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CULTIVATION OF OYSTER MUSHROOMS

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Oyster mushrooms are cosmopolitan, and belong to the genus *Pleurotus* (Fungi: Basidiomycetes). Their cap is normally shell-like (about 5-20 cm in diameter; 1.9-7.8 inches), fleshy, with eccentric or lateral stipe; and their color can be white, cream, yellow, pink, brownish, or dark gray. As primary decomposers having the ability to degrade lignocellulose, oyster mushrooms (*Pleurotus* spp.) are found growing in the wild on dead organic matter from tropical and temperate regions. Several species are also capable of acting as parasites of living trees, and attacking nematodes or bacterial colonies.

Empirical cultivation of *Pleurotus* started around 1917 in Germany, using natural spawn for inoculation of wood logs and stumps. The first large-scale cultivation on logs was achieved in Hungary in 1969. Later, a variety of lignocellulosic by-products from agriculture or forestry were also found to be good growing substrates, and several species were brought into cultivation throughout the world, such as the tree oyster (*P. ostreatus*), the gray oyster mushroom (*P. sajor-caju*), the abalone mushroom (*P. cystidiosus*), the white oyster mushroom (*P. florida* nomen nudum), the golden oyster mushroom (*P. citrinopileatus*), the pink oyster mushroom (*P. flabellatus*), and the black oyster mushroom (*P. sapidus*). At present, *Pleurotus* spp. is the second most important cultivated mushroom in terms of world production.

Taxonomy. The extraordinary genetic diversity of oyster mushrooms, involving adaptation to a broad range of environmental conditions and substrates, has placed taxonomists in a demanding situation as clear delimitation of *Pleurotus* species is difficult. Conventional methods (fruit-body morphology, microscopic observations, mating studies between populations, biochemical analyses) have not provided clear-cut results. Thorough molecular studies have been shown to be more informative: intra- and interspecific heterogeneity was determined using ribosomal and mitochondrial DNA analyses, and phylogenetic studies of ribosomal DNA sequences indicated geographic speciation in several groups. However, a present research hurdle is to have isolates that represent authentic indigenous populations, because many commercial strains, which are now widely distributed throughout the world, may have already escaped from cultivation. In general, the taxonomy and systematics of *Pleurotus* species is still far

from being solved, and requires not only the identification of effective classical and molecular characters, but also a basic consensus on speciation processes and species concepts within the genus.

Breeding potential. *Pleurotus* mushrooms show the typical life cycle of Basidiomycetes, a major fungal group (**Fig. 1**). It begins with the germination of a basidiospore in a suitable substrate, which gives rise to a monokaryotic mycelium containing genetically identical nuclei (n) and capable of indefinite independent growth. When two compatible monokaryotic mycelia are in close contact, they are able to establish a fertile dikaryon by hyphal fusion or plasmogamy. This dikaryon (n+n), having clamp connexions and binucleate in each hyphal compartment, contains two genetically different nuclei (one from each monokaryon) throughout the mycelium. When environmental conditions are appropriate (temperature, light, relative humidity), the dikaryotic mycelium will differentiate into fruit bodies having specialized structures called basidia. In these club-shaped, binucleate cells, which are formed in the lamellae (hymenium) of each fruit body, karyogamy

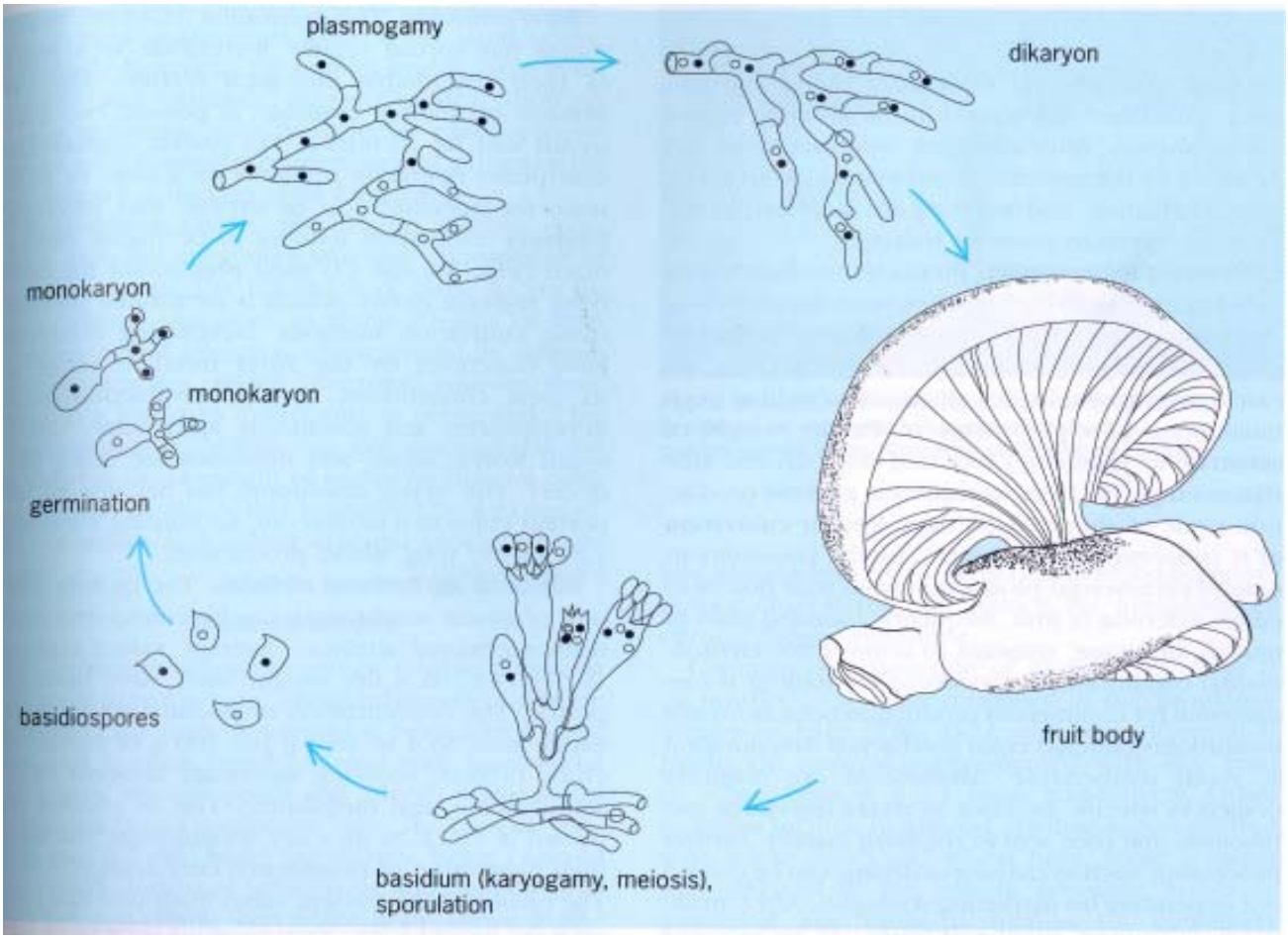

Fig. 1. Life cycle of the oyster mushroom *Pleurotus ostreatus*.

(fusion of the paired nuclei; 2n) and meiosis (recombination and segregation) take place. The four resulting haploid nuclei move to the sterigmata on the basidium, to form four new basidiospores. When the fruit bodies are mature, basidiospores are discharged, starting the sexual life cycle again. A few species have also an asexual cycle through the production of small structures (1-10 mm height; 0.03-0.39 inches) called synnemata, such as *P. cystidiosus*.

The pattern of sexuality of oyster mushrooms studied has been described as bifactorial heterothallism, in which two multiallelic mating-type factors (called *A* and *B*) act synchronously to control mating between monokaryons to produce a fertile dikaryon, in the absence of morphological differentiation. This pattern has advantages in breeding programmes, as compatible matings having different *A* and *B* factors are reliably recognized by the formation of clamp connexions in the dikaryotic mycelium. Oyster mushrooms are also easily handled in the laboratory on culture media, spore germination is close to 100%, and mutants can be obtained by direct mutagenic treatment (*e.g.*, X rays and UV radiation) of basidiospores or by hyphal fragments. These features allowed to obtain suitable combinations of selected characters through simple mating techniques. However, recombinant DNA technology is increasingly important in breeding oyster mushrooms, providing outstanding information and powerful tools at the molecular level. Most studies are concentrated in *P. ostreatus*, *P. cornucopiae*, and *P. sajor-caju*. The nuclear and mitochondrial DNA has been isolated and characterized. Uniparental inheritance and recombination of mitochondrial DNA has been shown to occur. Pulsed field gel electrophoresis revealed a chromosome number of 6-10, a genomic size of 20.8- >39.5 megabases, and a chromosome size of 1.1- >6 megabases. Reliable genetic markers were recently developed, such as enzyme markers and DNA restriction fragment length polymorphisms (RFLPs). DNA transformation has already been reported for *P. ostreatus*, and a molecular analysis to study the role and properties of important enzymes in the life cycle of oyster mushrooms is being carried out. A systematic combination of classical and molecular genetics will permit to deal with the main breeding challenges of oyster mushrooms: 1) Sporeless strains and/or strains with reduced sporulation, 2) Tolerance to elevated temperatures, and 3) Genetic resistance to competitor moulds, especially *Trichoderma*. Further genetic manipulation may be focused on increasing the nutritional value and post-harvest quality of fruit bodies, and increasing degradation efficiency of extracellular enzymes.

Spawn preparation. Commercial strains of oyster mushrooms with a range of fruiting temperatures (15°-30°C; 59°-86°F) are available. Modern methods of spawn preparation use cereal grains (*e.g.*, wheat, millet, rye), which are sterilized in glass jars or polypropylene plastic bags, inoculated with a

selected strain, and incubated at appropriate temperatures for complete colonization. An alternative small-scale method uses serial dilutions of basidiospores from a spore print to prepare grain spawn. Other organic raw materials (*e.g.*, straw, coffee pulp, cotton waste, sawdust), alone or combined in different mixtures, are also used to make spawn.

Substrate preparation. After homogenizing particle size, adjusting water content (about 70%) and pH (5-6), many substrates have been shown to be suitable for cultivation (*e.g.*, straw, coffee pulp, cotton waste, wood shavings, banana pseudostem, cotton seed hulls, waste paper, diverse plant leaves, cardamom pulp, sawdust mixtures, corn-cobs, tequila bagasse, pulp mill sludges, cocoa shell waste, and *Cassia* by-products). The direct use of some of these substrates, *i.e.* without any further treatment, for rustic cultivation in the field was reported from China. However, several methods have been developed to make substrates more suitable for growing oyster mushrooms on a large or small scale: 1) Sterilization, substrates are autoclaved at 100°-121°C (212° -250°F) for 1-2 h; 2) Pasteurization, substrates are placed in an appropriate room or tunnel and pasteurized with steam at 60°-100°C (140°-212°F) for 6-24 h, or immersed in hot water at 70°-90°C (158°-194°F) for 1-2 h; 3) Aerobic fermentation, substrates are aerobically fermented for a few days (2-6), and then pasteurized with steam at 60°-82°C (140°-180°F) for 12-24 h; 4) Semi-anaerobic fermentation, substrates are immersed in water (7-10 days) for inducing lactic acid fermentation; and 5) Xerothermic process, dry substrates are treated with steam at 100°C (212°F) for 1 h in a small tunnel, and then cool water is added. Supplementation may be carried out before treatment to increase nutrient content and yields.

Production systems. Prepared substrates are homogeneously inoculated with the spawn, either by hand or mechanically, at the rate of 0.5-3% of fresh substrate weight. The spawned substrate is placed in a variety of containers. In the tunnel process, the substrate is colonized in bulk under controlled conditions, and then transferred to smaller containers. The use of plastic bags of different sizes containing 7-30 kg (15-66 pounds) of spawned substrate is a common practice. Horizontal trays, shelves, vertical plastic sacks, and pressed rectangular blocks are also used. Containers are placed inside growing rooms for incubation. After complete colonization of the substrate by the mushroom mycelium (15-40 days), light, ventilation and watering are increased in the growing rooms to promote fruiting.

Harvesting and processing. Production of fruit bodies varies according to each species, spawn quality, substrate quality, environmental conditions (temperature, light, relative humidity, concentration of O_2/CO_2), and impact of pests (flies, mites) and diseases (fungi, bacteria, viruses). Average biological efficiencies (yield of fresh mushrooms as a percentage of the dry

weight of substrate at spawning) reported from diverse substrates range from 35-159%, considering a whole production cycle of about 70-80 days. Recent cultivation of *P. tuber-regium* has opened up the possibility to extend commercial production from fruit bodies to edible sclerotia (a firm, frequently rounded, mass of mushroom tissue, resistant to unfavourable environmental conditions). A processing technology is fundamental for commercial production as oyster mushrooms undergo rapid post-harvest deterioration at room temperature. Mushrooms are normally cooled down in specific facilities to retard fruit-body metabolism, and then sent to the fresh market. Further processing, *e.g.* canning or drying, can be carried out depending on marketing strategies. After mushroom cultivation, spent substrates can be recycled as organic fertilizer, animal feed, substrate for other cultivated mushrooms, as well as for biogas or enzyme production, paper manufacture, and production of cardboard.

World production. The cultivation of oyster mushrooms has spread out rapidly worldwide, as a natural result of their remarkable biological features and the versatile technology available. In general, two main trends can be identified: 1) Private commercial enterprises operating primarily on a large or small scale for accumulation of capital, and involving intensive cultivation tending to be highly mechanized (**Fig. 2**); and 2) Rural production for satisfying regional needs, which is performed through rustic cultivation methods. Developing countries have benefitted by the latter trend, in terms of its local contribution to food production, rural development, and sustainable agriculture. Recent figures have revealed that oyster mushroom production increased 442% during the period 1986-1991, reaching about 917,412 tons (fresh weight) in 1991. China, South Korea, Japan, and Indonesia are major producers. At present, *Pleurotus* spp. has become the second most important cultivated mushroom, accounting for about 22% of the total world production.

Nutritional and medicinal attributes. The protein content of oyster mushrooms can be considered as their main nutritional attribute. Average values ranging from 10.5-30.4%, on a dry weight basis, have been reported. The concentration of essential amino acids varies from 33.4-46.0 grams/ 100 grams of corrected crude protein, showing significant amounts of lysine, leucine, and methionine. The fat content reported is 1.1-2.2% on a dry weight basis, having a high proportion of unsaturated fatty acids (79.3%). The carbohydrate content varies from 46.6-81.8% on a dry weight basis. Main vitamins present in 100 g dry weight of oyster mushrooms are thiamine (1.16-4.80 mg), niacin (46.0-108.7 mg), and ascorbic acid (7.4 mg). Fibre (7.4-27.6% on a dry weight basis), and minerals (potassium, phosphorus, iron, copper, zinc) are also present in good proportion. Several compounds from oyster mushrooms, potentially beneficial for human health, have been

Fig. 2. Intensive cultivation of oyster mushrooms in a vertical container.

isolated and studied: 1) Polysaccharides showing strong antitumor activity, 2) A lectin called pleurotolysin with hemolytic properties, and 3) Extracts with hypotensive action on renal functions.

Disadvantages. Oyster mushrooms release enormous amounts of spores into the atmosphere of growing rooms. The inhalation of these spores induces allergic reactions in about 30-40% of farm workers. Spore allergy is characterized by influenza-like symptoms, which disappear without treatment in a few days if exposure is prevented. This major drawback, which has not discouraged the worldwide development of oyster mushroom cultivation, can be reasonably controlled wearing effective masks, and having efficient ventilation systems.

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