PROPAGATING MUSHROOMS

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The major world producers of mushrooms are France, U.S.A., China, Holland, United Kingdom, Taiwan, Italy, and South Korea. The world annual production of cultivated *Agaricus bisporus* exceeds one million tonnes.

In Australia, mushroom cultivation began about 1930 when Dr. Noble, then Chief Biologist of the N.S.W. Department of Agriculture, was able to establish mushroom growing in outdoor ridge beds in the County of Cumberland. Production became established in disused railway tunnels at Wynyard, Helensburgh, Lithgow, Bowral, and Glenbrook. Nowadays mushrooms are grown in specially insulated buildings where temperature, humidity, and CO_2 are carefully controlled. The Australian industry at the present time has about 80 growers producing 15 thousand tonnes of mushrooms per annum with a gross value of A$50 million.

Mushroom Spawn. "Spawn" is the name given to the mycelium of the mushroom. Spores are produced from special structures called basidia on the gills of the mushroom. These spores, when germinated, give rise to the mycelium. Until 1900 spawn for cultivation of mushrooms was obtained from fields or horse manure stables where mushrooms had been produced. Since 1900 a sophisticated and technical spawn making industry has developed. The original commercial spawn was made by sterilizing horse manure and adding a suspension of mushroom spores. The spores would germinate and colonize the manure. In 1932 Dr. James Sinden of Pennsylvania State University was able to produce spawn on sterilized cereal grains. Grain spawn is now the major source of mushroom spawn worldwide. Its advantages over manure spawn are its ease of handling, production, and its reliability.

Spawn Preparation. Spawn is made by growing mushroom mycelium on sterilized cereal grains. Wheat, rye, sorghum, and millet are the most commonly used grains. The grain is boiled in water to obtain a moisture content of 45 to 50% by weight. Excess water is drained off and the grains mixed with gypsum and limestone. Gypsum prevents the grains sticking together and results in a product that is easy to handle. Lime is added to adjust the pH of the grain to around 6.5. It is important not to overcook the grain allowing it to split as this results in fluffy growths of mycelium called sectors which are undesirable in mushroom spawn. The cooked grain is filled into containers, either autoclavable polypropylene bags and bottles, or glass jars, and sterilized for 1½ to 2 hours at 121°C. The extended autoclaving time is necessary to kill

"flat sour" bacteria that are present naturally on grain.

When the grain is cooled after sterilizing it is inoculated with a pure culture of inoculum, shaken, and incubated at 25°C. Colonization takes 10 to 14 days.

Spawns And Their Characteristics. Present day spawns, unlike varieties of green plants, are known by code numbers rather than by names e.g. U1, X1, B92, S53. There are 4 types of spawns that are classified on the basis of the colour of the mushroom cap and on the tendency of the cap to produce scales. The four types are:—

1. Smooth White Strains
2. Rough White or Off White Strains
3. Cream Strains
4. Brown Strains

The demand for use of each type of strain varies from country to country and whether the mushrooms are destined for fresh or canned sales. Environment plays an important part in the growing of strains, e.g. a rough white strain will be similar to a smooth white strain under conditions of high humidity and low air movement. A brown strain will be cream in colour under conditions of low humidity. Off white strains are the major strain type grown throughout the world. They have good keeping quality and lower costs of picking than white strains. They are grown on farms infected with mushroom virus disease because of the widely held view that virus is not transmitted from smooth white to off white strains.

Maintenance of Mushroom Strains. Various techniques are used to maintain mushroom strains. The simplest way to maintain strains is to subculture the mycelium onto a suitable agar medium. Lambert (10) stated that strains can be maintained in this way for many years and have a fairly good chance of keeping their vigor. Other workers (3, 4, 8) have confirmed this. It is important to monitor the type of mycelium that is produced and eliminate any culture whose growth appears abnormal. One of the abnormalities is a slow appressed mycelial growth, another is a fluffy mycelium that produces sectors on agar and stroma on the cropping beds. Lambert (10) anticipated that spawn grown on grain over a long period might change into the fluffy type. Mycelium stored on an agar medium e.g. Malt Agar, Potato Dextrose Agar, Compost Agar, needs to be subcultured occasionally to prevent drying out.

The storage of mycelium by immersion in liquid nitrogen (−196°C) has been shown to be an ideal method for storage of *Agaricus bisporus* whose mycelia do not produce asexual spores. Liquid nitrogen was first used by San Antonio and Hwang (13) to store mushroom cultures. Mushroom strains have now been stored for up to 10 years without any change in their character (2, 14).

Obtaining New Strains of *Agaricus bisporus*. Until 1972 when a proper understanding of the breeding system of *A. bisporus*

became known, spawn makers obtained new cultures through selection of mushroom sporophores. Multispore cultures of *A. bisporus* are known to have differences in yield, growth rate and other characteristics. (4, 10, 15). The start of the pure white mushroom in commercial cultivation began when in 1927 a clump of pure white mushrooms were found in beds that had been spawned with a cream strain (7). These white fruit bodies were propagated by multispore culture. Spores are collected by standing a mushroom with a stretched veil on a sterile petri dish or filter paper. The mushroom matures and drops spores onto the dish or filter paper. The spores are then germinated on agar media producing hyphae that fuse to form the multisporous culture.

Cultures derived from single mushroom spores show much greater variations than multispore cultures. Lambert (9), noticed that monosporous cultures of the cultivated mushroom are usually fertile but rather variable. This variability in different properties such as the appearance of the mycelium on agar, growth rate, shape of the fruiting bodies, and productivity makes it possible to develop new strains of *A. bisporus* by the isolation and selection of monosporous cultures. (5, 6, 8, 10). Single spore cultures are obtained by dilution of a suspension of spores similar to the classical technique for obtaining single spores of bacteria.

In 1972 the breeding cycle of *A. bisporus* was elaborated by Raper and Raper (12), Miller and Kananen (11), and Elliot (1). *A. bisporus* was described as a secondarily homothallic basidiomycete with a bipolar system of sexuality. This knowledge meant that interstrain breeding was possible. *A. bisporus* differs from other Basidiomycetes in that there are two spores on each basidium and when two nuclei of the different sexual factors occur in the one spore then this monospore is fertile. For breeding of *A. bisporus* it was necessary to work with cultures from basidia, which as an exception bear four spores instead of two and consequently have only one nucleus each. Obtaining much monosporous cultures is however a long and difficult process. In 1981 Dr. Fritsche of the Dutch Mushroom Experimental Station introduced the first hybrids produced by this process. The hybrids called U1 and U3 have since enjoyed spectacular success throughout the world. These new hybrids combined the desirable qualities of the off white and pure white spawn types. The pure white strains have the advantage of a smooth white cap but tend to lack size and weight; the off white strains, by contrast, produce mushrooms of a better weight and therefore less cost to pick. These new hybrids produced higher yields, lowered picking costs, and had better shelf life.

The development of successful protoplasting methods in *Agaricus* have taken some time but they now offer the prospect of producing inter-species hybrids. There is clearly potential for the use of the new recombinant DNA technology from other fields to

produce new mushroom strains with desirable characteristics.

The mushroom industry today, as in the past, depends for its success and continued survival on the ready availability of good quality spawn.

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