FRONTIERS IN INDUSTRIAL MYCOLOGY

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Preface

Fungi have often been viewed during past centuries as pathogenic or damaging organisms that killed plants and rotted away their fallen litter. Driven by images of decaying plants and fear of poisonous and hallucinogenic mushrooms, early folklore abounded with misconceptions about the nature and value of fungi. The "dark ages of mycology" fell away as mankind began to realize the positive attributes of many fungi. During this century, a firm appreciation of fungi developed as we began to understand the important ecological roles of saprophytic fungi in recycling plant biomass nutrients and mycorrhizal fungi in nourishing and protecting tree roots. Our realization of the benefits of fungi broadened as we increased consumption of them as food, established the widespread use of fungal enzymes in food manufacture, and discovered a few of the important antibiotics produced by them. During the last two decades, positive expectations developed for harnessing fungi for expanded use in both established and wholly new applications. *Frontiers in Industrial Mycology* describes the current efforts underway to create a broad range of exciting large-scale applications using filamentous fungi.

The first important use of fungi by mankind was probably as a food crop harvested as the large edible mushrooms produced by higher filamentous fungi. Widespread domestication of various mushrooms was slow compared to that for higher plants. This was primarily due to technical reasons resulting from a lack of basic information. Over 300 years passed during the discovery, development, and refinement of the highly efficient methods now typically used to convert composted horse or poultry manure and straw into the commonly cultivated white mushroom (*Agaricus bisporus*). Methods are now being developed to cultivate efficiently new desirable mushrooms with bold memorable flavors and large market potential. The status of the current methods to produce shiitake (*Lentinula edodes*) and morels (*Morchella* sp.) from underutilized wood particles and low economic value grain are outlined in Chapter 1.

In many areas of the world, fungal fermentations have been an important method of upgrading plant protein and oilseed crops. The fermentations can improve flavor or palatability, increase digestibility or nutrient content, reduce processing energy, or increase shelf life. Relocation of ethnic groups and increased importation of their unique foods have markedly expanded the use of traditional fermented foods. Foods previously unknown in North America and Europe, such as soya sauce (fermented by *Aspergillus* species and yeasts from koji starter cultures), are now common household products. Historically, when a food has been introduced and becomes popular within a new region, the local inhabitants typically develop ways to produce it and often create new varieties. For fermented foods, this requires mastering the use of the microorganisms involved. Throughout the Eastern hemisphere, ragi-type starter cultures are commonly used to carry out a variety of different fermentations. The species of mucoraceous fungi present in ragi-type starters are described in Chapter 2.

During World War II, the timely discovery and successful use of penicillin produced by *Penicillium* species provided an early dramatic model guiding the production of new antibiotics for the health care profession. Today, penicillin analogs and related sulfur-containing beta-lactam antibiotics (*e.g.*, cephalosporins), produced either by fungi or bacteria, continue to be important for curing an impressive range of bacterial infections. Methods to increase yields or develop new analogs, including collection of new wild strains of targeted commercial species, mutagenesis, and media optimization, have been successfully exploited for decades. The benefits to be gained from these methods alone are dwindling however; further yield increases are proving minimal. The use of modern recombinant DNA technology with filamentous fungi to directly increase yields and produce new analogs is described in Chapter 3.

There have been literally thousands of antibiotics isolated since the discovery of penicillin, the majority of which are from actinomycetes (filamentous bacteria). Most of these are antibacterial. Unfortunately, only a handful of the known antifungals are sufficiently non-toxic to animals to be considered for widespread use with humans. In the search for new desirable antifungals, many organizations have intensified their screening of fungi. The promise of developing new antifungals by chemically modifying compounds produced by *Aspergillus* species is described in Chapter 4.

Since the advent of modern recombinant DNA technology, there has been extensive commercial interest in expressing proteins in many different microbial hosts. Expressing foreign proteins in filamentous fungi shows much promise. Fungi can be grown on low cost media, and their hyphae are inexpensively harvested or removed. And fungi exhibit remarkable permissiveness for expressing foreign genes. Heterologous expression in filamentous fungi is reviewed and key areas requiring further study are outlined in Chapter 5.

Recently, widespread interest has developed for better maintaining and significantly expanding forest timber stands. This is due primarily to our increasing need for fiber and wood products, to the desire to use wood as a renewable biomass source for chemical and fuel production, and to the hypothesis that the extent of global warming can be minimized by using large-scale plant growth to remove carbon dioxide from Earth's atmosphere. The economics driving successful (re)forestation programs are highly dependent on the survival and vigor of the young fragile trees that we plant. The dramatic increases in survival and productivity that can result from the inoculation of trees with certain ectomycorrhizal fungi are shown in Chapter 6. Especially encouraging is the marked benefit to trees planted in otherwise marginal soils.

The pulp and paper industry is a tremendously large-scale forest products industry having marked environmental impact. The industry currently uses certain chemical wood pulping and pulp bleaching processes that generate undesirable or potentially hazardous effluents. For the last two decades, researchers have speculated that lignin-degrading fungi, which exhibit pulping and bleaching action on wood in nature, could be used to develop less polluting biopulping and biobleaching processes. Research underway to develop a commercial-scale biopulping process using white-rot fungi with wood chips is discussed in Chapter 7. Efforts to develop a biobleaching process using fungal enzymes with conventional chemical pulp, which is capable of generating less chlorinated aromatic compounds, are described in Chapter 8.

Many industries involved in wood preservation, in manufacturing or processing, and in chemical production, and farms and plantations using certain pesticides, have contributed to the widespread release of hazardous toxins into the environment. Aromatic chlorinated compounds, such as the now infamous PCB's (polychlorinated biphenyls), are proving to be particularly troublesome, due to their high toxicity and difficulty in being degraded by microbes in the environment. Certain lignin-degrading fungi are well known for their relatively non-specific ability to attack complex aromatic compounds. The potential use of white-rot fungi to degrade the wood preservative pentachlorophenol and other chlorinated aromatic contaminants present in either soil or liquid effluents is discussed in Chapter 9.

Over the last two or three decades man has attempted to help protect the environment by developing less toxic products to combat troublesome insects, pathogens, and weeds that plague our crops and forests. Insecticides comprised of pathogenic bacteria, viruses, or protozoa have been on the market for several years. Unfortunately, many sucking or burrowing insects ingest so little material from plant surfaces that current bioinsecticides fail to kill them. And many soil-inhabiting insects remain protected from economically feasible aerial sprays. In contrast to the above, fungal pathogens are generally effective without ingestion. They possess hyphae that directly penetrate insect cuticles. Many also appear capable of colonizing or persisting in soil for periods sufficient to be effective against terrestrial insects. The extensive range of entomopathogenic fungi potentially effective for bioinsecticides and their use are outlined in Chapter 10.

Although fungal spores (e.g. conidia) are the natural and perhaps most effective form in which to apply fungal biocontrol agents, many organizations have had difficulty in producing sufficient concentrations of spores to be economically viable. This has slowed progress in the field and forced researchers to develop inoculants based on more fragile vegetative hyphae. Several different methods for producing conidia of entomopathogenic fungi are discussed in Chapter 11. These include a new and simple solid-substrate fermentation method that produces abundant conidia of several important fungal species. The method is illustrated in detail with *Beauveria bassiana,* which is active against potato beetle, grasshoppers, Gypsy moth, and certain other troublesome insect pests.

Seed rots, damping off, wilts, fruit rots, and root rots are very damaging costly agricultural problems caused by fungi. Traditionally these have been combated by the application of chemical fungicides. That certain filamentous fungi characteristically attack other fungi in nature, however, suggests that effective fungal-based fungicides might be possible. Advances in developing fungicides, using nonplant-pathogenic *Trichoderma* species highly competitive against other fungi, are discussed in Chapter 12.

Throughout history, fungal pathogens, often inadvertently introduced by humans into new areas, have exhibited devastating effects on certain plants. This is most frequently remembered in its darkest light, when foods crops or flora essential for the welfare of the local inhabitants were wiped out. Due to the often specific nature of fungal pathogens, however, there is the possibility of intentionally using fungi to selectively remove undesirable plants. Strategies involved in developing bioherbicides, and the current status, and recent advances in the field are described in Chapter 13. Specific examples are given showing the astounding ability of fungal bioherbicides to remove some particularly troublesome weeds inadvertently introduced into the Hawaiian islands.

The chapters mentioned above give but a brief glimpse of some important environmentally sound applications currently being developed based on the use of filamentous fungi. The research and process developing efforts underway to harness these organisms are exciting and challenging. Realization of each application will require the cooperation of a wide range of different professionals from specialized areas of science, engineering, and business. In spite of the challenges involved, our marked recent advances suggest that a rewarding future lies ahead on the *Frontiers of Industrial Mycology*.

Acknowledgments

Frontiers in Industrial Mycology represents the goals and aspirations of the many dedicated researchers here who contributed their excellent research, writing, and timely peer reviews. Their efforts in developing important applications serve as an excellent example of how science can be used to benefit mankind and protect the environment.

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1

Production of Specialty Mushrooms in North America: Shiitake and Morels

Thomas J. Leonard and Thomas J. Volk

There is increasing interest in the American marketplace for mushrooms other than the common white button mushroom. The trend is toward species with more flavor. Among the new mushrooms making common appearances are the oyster mushroom and shiitake, more formally known as *Pleurotus spp*. and *Lentinula* (=Lentinus) *edodes*, respectively. A third type of mushroom, although less common, is the morel, *Morchella spp*, which is just beginning to be developed commercially. Since morels and shiitake are the more flavorful of the three mushrooms and are more difficult to produce, we focus our discussion on commercial cultivation practices for these two mushrooms and the challenges ahead for making them more readily available.

General Features of Shiitake Cultivation

An attractive alternative to producing shiitake mushrooms on hardwood logs is the "artificial log procedure," which involves inoculating a sterilized or pasteurized supplemented sawdust mixture in a polypropylene bag with shiitake spawn. The shiitake mycelium colonizes the sawdust mixture relatively rapidly, thanks to the abundance of air spaces and the uniform distribution of nutrients. Within a 2-month period the loose medium coheres into a synthetic log. When the plastic is removed and the artificial logs are placed in climate-controlled production rooms, the first mushrooms usually develop after only 2–3 weeks; subsequent flushes may be completed within 6 months. By contrast, the natural log cultivation cycle usually takes about 1 or 2 years from inoculation to the first mushroom flush and up to 7 years for completion of the subsequent flushes.

There is a significant difference in the biological efficiency (BE) of the natural and artificial log methods. BE is the percent fresh weight of mushrooms produced from a given dry weight of logs or supplemented sawdust mixture; it is an indication of the efficiency underlying the bioconversion process that transforms wood and supplements into mushrooms. For the natural log method, the maximum BE can reach as high as 33%, but it usually averages less than 20%. The BE of an artificial log, however, may range from 50% to 145% (San Antonio, 1981; Leatham, 1982; Royse et al., 1985). (Biological efficiency can exceed 100% because it is based on the wet weight of mushrooms, which can contain 85% to 90% water.)

Supplemented sawdust, therefore, enjoys important advantages over the natural log method with respect to both time and efficiency of production: it is completed in one-tenth the time and with at least double the BE of the natural log methods. Artificial log cultures can also be grown under controlled conditions year round and are handled more easily. Nevertheless, this method is not without its drawbacks. It is labor intensive and entails many stages, each of which requires a special set of often ill-defined growth conditions, each depending on the strain being employed. Consequently, it is easy to mishandle some of the stages unwittingly and thereby to affect adversely the yield and/or quality of the mushrooms produced. The relatively thin binding surface or skin of the artificial logs makes them more vulnerable to both microbial and insect pests and damage during handling.

The flavor of fresh and dried shiitake mushrooms differs distinctly even when mushrooms are from the same strain, but there is little, if any, flavor difference in fresh mushrooms produced from sawdust and natural logs when growth proceeds under the same environmental conditions. Mushrooms grown outdoors on natural logs, however, generally exhibit higher dry weight because of the increased surface evaporation from the developing mushrooms. The nutritional value of mushrooms produced on supplemental sawdust is higher owing to both the inherently richer substrate and the fact that mushrooms act as nutrient sinks translocating nutrients from their substrates up into mushroom tissues (Thrower and Thrower, 1968).

What is presently needed in the shiitake industry is to render the sawdust cultivation procedure more rational and controllable by identifying the important factors in each stage of cultivation that affect yield and quality. The following analysis of the artificial log procedure includes an introduction and general description of the stages of shiitake development as it occurs using the plastic bag method. We also discuss problems with the method and outline possible programs of research and development that would make cultivation more "user-friendly."

The plastic bag approach to cultivating shiitake was developed independently in Japan, China, and Taiwan about 20 years ago (Ando, 1974; Han *et al.*, 1981). A clear and concise description of what can be taken as the standard procedure was recently reported by Miller and Jong (1987). Each of the developmental stages leading to mature shiitake mushrooms can serve as a subject for experimentation in order to perfect the sawdust cultivation procedure. Recently there have been several interesting modifications of the basic procedure that do much to reduce time and effort. A discussion of some of the more innovative procedural changes follows.

Brief Description of the Sawdust Cultivation Procedure with Plastic Bags

Preparation of Spawn

Selected strains of *Lentinula edodes* are grown on a potato dextrose or malt agar medium in petri dishes (and subsequently transferred to sterile rye in plastic bags provided with aeration filters.) The grain has been boiled for 20 minutes to soften the seed coats slightly and to hydrate the grain, allowing more rapid penetration by the mycelium. The bags are incubated at room temperature (22°C) and are briefly shaken several times over the 2-week incubation period to prevent clumping of the mycelium. Some spawn growers use the same substrate recipe for growth of the spawn and for the spawn run or incubation phase, which makes sense physiologically, since the mycelium of the spawn has already become adapted to producing all of the wood-digesting enzymes necessary for immediate growth.

Spawn-Run Phase

Vigorously growing spawn is added to sterile nutritionally-supplemented sawdust in plastic bags provided with a filter for gas exchange. During this time the mycelium grows through the sawdust substrate and releases a battery of lignocellulolytic enzymes. The extracellular enzymes degrade the wood into smaller more soluble molecules that can be readily absorbed by the hyphae, thereby providing the mycelium with stable nutrients to support growth (Leatham, 1985). The incubation temperature at this stage is generally 25°C. By the end of the spawn run, generally 60–120 days after inoculation, depending on the strain and substrate formula, the sawdust log is fully colonized and has developed a fairly thick mycelial skin around the culture surface. It is also during this phase that the mycelium stores essential nutrients in quantities sufficient to support subsequent mushroom formation.

Early Fruiting Phase

Nodules of various sizes and shapes may develop on the thick mycelial skin surface in plastic bag cultures of many shiitake strains. The nodules are compacted mycelium generally assumed to contain potential mushroom primordia (Chang and Miles, 1989). Although most nodules abort, presumably owing to competition for nutrients (Madelin, 1956), the tissues within some nodules go on to differentiate and develop viable primordia. The timing and extent of nodule formation, for the most part, depend on the genotype of the strain and the environmental parameters of temperature and light (unpublished observations).