisporus* strains is used for commercial cultivation and potential to generate new products for growers and consumers is left unused. As a result all currently used strains are susceptible to the same extent to pests and diseases.

Substrate utilization

The trays used for the assessment of compost utilisation contain 8 kg of compost at spawning representing 2.66 kg of dry matter. This dry matter consists of 0.84 kg of ash and 1.83 kg of organic matter. The organic matter consists of 1.23 kg of lignocellulosic fibers (0.22 kg of hemicellulose, 0.66 kg of cellulose and 0.36 kg of lignin) and an undefined fraction (0.6 kg).

Yields of mushrooms ranged from 0 to 218 grams of dry matter per tray. As shown in Fig. 2 higher yields are accompanied by a larger drop in compost pH at the end of the second flush. Apparently mushroom production is accompanied by the production of acids. It is not clear however, which acid would be responsible for the pH drop. Oxalic acid is produced in large quantities by *A. bisporus* and may be a candidate. According to Gadd [7], oxalic acid plays an important role in degradation of lignocellulosics. Baldrian and Valášková [8] suggest a number of different roles for oxalate in degradation of cellulose, such as chelating metal-ions, as in Fig. 5. Relation between the amounts of consumed cellulose and hemicellulose. The coloured data points represent commercial strains. The red data point represents A15.

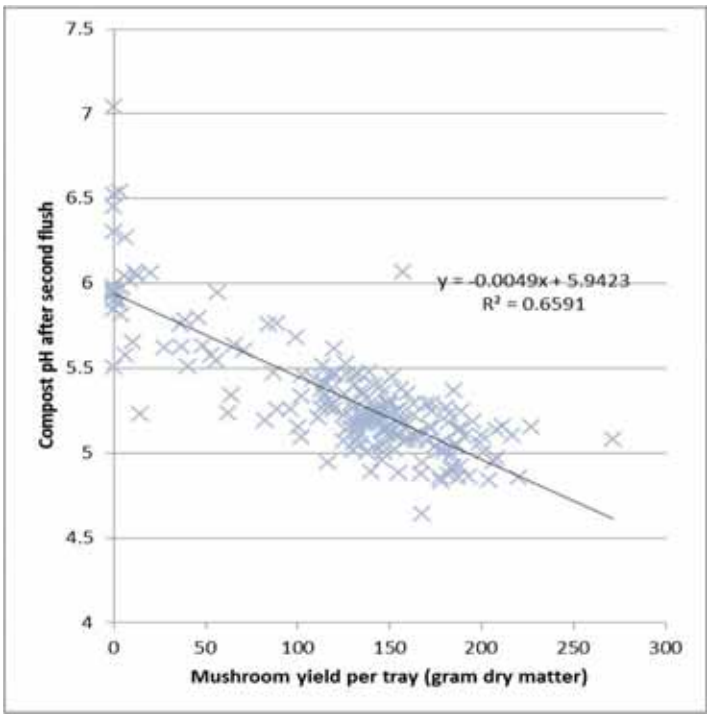

Figure 2. Relation between mushroom yield and drop in compost pH

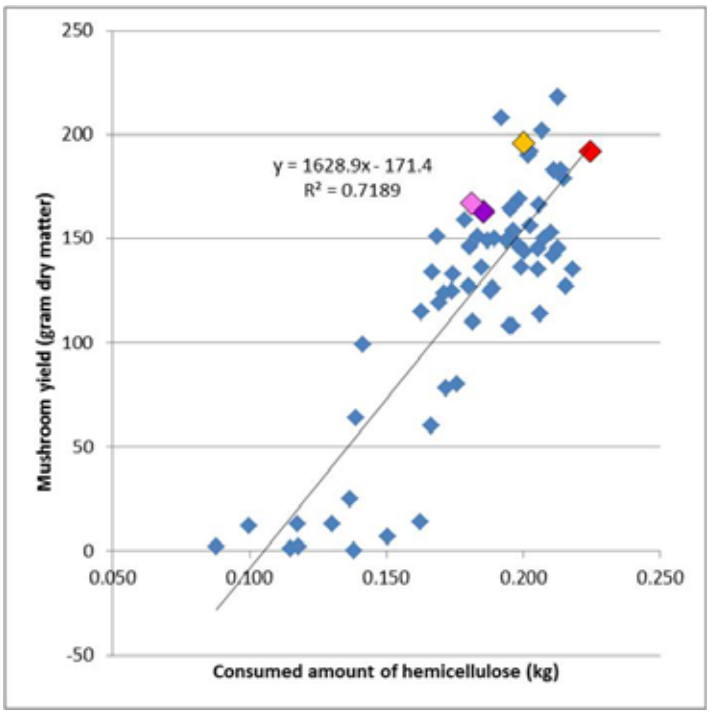

Figure 3. Relationship between mushroom yield and consumption of hemicellulose. The coloured data points represent commercial strains. The red data point represents A15

Mushroom yield (expressed in gram dry matter) correlates well with consumption of hemicellulose from the compost (Fig. 3). A less significant correlation is found for mushroom yield and consumption of cellulose (Fig. 4). Nevertheless, consumption of hemicellulose is closely linked to the consumption of cellulose (Fig. 5). High yielding strains consume cellulose and

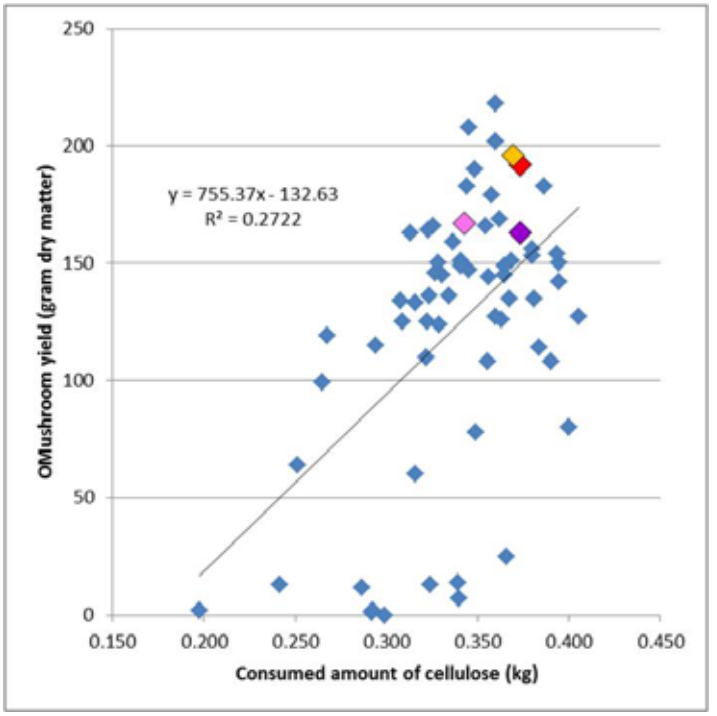

Figure 4. Relationship between mushroom yield and consumption of cellulose. The coloured data points represent commercial strains. The red data point represents A15

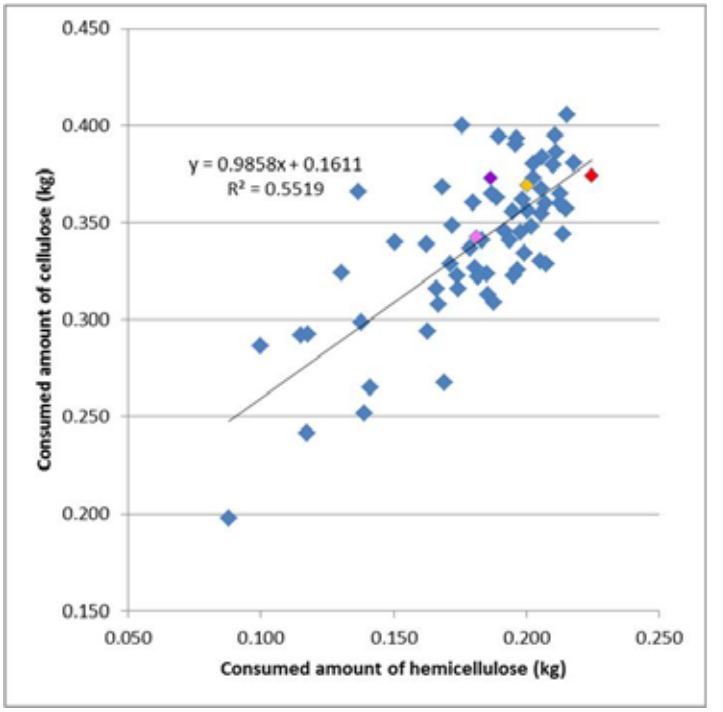

Figure 5. Relation between the amounts of consumed cellulose and hemicellulose. The coloured data points represent commercial strains. The red data point represents A15

hemicellulose in a fixed ratio (Fig. 6). Strains that yielded more than 100 gram of mushroom dry matter per tray, consumed cellulose and hemicellulose in a ratio of about 1.7. Strains that yielded between 50 and 100 gram of mushroom dry matter per tray, consumed cellulose and hemicellulose in a ratio close to 2. Strains that produces only low amounts of mushrooms (less than 50 gram of mushroom dry matter per tray) consumed cellulose and hemicellulose in a ratio between 2 and 3. As can be seen in the right graph in Fig. 3, the low yielding strains consumed up to 0.35 kg out of the 0.66 kg of cellulose present in a single tray, while producing only about 25 gram of mushroom dry matter.

Biological efficiency

Biological efficiency of mushroom production is here expressed as the amount of dry weight mushrooms produced per kilogram of organic matter consumed, with values ranging from 0 to 395 g DM/kg OM consumed. Analysis by ANOVA has shown differences to be statistically significant. At p=0.05 the least significant difference (l.s.d.) was 64.

Values for biological efficiency show a more or less normal distribution (Fig. 7). A number of strains produced no or barely any mushrooms, producing a rather large lowest bin. The 4 commercial strains tested showed biological efficiencies of 269, 280, 317 and 325 g DM/kg OM consumed, for a commercial brown strain (Somycel 856), two traditional white strains (Somycel 53 & Le Lion B80), and a hybrid strain (Sylvan A15), respectively. Given a l.s.d. of 64, the value of 317 g DM/kg OM consumed for Sylvan A15 is significantly lower than the highest biological efficiency recorded in this experiment (395 g DM/kg OM). Repeating the experiment will likely make the determination of the biological efficiency more accurate. The large variation in biological efficiency indicates that this collection has potentials to improve compost utilisation via breeding.

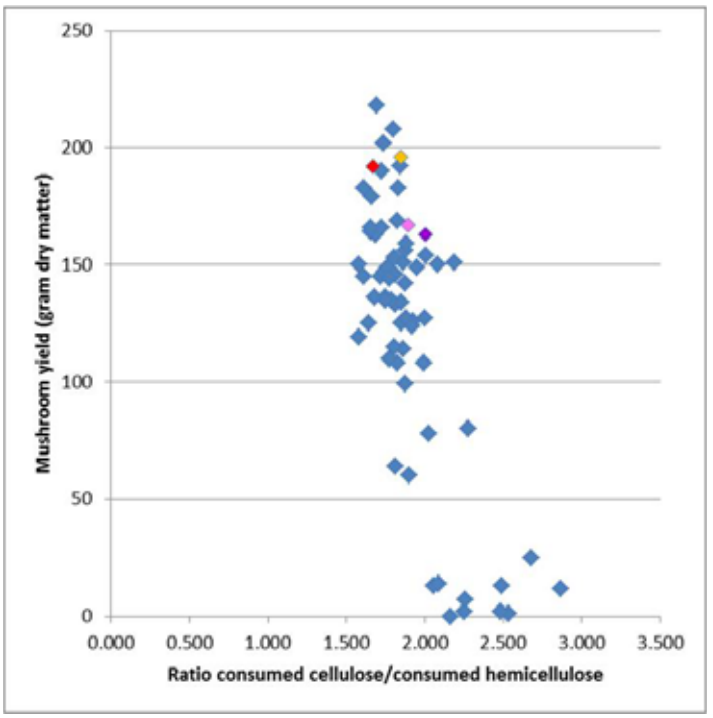

Figure 6. Relation between the ratio consumed cellulose/hemicellulose and yield. The coloured data points represent commercial strains. The red data point represents A15

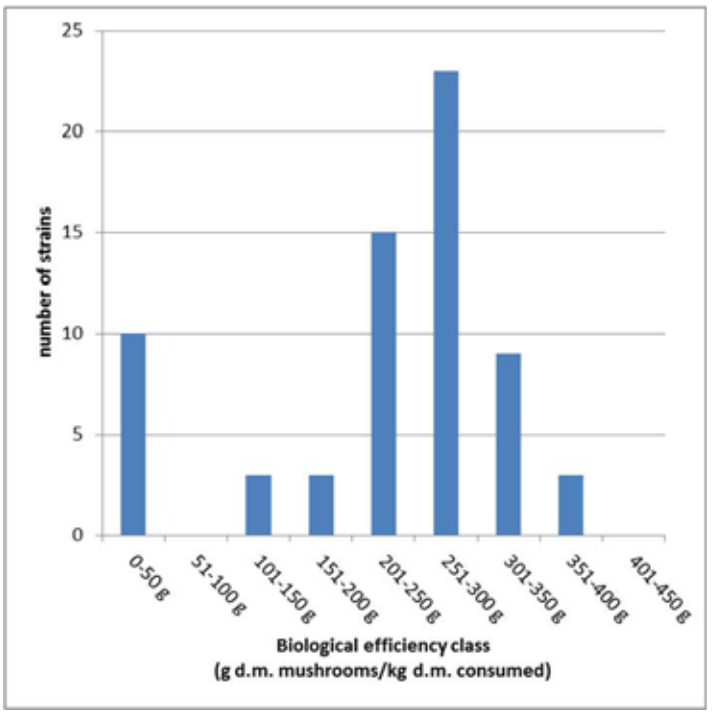

Figure 7. Distribution of biological efficiency among strains

CONCLUSION

In conclusion, it can be said that the currently commercially used hybrid strains of *A. bisporus* represent a narrow genetic base. Traditional commercial strains (whites, off-whites and browns) represent a wider genetic diversity. Wild collected strains show an even wider genetic diversity.

Analysis of compost utilization by a range of 66 genetically dissimilar *A. bisporus* strains has shown that mushroom production in *A. bisporus* is strongly linked to the consumption of hemicellulose and to a lesser extend to cellulose with a remarkable fixed ratio in cellulose/hemicellulose consumption. The yield in mushroom production is also correlated with pH of the compost, i.e. the higher the yield, the lower the pH of the compost after two flushes.

Analysis of biological efficiency of 66 genetically dissimilar strains has shown that in this experiment mushroom production can reach a yield of 395 g of dry weight mushrooms per kg of organic matter consumed, which is higher than the production of the currently used hybrid strain A15 in the same experiment.

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