A COMPLEX WEB OF LIFE: BACTERIAL-FUNGAL INTERACTIONS

In his book Entangled Life, Merlin Sheldrake imagines the soil as a "horizonless external gut – digestion and salvage everywhere, with flocks of bacteria surfing waves of electrical charge... like the Wild West with all those bandits, brigands, loners, crap shooters... and the seething intimate contact on all sides by fungal hyphae."

Getting up close and personal to the community of biota within compost reveals a hustle and bustle that could rival Tokyo central station at peak hour. Mushroom compost thrums with life and activity.

The fermented and pasteurised substrates that support mushrooms are home to countless microorganisms, interacting with each other in a series of physiological and biochemical reactions to create ideal growing conditions for the *Agaricus* mycelia.

Understanding these bacterial interactions in mushroom compost will likely underpin future developments in the industry as it searches for more sustainable sources of substrates.

University of Sydney honours student Shivagami Shamugam has been investigating the status of current research, and opportunities to exploit bacterial interactions, as part of a levy-supported research project with Dr Michael Kertesz. Her review has been accepted (with minor changes) for publication in the Journal of Applied Microbiology – a significant achievement for an honours student. The following attempts to summarise this review.

Bacterial succession planning

Optimising the composting process is a key challenge for the Australian mushroom industry. Microbiological research helps us understand bacterial interactions, the role of bacteria at each phase of composting, and bacterial responses to different materials.

Much like forested landscapes recovering after a bushfire, there is bacterial and fungal species succession that occurs within mushroom substrates as compost develops and the conditions change (Figure 1).

Figure 1. University of Sydney honours student Shivagami Shamugam has been studying bacterial – fungal interactions

Regular readers of the *Australian Mushroom Journal* would have seen Professor Micahel Kertesz' previous results demonstrating how populations of different microbial species change during maturation of Australian compost. For example, this research has identified over 30,000 different microbes at the end of Phase 1, all interacting with each other, the mushroom mycelia, and other fungi.

The processes in Phase I composting are biochemically complex, but relatively well understood. As compost temperature rises, heat-loving bacteria are favoured, resulting in high populations of *Firmicutes*, *Proteobacteria*, and other species.

During Phase II, temperatures transition from 58-60°C, to 48-51°C and finally around 25°C before introduction of mushroom spawn. This is when the heat-loving fungus *Mycothermus thermophilus* (previously *Scytalidium thermophilum*), comes into its own, combining with several bacterial species to break down cellulose and hemicellulose in the compost and absorb the excess ammonia otherwise toxic to *Agaricus* mycelium.

By the end of Phase III, *Agaricus* is the dominant species, having absorbed most of the *M. thermophilus*, as well as its bacterial companions.

Nomad Pseudomonads

As *Agaricus* continues to develop, various bacteria attach to the growing mycelia. Perhaps most important are the 'good' bacteria *Pseudomonas putida* – which helps initiate mushroom formation – and the 'bad' bacteria *Pseudomonas tolaasii* – cause of brown blotch.

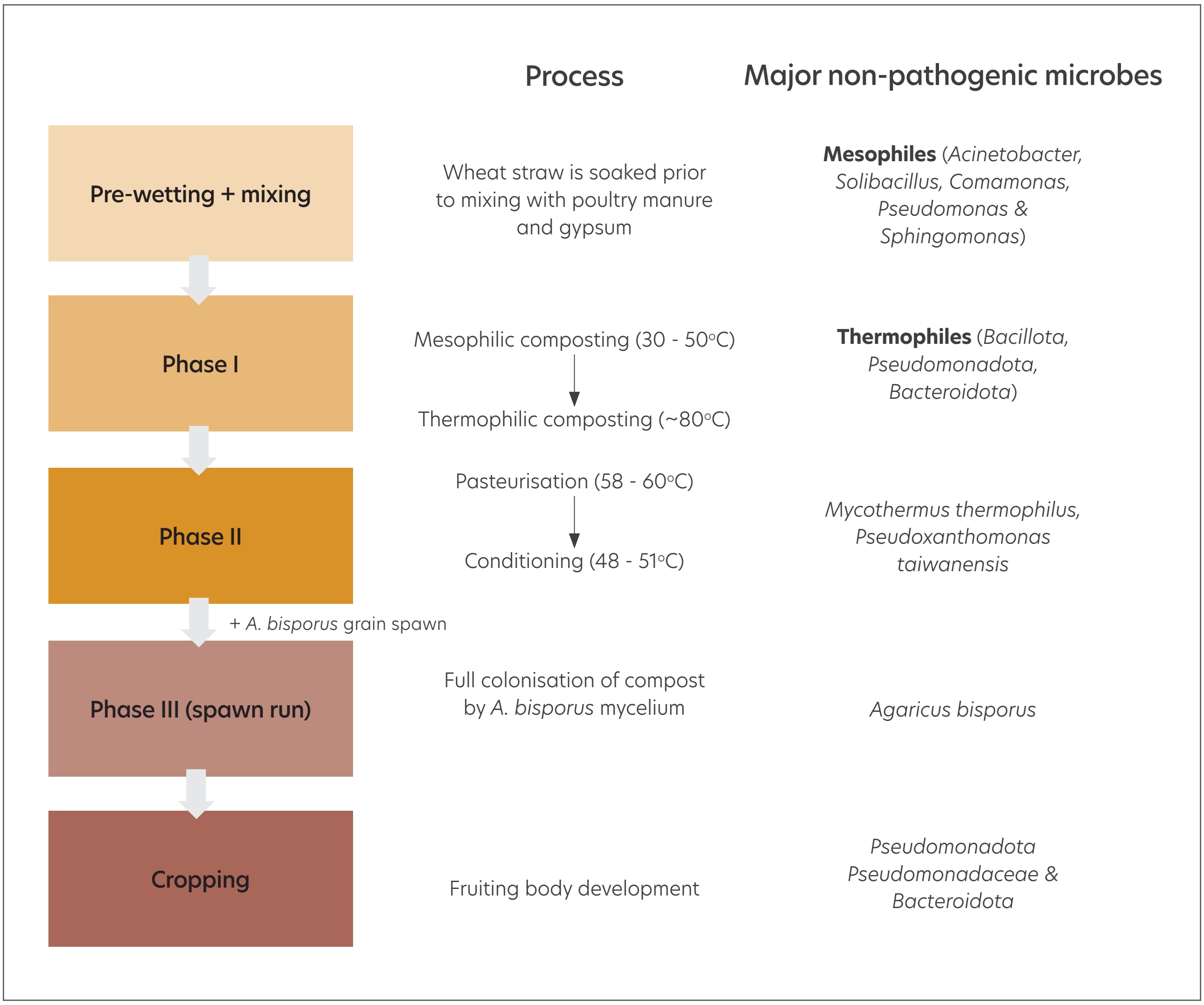

Figure 1. Shamugam and Kertesz

Nutrient-poor conditions, such as occur in the casing, stimulate *Pseudomonas putida* to respond more quickly to attractants produced by the *Agaricus* mycelia. There it 'feeds' on ethylene as well as various 8-carbon compounds (VOCs), particularly the inhibitor 1-octen-3-ol. Removing these 'blockers' is what allows the mycelia to pin and form mushrooms.

Many strains of *P. putida* also secrete organic acids, increasing availability of microelements for *Agaricus*. Some also secrete cellulase; although *Agaricus* can degrade celluloses itself, it seems possible that this makes it easier for the hyphae to absorb carbon from these materials.

However, the situation is clearly complex, as *P. putida* cannot **always** be detected in either compost or casing, and inoculating additional *P. putida* has not been demonstrated to increase yield. Nevertheless, identifying *P. putida* strains which respond more quickly to nutrient gradients and VOCs could potentially increase the efficiency of this bacteria's important effects.

Bacteria, nutrient uptake, and the promotion of growth

How fungi take up nutrients and use bacteria as a nutrient source is as complex as it is ingenious. Mushrooms are opportunists, developing a number of mechanisms to take advantage of the carbon and nitrogen available in their vast bacterial pantry.

Three such interactions are illustrated in Figure 2.

System A, known as bacteriolytic enzyme activity, works a little like our own digestion, in that the mycelia produce and releases enzymes (key enzymes are muramidase and NAG) to break down bacteria in the compost into components that are readily absorbed by the fungal hyphae.

In **System B**, simple sugars and other compounds produced by the mycelia attract bacteria to the environment around the mushroom mycelium, where they attach directly onto the hyphal surface. Some may then be broken down as food, whereas others (e.g. *P. putida*) provide benefits such as absorbing VOCs.

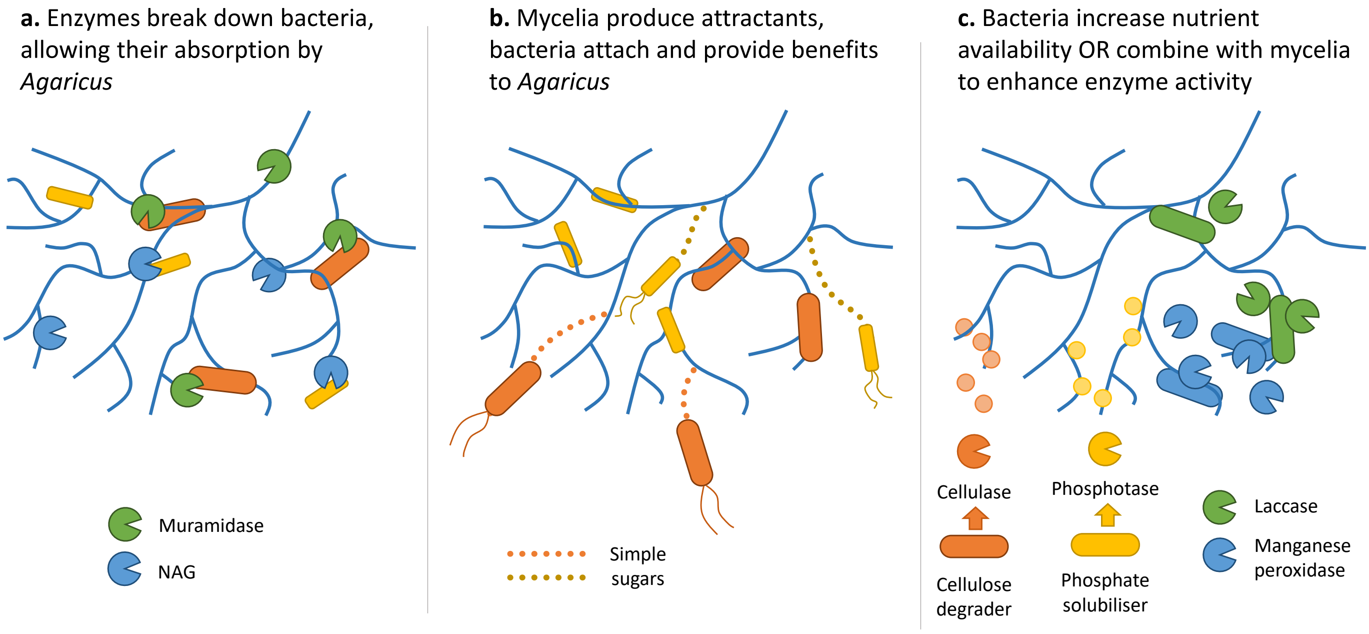

Figure 2. Three systems of interaction between bacteria and Agaricus mycelium. A. Bacteriolytic enzyme production (muramidase and NAG) breaks down bacteria into forms that can be absorbed directly B. Bacteria are attracted by hyphal exudates and attach, where they may provide benefits such as breakdown of ethylene and C8 compounds C. Bacteria produce enzymes that break down cellulose or phosphates into forms readily absorbed by the mycelia OR combine with the mycelia to enhance enzyme activity, increasing absorption from the substrate.

In **System C**, bacteria work for the mushroom! Bacteria release enzymes such as cellulase and phosphatase, which break down nutrients in the substrate (cellolose, hemicellulose, phosphate) into forms readily absorbed by the hyphae. Alternatively, attached bacteria combine with the mushroom mycelia, enhancing production of enzymes such as manganese peroxidase and laccase, thereby improving uptake from the substrate.

For those looking for further reading, a review that includes the role of bacteria in compost production is available on the MushroomLink website https://www.mushroomlink.com.au/resources-1/review-pre-and-postharvest-management-of-mushrooms.

Deep dive: Like Dr Michael Mosley selecting from a buffet, mushrooms release different enzymes to break down bacteria according to need.

- Researchers believe that *Agaricus bisporus* can sense differences in substrate and secrete different concentrations of extracellular enzymes to balance its carbon and nitrogen requirements
- Bacterial biomass represents a major nutrient source for mycelial growth of *Agaricus*
- *A. bisporus* can degrade both living and dead bacteria cells by secreting enzymes that degrade the bacterial cell walls, releasing essential nutrients. This may be why *A. bisporus* can outcompete other fungi, which rely on the availability of free nutrients within the compost
- Carbon and nitrogen can be absorbed very efficiently from bacteria by the Agaricus mycelium due to two key enzymes: *β*-N-acetylmuramidase (muramidase) and *N*-acetyl-*β*-D-glucosaminidase (NAG)
- The presence of bacteria stimulates production of muramidase, especially if carbon sources such as glucose and fructose are not available
- Modifying the substrate to increase the biomass of bacteria readily degraded by *Agaricus* could potentially increase growth, especially during spawn run
- However, interactions in compost are complex, and the mechanisms by which *A. bisporus* breaks down some bacterial taxa more than others are uncertain

Bacterial interactions with the mycelium of the cultivated edible mushrooms Agaricus bisporus and Pleurotus ostreatus. Shivagami Shamugam and Michael A. Kertesz*The University of Sydney, School of Life and Environmental Sciences, Faculty of Science, Sydney, NSW 2006, Australia

Pre and post harvest management of mushrooms Jenny Ekman, Applied Horticultural Research.

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