Horticulture Innovation Australia

Final Report

Improving consistency of mushroom compost through control of biotic and abiotic parameters

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Summary

The yield and quality of a mushroom crop depends critically on consistent quality in the compost used to grow the mushrooms. Fluctuations in availability and quality of wheat and poultry manure for production of this compost are a major risk for the Australian Mushroom Industry. Alternative agricultural byproducts can be used as feedstocks, but an in-depth understanding of the biology and chemistry of the composting process is needed before they can be used with confidence.

A detailed analysis of the biology of a standard composting process in the Australian composting industry was undertaken. This was the most detailed study of its kind to date, and identified over 30,000 different microbes in compost, starting from feedstocks and continuing to the final stage of mushroom harvesting. It also characterized the biological activity of these microbes, and the chemical and physicochemical changes occurring in the compost throughout the process. The dominant microbes change regularly as straw becomes compost, and a mixture of these microbes has the potential to be used as a supplement, to promote reliable composting of alternative feedstocks.

Further productivity risks in the mushroom composting process are associated with the annual intake of new season straw and the use of recycled process water for straw wetting. New season straw decomposes more slowly than weathered straw, and provides reduced mushroom yields. Analysis showed different microbe populations present on weathered straw, which can potentially be used to condition the new straw and accelerate composting. Recycled process water was shown to be beneficial, since its microbial population is dominated by one of the key bacteria in the composting process and it therefore acts as a natural supplement.

Several approaches to test compost supplementation were trialed. The aim was to develop a method for incorporating small-scale composting trials within the full-scale industrial process. Achieving this aim proved elusive, partly because of conflicting demands between the experimental requirements and workload limitations in the industrial setting. A more collaborative effort is needed to develop a suitable testing method to ensure that results from small-scale manipulations are a strong reflection of large-scale conditions.

Keywords

Mushroom, compost, microbial communities, enzyme, PCR, T-RFLP, DRIFTS, next generation sequencing, amendments, weathering, goody water.

Introduction

The mushroom industry in Australia is estimated to produce 67,000 tonnes of mushrooms per annum with a retail value of almost $700 million. However, the industry faces risks due to increases in price and fluctuations in the availability and quality of raw materials for compost production. As all mushroom growers know, the yield and quality of a mushroom crop depends critically on maintaining consistent, reliable quality in the mushroom compost used as a growth substrate. Achieving this consistency of compost is an ongoing issue for the industry with problems arising due to fluctuations in availability, quality and cost of both of the main components, wheat straw and chicken manure. New season straw, for example, is slow to break down and often gives low-yielding composts. During drought years, wheat straw is often difficult to obtain and costly, but even in non-drought years, compost manufacturers are starting to feel price competition from the biofuel sector. Changes to poultry farming practices have led to lower nitrogen levels in the chicken manure supplied which directly affects the composting process and the nutrients available to the mushroom crop. In addition, occasional batches of compost afford unexplained, low crop yields which growers and manufactures are currently at a loss to understand or remedy, despite years of experience.

The past 40 years have seen extensive research into the chemical, physical and biological characteristics of mushroom composting largely with the aim of understanding and optimizing the composting process in order to minimize or avoid the risks outlined above. Biological activities change throughout the composting process as different chemical components of the straw and manure are broken down by microbes (fungi and bacteria). We now understand how chemical parameters such as pH, temperature, carbon:nitrogen ratio and moisture can affect yield. Different microbes are active at different stages of the process and a wide range of microbes have been isolated and characterized. For example, 33 species of bacteria and 82 species of fungi have been found in mushroom compost and have been grown in the laboratory (Fergus, 1964; Ryckeboer *et al.*, 2003). These and other microbes produce a range of biological enzymes, such as cellulase, xylanase, amylase and protease, which break down specific components of straw (Mondini *et al.*, 2004; Tiquia, 2002; Zhang *et al.*, 2014). Some of the microbes are active at low temperatures; some are more active at higher temperatures. Accurate prediction of the success of the composting process requires a deep understanding of microbial activity and how the enzymes they produce contribute to the decomposition of straw at any given time.

Despite this extensive bank of knowledge, however, there are important gaps in our understanding of the biology of the composting process which have become apparent due to recent scientific advances in related fields. In particular, the range of compost microbes identified in earlier studies is limited to those that can be grown in the laboratory. Modern molecular microbial ecology studies have demonstrated that over 90% of soil and compost microbes do not grow well in culture, although they flourish in their native environment. This makes it likely that the key microbes required for successful mushroom composting have not previously been identified. The diversity of microbes in domestic waste stream composting has been studied using molecular fingerprinting techniques (Li *et al.*, 2013; Narihiro *et al.*, 2004; Neher *et al.*, 2013; Takaku *et al.*, 2006). Significant changes in microbial diversity during the composting process of waste have been noted (Ishii *et al.*, 2000; Li *et al.*, 2013; Peters *et al.*, 2000; Ryckeboer *et al.*, 2003; Yamada *et al.*, 2008) but identification of key organisms has not yet been attempted. An initial molecular sequencing study using small-scale composting facilities (Partanen *et al.*, 2010) estimated the total bacterial diversity of over 2000 phylotypes in the composts studied, and although many of these were similar to compost bacterial species reported earlier (including bacteria from the phyla *Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria* and *Deinococcus-Thermus*), many others were previously unknown. Only a few molecular sequencing studies have been done using substrates for *Agaricus* and *Pleurotus* (Szekely *et al.*, 2009; Vajna *et al.*, 2010; Vajna *et al.*, 2012).

In addition, much of this research has been done overseas. However, because of the variation in composting process and ingredients used, the results are not always directly useful for predicting the

composting process in Australia. This is particularly relevant when one considers that the active microbes are always introduced with the feedstocks, and a key feature of this research is to understand how alternative feedstocks impact on the composting process.

The current project aimed to provide detail about the key microbes involved in mushroom composting and the successional changes that occur during composting and cropping processes. Using this knowledge, biomarkers that can be used to assess compost quality and to identify microbial and biochemical differences in raw materials were identified. An important objective was to provide growers with the information to test substrate quality if alternative feedstocks were used to supplement or partly replace the traditional wheat straw/poultry manure raw ingredients used in the Australian mushroom industry. This knowledge will allow growers to respond to changes in feedstock availability, quality and price by changing or substituting feedstocks with more confidence.

Methodology

Detailed methodology is provided in the report included as an appendix to this report, however the methodology is briefly summarised below.

Literature review:

A literature review was conducted from published and "grey" literature sources covering:

- compost sources,
- variation in compost quality,
- abiotic and biotic assessment of different compost types,
- commercially practical tests and testing facilities available worldwide to assess parameters of compost quality,
- enzyme activity of soil microbes and what co-factors are essential for functioning of these enzymes,
- commercially available supplements for mushroom compost and microbial growth and alternative sources of such supplements.

Pilot Study:

In the Pilot Study of the project (initial project phase), traditional commercial composting materials and methods were trialled and characterised using large-scale composting systems. Periodic sampling of substrate or continuous collection of certain data occurred before, during initial composting and after pasteurisation and conditioning.

The enzymes present in the compost substrate and throughout the growing process were characterised. Measurement of physical, chemical and biological parameters (i.e. biotic and abiotic) through the composting and cropping process were also conducted.

Experimental Studies:

The second stage of research was guided by results from the Pilot Study. Once it was known how widely nitrogen and carbon varied during the composting phase, additions of both nutrients were made. Nutrient supplements including sugar cane bagasse, new straw and sorghum stover were investigated. Microbial characteristics of goody water, model systems to trial alternative compost substrates and weathering of wheat straw were also examined.

Outputs

Briefly these included preparation &/or delivery of industry and scientific-focused publications and presentations related to project activities and outcomes in addition to ongoing discussions between the project team and representatives of mushroom composters.

Outcomes

Detailed chemical and biological data were obtained for a standard composting run and mushroom production run under conditions typical for the Australian mushroom industry. This represents the most detailed biochemical and molecular study yet undertaken of the mushroom composting process. Activities of eleven different compost enzymes were determined, along with physicochemical measurements and analysis of the microbial community (T-RFLP and Illumina sequencing) at 37 timepoints from raw materials through composting to the end of the mushroom production process, examining both casing and compost during three flushes of mushroom cropping. The microbial ecology of recycled process water ("goody water") was similarly studied, as was the biology of new season straw and weathered straw. Several approaches to test compost supplementation were tested, using sugar cane bagasse and sorghum stover as potential wheat straw replacements.

Major research findings

The major findings obtained concern the standard composting run. Enzymatic activities in compost were highest during the pre-wet phase, and decreased by the end of rick turn, sharply increased during Phase 2 (after pasteurization), only to decrease again throughout spawn run. Near Infra Red fingerprints, a technique used for characterizing organic samples, revealed clear differences between casing and compost at various stages of composting and mushroom production, suggesting this may be a useful methodology may be of use in characterizing compost and casing in future research. Over 30,000 different microbes were identified in compost with highest diversity at the end of Phase 1 and an obvious biological succession throughout the whole process. The bacterial community in Phase 1 was dominated successively by an *Acinetobacter* followed by *Bacillus*, an unidentified *Proteobacterium* and *Thermus*. In Phase 2 and Phase 3, the main bacteria were a *Pseudoxanthomonas* strain together with *Steroidobacter* and *Chitinophaga*. The fungal community also showed a strong biological succession during pre-wet and Phase 1, with a strain of *Lewia* dominating initially followed by *Myceliophora*, an unidentified fungus, a strain of *Penicillium* and *Scytalidium thermophilum*. *Scytalidium thermophilum* dominated throughout Phase 2 and Phase 3 until it was overgrown by *Agaricus* during the spawn run.

Key risks to productivity in the mushroom composting process are associated with the annual intake of new season straw and the use of recycled process water for straw wetting. Molecular analysis showed that the microbe populations present on weathered straw are different from those on new season straw, suggesting that the former could potentially be used to condition the new straw and accelerate composting. Recycled process water was shown to be beneficial, since its microbial population is dominated by *Thermus thermophilus,* one of the key bacteria in the composting process, and it therefore acts as a natural supplement.

Several approaches to test compost supplementation were trialed, with the aim of developing a method for incorporating small-scale composting trials within the full-scale industrial process. Achieving this aim proved elusive, partly because of conflicting demands between the experimental requirements and workload limitations in the industrial setting. More research is urgently required in this field, and the testing method adopted must be actively accepted by industry to ensure future research success

Evaluation and Discussion

The main initial objective of this project was to gain a comprehensive understanding of the composting process by documenting the changing nature of compost during all phases of commercial production. This required analysis of chemical and microbiological properties of compost at key points in the process from raw materials through to Phase III compost. This first objective was then linked to a second one, which was to extend the understanding of the composting process into the mushroom cropping process, examining in detail the chemical and biological properties of both casing and compost during three flushes of mushroom cropping, and correlating the data with the crop yield obtained.

The project aimed to use this baseline information to design further experimental manipulations, in which the key wheat straw and poultry manure feedstocks would be substituted with alternative substrates to determine critical impacts on the composting process. Overall, the aim was to gain better understanding of two aspects of cropping, namely how to reduce the risks associated with changed quality and supply of raw materials used in compost production, and how to improve yields by using biological compost supplements.

Defining the norm - the Baseline experiment

The baseline experiment was designed to obtain detailed chemical and biological data for a standard mushroom composting run and cropping run under conditions typical for the Australian mushroom industry. To our knowledge, it represents the most detailed molecular study yet undertaken of the mushroom composting process. Compost samples were taken at thirty-seven time points during composting and cropping, with up to nine-fold replication at each timepoint. In addition, casing samples were taken at sixteen timepoints during the cropping phase. The mushroom yields obtained were statistically comparable with yields obtained on commercial farms in Australia, demonstrating that the facilities at the Marsh Lawson Mushroom Research Unit are an excellent model system for study of large scale mushroom cropping systems.

Although the MLMRU accurately models mushroom cropping, it does not include a composting facility. To ensure that the results obtained accurately represented conditions in a large scale composting yard, the experiment was done with samples taken directly from a full bunker-scale operation at ELF Farm Supplies in Mulgrave, NSW. This is an important distinction, as small scale composting (e.g. in a model compost pile) must be very carefully monitored to avoid artefacts due to changed temperature, pressure, and bulk density relative to the full scale industrial process. A previous molecular study of domestic waste composting, for example, compared a pilot reactor (5 m^3) with a full scale reactor (160 m^3), and found significant differences in microbial diversity (Partanen *et al.*, 2010). Spawn run was done either in the ELF Phase 3 facility, or at the MLMRU, and the yields obtained were near-identical, though with slightly lower yields in the MLMRU.

The chemical results obtained from compost in the baseline experiment (pH, ash, N, C-content) were comparable with previous studies, confirming that composting proceeded as expected. Substrate analysis using near-infrared spectroscopy (DRIFTS) showed a clear progression in compost chemical composition from the feedstocks. Although DRIFTS only provides carbon fingerprints, and does not identify specific compounds, this corresponds well with the known chemistry of composting, with sequential degradation of monomeric and oligomeric carbohydrates, followed by polymers such as cellulose, hemicelluloses and lignin. This succession was also seen in the microbiological population.

Clear successional changes were seen in both the bacterial and fungal populations through pre-wet and phase 1, with the dominant species in the population changing on a rapid basis. With the exception of *Thermus thermophilus,* which became important at the end of phase 1, most of the dominant bacterial taxa in phase 1 were not previously well-characterized species, but were largely unrelated to microbes that have been studied before. Likewise, the fungal species in phase 1 were largely unknown, though the well-studied *Scytalidium thermophilum* dominated in phase 2 and the initial stages of spawn run.

If key microbes from phase 1 are to be studied as potential inocula in the future, then most will first need to be isolated and characterized. However, the importance of this strategy as a means of obtaining reliable composting is diminished by two observations. First, most of the important taxa in phase 1 were only dominant for a brief period during composting, and were rapidly overgrown by other taxa. Second, it was observed that although many potentially pathogenic bacteria were introduced with the poultry manure, these did not persist at detectable levels beyond the pre-wet stage, casting doubt on how well other bacteria introduced as inocula may survive the composting process.

Developing methods for supplementation experiments

One of the important objectives of the project was to test the effect of supplementing compost either with alternative feedstocks or with microbial/enzyme additives. Supplementation of this nature cannot be done on a large scale because of the inherent commercial risk involved, but it needs to be done in a system that accurately models the industrial scale, in order that reliable predictions can be made. Repeated consultation with industry partners was used to try and to design an effective model that could be used for supplement testing within the full scale process. The research model needed to yield a sufficient volume of modified compost for reliable yield analysis in the MLMRU, which requires about a ton of compost. In addition, because of the very heterogeneous nature of compost, the model also needed to provide enough replicates for statistical validation of the results. Two main strategies were used to contain small amounts of test compost within the large composting facility, inserting either 20 kg bags ("onion bags") or a medium scale (3-4 tonne) stainless steel mesh bin into the bunker full of untreated compost. The first of these strategies proved unsuccessful because the compost within the bags was not mixed during the process to the same extent as bulk compost, and yields were therefore reduced. In addition, the number of bags required for statistically valid experiments required too much manual handling to be practical. Use of the mesh bin was more successful, but its use highlighted the important issue that reproducible experimentation required consistent handling by the composting team. It became apparent during the project that inclusion of any statistically useful test method within the normal composting procedure placed additional workload requirements on the compost yard technical operatives that were not readily accepted, and was therefore unachievable. There is clearly a need for more extensive trials using e.g. a bin technique to enclose test compost with the bunker, but successful experiments will require committed assistance from the workers on site. Alternatively, it may be more practical to test supplementation mixtures in a small scale composting unit, but this was not available for this project.

Despite the limitations described above, two alternative substrates were tested during the course of the project. Inclusion of different proportions of sugar cane bagasse gave lower yielding compost, but it was concluded that this result was largely due to the onion bag method used. Supplementation with small amounts of sorghum stover gave enzyme results that suggested that this may enhance yields, but further research is required to confirm this.

Process problems – new season straw and goody water

A key problem that the mushroom industry faces every year is the reduction in composting efficiency caused by the introduction of new season straw. The industry currently remedies this by staggering the introduction of new straw (i.e. mixing the new season straw with old season straw), and by allowing the new straw time to weather before it is used. In principle, the better composting of old straw may be due to partial chemical degradation of the straw in the field, or due to colonization by organisms that allow more rapid composting when the straw is used. This project has confirmed that there are chemical changes in the straw during weathering, but also that there are significant changes in the microbial community with weathering. The observed effects were determined in the very early stages of composting, suggesting that if the "old straw" organisms can be isolated and cultured, then treatment of "new straw" with these organisms might be effective in accelerating the start of the composting process.

A second problem that faces all composters is the amount of process water used. Most composters recycle their process water, and use the recycled water ("goody water") primarily for straw wetting. If this water is allowed to become anaerobic there are often associated odour problems, because the high levels of sulfate present (introduced as gypsum) are readily reduced to the odiferous sulfide under anaerobic conditions. With good aeration, however, this problem is avoided (Noble *et al.*, 2009). Our results show that the dominant bacterial organism present in the aerated goody water is *Thermus thermophilum*, which is known to be active and dominant in late phase 1 and phase 2 composts. It is not yet known, however, whether the use of goody water is critical in introducing this organism at the desired levels. The goody water also contained a number of potentially pathogenic organisms, which were shown to be associated primarily with the pre-wet phase and may derive from the chicken manure, since they are closely related to known poultry pathogens. However, they do not persist past mid phase 1, and are therefore not of further concern.

Design of an inoculum to accelerate composting

As noted above, frequent changes in the dominant microbiota during pre-wet and phase 1 composting (microbial succession) mean that no single bacterial or fungal species is an obvious candidate to use as an inoculum to accelerate composting. Previous commercial products of this nature have used *Scytalidium*, which dominates the fungal community in phase 2, but these products had varied success, and have now largely fallen into disuse. There is also a widely held view in the Australian mushroom composting community that process variation between composting yards is too great for a single product to be effective at a range of locations, even within Australia. However, this has not been confirmed at the molecular level, and there is scope for detailed study to confirm this hypothesis. Discussions with Sylvan, one of the world's largest spawn producers, suggest that they are no longer actively developing an effective compost inoculum.

Our results indicate that although there is no single organism that is active and present throughout the process, and may be limiting in low-yielding composts, there is nonetheless potential to develop inocula that either (a) contain a cocktail of the dominant organisms known to be present (b) are applied at different stages of the process, and (c) are present in weathered straw but not yet available in freshly harvested straw. All of these need further research to characterize survival and activity of inocula, effectiveness in yield promotion, and uniformity of these or related organisms in multiple runs at a range of composting sites across the country. Most importantly, success of this research depends on development of a model composting system that is supported by industry, or acceptance of small-scale composting results as a model of large scale bunker-based composting.

Recommendations

Recommendations - scientific and industry

- This project has identified a range of microbes that are present at high levels in a standard composting run. Further research needs to confirm these findings in repeated runs, and at a range of different composting yards, using next generation sequencing techniques.
- Comparative sequence analysis of multiple commercial composting runs is needed, in an attempt to correlate the presence/absence of particular organisms (or consortia) with improved (or reduced yield). This may lead to the way to development of biomarkers that can be used as quality markers for composts, and reduce the hazards of poor yields.
- It is imperative that a method is developed whereby small scale composting trials can be incorporated within full scale technology. This method must be accepted by industry, or future investigations on the effect of substrate variation will also be unsuccessful.
- A small scale composting system should be developed and used for substrate supplementation experiments. However, this needs to be validated in detail against several commercial yards, in order for the results to be accepted by industry.
- Dominant bacteria identified in the composting process (*Acinetobacter, Bacillus,* specific uncultured Proteobacteria and *Thermus*), need to be isolated and characterized in vitro, as do the phase 2 *Pseudoxanthomonas, Steroidobacter* and *Chitinophaga* organisms. This will allow the development and testing of consortia that may stabilize composting quality.
- The effect of incorporating larger amounts of alternative substrates needs to be investigated, using the model composting systems developed above. These should include traditional alternative substrates (e.g. sorghum, peastraw, rice straw etc), but also new alternatives such as waste-streams from the food industry, or products derived from municipal green waste, or even municipal domestic waste. This should also include the effect of supplementing poultry manure with other N sources such as soy products, feathermeal and seed products.
- Detailed studies are required to characterize fully the effect of wheat straw weathering at a range of locations, and how this impacts the chemistry and biology of the weathered product.
- More thorough characterization of goody water microbiology is required at a range of locations, to confirm the results obtained here for a single composting yard.
- Nitrogen cycling during the cropping phase should be investigated in more detail. Since nitrogen is a key limiting nutrient in mushroom growth, understanding N transformations may allow extended cropping and improve yields.
- Explore the use of IR analysis (DRIFTS) to monitor compost composition in more detail. This will require detailed chemical work, as even the most recent techniques use proxy methods for compost components (cellulose, hemicelluloses).

Scientific Refereed Publications

Four peer-reviewed scientific publications are in preparation:

- Safianowicz, Bell and Kertesz, *Microbial communities in the mushroom composting process*
- Safianowicz, Bell and Kertesz, *The influence of new season straw in the mushroom composting process – a microbial approach*
- Safianowicz, Bell and Kertesz, *The role of goody water in the mushroom composting process*
- Safianowicz, Bell and Kertesz, *Recent advances in understanding of molecular, biochemical and physiological processes regulating growth and development of* Agaricus bisporus

Intellectual Property/Commercialisation

No commercial IP generated.

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Processes involved in mushroom production were studied at the Marsh Lawson Mushroom Research Unit at the University of Sydney, which is co-funded by the Australian Mushroom Growers Association.